ANNEXURE I
RESEARCH PUBLICATIONS

A. PUBLICATIONS ASSOCIATED WITH THE THESIS

1. **Paul, S.**, Dutta, S., Chaudhuri, T. K. & Bhattacharjee, S. 2014. Anti-inflammatory and protective properties of *Aloe vera* leaf crude gel in carrageenan induced acute inflammatory rat models. ***International Journal of Pharmacy and Pharmaceutical Sciences*** 6(9): 368-371.
   [Innovare Academic Sciences, MP, India] **(ISSN: 0975-1491).**
2. Guha, P., **Paul, S.**, Das, A., Halder, B., Bhattacharjee, S., Chaudhuri, T. K. 2014. Analyses of human and rat clinical parameters in Rheumatoid Arthritis raise the possibility of use of crude *Aloe vera* gel in disease amelioration. ***Immunome Research*** 10: 081.
   [Published from Longdom Publishing SL, Spain under Longdom group, Belgium] [doi: 10.4172/17457580.1000081]; **(ISSN: 1745-7580).**
3. **Paul, S.**, Das, A. P. & Bhattacharjee, S. 2015. Rheumatoid arthritis: molecular basis and cures from nature. ***International Journal of Pharmacy and Pharmaceutical Sciences*** 7 (7): 30-39.
   [Innovare Academic Sciences, MP, India] **(ISSN: 0975-1491).**
4. **Paul, S.**, Dutta, T. & Bhattacharjee, S. 2015. Identification of phytocomponents from hexane soluble fraction of wild *Aloe vera* (L.) Burm. F. gel collected from University of North Bengal campus through GC-MS analysis. ***North Bengal University Journal of Animal Sciences*** 9: 9-13.
   [Department of Zoology, University of North Bengal, West Bengal, INDIA] **(ISSN: 0975-1424).**
5. **Paul, S.**, Dutta, T., Chaudhuri, T. K. & Bhattacharjee, S. 2017. Curative and protective properties of crude gel of *Aloe vera* from sub-Himalayan West Bengal in chronic and acute inflammatory rat models. ***Indian Journal of Traditional Knowledge*** 16(1): 121-127.
   [NISCAIR, CSIR, INDIA] **ISSN: 0975-1068 (Online); 0972-5938 (Print**).
6. **Paul, S.,** Modak, D., Chakraborty, A. K., Sen, A., & Bhattacharjee, S. 2018. *In vivo* and *in silico* approaches to investigate the toxicological and analgesic properties of unprocessed *Aloe vera* gel in experimental rat models. ***Archives of Biological sciences*** 70(4): 727-735.
   [Serbian Biological Society, Serbia]; **ISSN: 1821-4339 (online).**
7. **Paul, S.,** Modak, D., Nandi, D., Sarkar, A., Roy, J., & Bhattacharjee, S. 2021. *Aloe vera* gel homogenate shows anti-inflammatory activity through lysosomal membrane stabilization and down-regulation of TNF-α and Cox-2 gene expressions in inflammatory arthitic model. ***Future Journal of Pharmaceutical Science***. 7: 12.
   [Springer Nature, affiliated with Future University, Egypt;] **ISSN: 2314-7253 (online).**

B. OTHER PUBLICATIONS

1. **Paul, S.**, Sarkar, S., Dutta, T. & Bhattacharjee, S. 2016. Assessment of anti-inflammatory and anti-arthritic properties of *Acmella uliginosa* (Sw.) Cass. based on experiments in arthritic rat models and qualitative GC/MS analyses. ***Journal of Intercultural Ethnopharmacology*** 5(3): 1-6.
   [SAGEYA Publishing Company, Ankara, Turkey] **(ISSN: 2146-8397).**
2. Modak. D., **Paul, S.,** & Bhattacharjeee, S. 2017. Anti-inflammatory activity of *Acmella uliginosa* (sw.) Cass. flower methanolic extract on membrane stabilization and protein denaturation: an *in- vitro* evaluation. ***North Bengal University Journal of Animal Sciences*** 11: 61-69.
   [Department of Zoology, University of North Bengal, West Bengal, INDIA] **(ISSN: 0975-1424).**
3. **Paul, S.,** Modak, D., Dutta, S., Chaudhuri, T.K., & Bhattacharjee, S. 2019. Evaluation of the Effectiveness of *Acmella uliginosa* (Sw.) Cass. Flower methanolic extract in pain amelioration and memory impairment in the experimental rat models: Search for an Alternative Remedy Over Opioid Pain-Killers. ***Pharmacognosy Magazine*** 15: S335-345.
   [Wolter-Kluwer Health Pvt Ltd, India]; **ISSN: 0973-1296 (Print), 0976-4062 (online).**
4. Das, K., **Paul, S.,** Modak, D., Mahato, B., Bhattacharjee, S., & Roy, M.N. 2019. Synthesis characterization and comparison of host-guest complexes of β-CD with vitamins explored through their biological activities. ***ACS Omega***. 4(4): 7151-7175.
   [American Chemical Society, USA] **ISSN: 2470-1343 (online).**
5. Lala, M., Modak, D., **Paul, S.,** Sarkar, I., Dutta, A., Kumar, A., Bhattacharjee, S., & Sen, A. 2020. Potent bio-active methanolic extract of wild orange (*Citrus macroptera* Mont.) shows anti-oxidative, anti-inflammatory and anti-microbial properties in in vitro, in vivo and in silico studies. ***Bulletin of National Research Centre.*** 44: 81.
   [National Research Centre, Egypt; Springer Nature] ISSN: **2522-8307 (online).**
6. Modak, D., Paul, S., Sarkar, S., Thakur, S., & Bhattacharjee, S. 2021. Validating potent anti-inflammatory and anti-rheumatoid properties of *Drynaria quercifolia* rhizome methanolic extract through *in vitro, in vivo, in silico* and GC-MS based profiling. ***BMC Complementary Medicine and Therapies***. 21: 89.
   [Biomed central limited; Springer Nature] **ISSN: 2662-7671 (online).**

ANNEXURE II

APPROVAL OF ANIMAL ETHICAL COMMITTEE

CERTIFICATE

This is certified that the Project Title
Exploration of Anti-Rheumatoid products from Herbal Resources of North-East India and Sub-Himalayan Region of West Bengal

has been approved by the IAEC.

Min Bahadur
Name of the Chairman/Member Secretary, IAEC

PRABIR KUMAR MI
Name of CPCSEA Nominee

14/8/13
Chairman/Member Secretary, IAEC

HEAD
Department of Zoology
University of North Bengal
District Darjeeling
West Bengal, Pin 734013

CPCSEA Nominee 14/8/1

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by the Office)

CERTIFICATE

This is certified that the Project Title

Exploration of anti inflamatory and anti arthritic properties of the rem(L) Burm.f. from Sub Himalayan west bengal, India

has been approved by the IAEC. vide no. IAEC/NBU/2013/02.

Min Bahadur
Name of the Chairman/Member Secretary, IAEC

Dr. Nihar Ranjan Bhuyan
Name of CPCSEA Nominee

12/9/18
Signature with date

Chairman
Institutional Animals
Ethics Committee (IAEC)
Department of Zoology
University of North Bengal
Siliguri, West Bengal

Chairman/Member Secretary, IAEC:

CPCSEA Nominee 12/9/18

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by the Office)

12/9/18 12/09/18 12/9/18 12/9/18 12/9/18

Chairman
Institutional Animals
Ethics Committee (IAEC)
Department of Zoology
University of North Bengal
Siliguri, West Bengal

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Original Article

ANTI-INFLAMMATORY AND PROTECTIVE PROPERTIES OF ALOE VERA LEAF CRUDE GEL IN CARRAGEENAN INDUCED ACUTE INFLAMMATORY RAT MODELS

SUBHASHIS PAUL[1], SOMIT DUTTA[2], TAPAS KUMAR CHAUDHURI[2], SOUMEN BHATTACHARJEE*

[1]Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, P. O. North Bengal University, Raja Rammohunpur, Siliguri, District- Darjeeling West Bengal 734013, India, [2]Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, P.O. North Bengal University, Raja Rammohunpur, Siliguri West Bengal 734013, India.
Email: soumenb123@rediffmail.com

Received: 29 Jul 2014 Revised and Accepted: 26 Aug 2014

ABSTRACT

Objectives: Current clinical treatment regimes for inflammatory diseases have different drawbacks including side effects of the drugs and the high cost of long term treatment. In the last few decades different promising herbal medicines have been explored for their anti-inflammatory and anti-rheumatic effects, but conclusive evidences are not available in the case of crude *Aloe vera* gel for its anti-inflammatory effects. The objective of the study was to document the protective and curative roles of orally administered and peritoneally injected crude wild *Aloe vera* gel in carrageenan-induced inflammation in a rat model.

Methods: Inflammation was induced by injecting 1% carrageenan in the left hind paw of Wistar albino rat. Crude, unprocessed *Aloe vera* gel was peritoneally injected and orally fed to experimental and control rat groups to investigate its effect on paw joint edema by measuring the paw circumference with vernier caliper. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell viability assay was performed to investigate the cytotoxic effect of the gel.

Results: Paw edema was brought to near normal levels in the experimental groups after the treatment with crude *Aloe vera* gel. Orally fed gel showed no cytotoxicity on macrophages and spleenocytes. Protective property of crude *Aloe* gel was also evident in both the experiments.

Conclusion: *Aloe vera* crude gel has both protective and curative properties against inflammation.

Keywords: *Aloe vera*, Carrageenan, Indomethacin, Medicinal plant, Paw edema, Pharmacology.

INTRODUCTION

Carrageenan induced inflammatory rat model is a standard model system for experimentation on acute inflammatory conditions. Generally non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressive drugs used in the relief of inflammatory diseases worldwide are often associated with severe adverse side effects like peptic ulcer and gastrointestinal bleeding [1]. In traditional medicine and Ayurveda, many plant products are used as anti-inflammatory agents to cure the inflammatory pain and swelling which still lack a proper screening process. In the northern region of West Bengal, traditional Ayurveda practice uses *Aloe vera* as a potent anti-inflammatory agent. The gel-like layer under the leaf of the plant, actually the parenchyma cells, is traditionally known to decrease inflammatory pains. *Aloe vera* (Family Xanthorrhoeaceae) is a stem-less or short-stemmed succulent plant growing upto 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up [2]. *Aloe vera* contains different phytochemical agents which are able to cure different disease symptoms [3]. The plant is used widely in dermal diseases and is a good laxative agent [4]. It also represents compounds responsible for anti diabetic [5], anti-oxidant [6], antimicrobial [7] and wound healing activities [8]. However, the experiment-based proof is still lacking regarding the anti-inflammatory properties of the crude unprocessed gel of this plant. Our study was aimed to evaluate the anti-inflammatory properties of different doses of aqueous crude extract of *Aloe vera* leaf gel to give a scientific base to the traditional claim.

MATERIALS AND METHODS

Collection of Plant Specimens

Wild *Aloe vera* (Class Magnoliopsida, Order Asparagales, Family Xanthorrhoeaceae) plants were collected from the sub-Himalayan Terai areas of northern West Bengal and were identified by Prof. A. P. Das, plant taxonomist in the Department of Botany, University of North Bengal [Accession no. 09884 (NBU)].

Preparation of Extract

Crude gel was collected by peeling out the outer cuticle and cutting out the gel aseptically into small pieces. The gel was weighed, mixed with distilled water (1:5 w/v) and then homogenized to create a homogenate. The sample was freshly prepared every time before use. It contained all the ingredients of the crude gel in the same proportion as it appears in the leaf. To know the dry weight of the gel (weight without water parts), each piece was dried separately in an air oven at 37°C for 48 hours and was then weighed.

Chemicals

Carrageenan was purchased from Hi-Media Laboratories Pvt. Ltd., (Mumbai, India). Indometacin or Indomethacin (Jagsonpal Pharmaceutical Ltd., New Delhi, India), the non-steroidal anti-inflammatory drug was purchased from local drug suppliers and used as the control drug of inflammation. RPMI-1640, fetal bovine serum (FBS), antibiotics and EZcount™ MTT assay kits were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

Experimental design

Studies were carried out using Wistar albino rats of either sex weighing 60 ± 15 g. They were maintained in the animal house of the Department of Zoology, University of North Bengal. The animals were clustered in six groups; each contained six rats for anti-inflammatory activity study (PC, NC, 125D, 250D, STDG, and PROG). Rats were maintained under standard laboratory conditions (temperature 25 ± 2°C) with normal daily cycle (12/12h). The rats were acclimatized to laboratory condition for 10 days before commencement of experiments. The study was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of

Experiments on Animals) of University of North Bengal, West Bengal, India. PC refers to Positive Control i. e. Normal rats, NC refers to Negative Control where inflammation was induced by 1% carrageenan but no treatment was done. In the anti-inflammatory test, the group 125D represents the group injected with 1% carrageenan and treated orally with 125 µl aqueous extract of *A. vera* corresponding to approximately 25 gm wet gel/kg body weight (20 mg dry weight/kg body weight), 250D represents 1% carrageenan injected rat treated with 250 µl aqueous extract of *Aloe vera* gel orally corresponding to approximately 50 gm wet gel/kg body weight (40 mg dry weight/kg body weight). PROG or protection group was treated with *A. vera* 125 µl doses orally once a day for 7 days prior from the day of injection. STDG or Standard group represents carrageenan-induced inflammatory rats treated with 60 µl of 10mg/ml Indomethacin dose equivalent to 10mg/kg b. w., known to be a standard drug of inflammatory conditions [9].

Determination of the cytotoxic effect of the extract on peritoneal macrophages and spleenocytes were performed by MTT assay. Same amounts of crude gel, present in the 125 µl and 250 µl homogenized solution of 1 gm/5 ml (w/v) in dH_2O were applied to experimental rat groups for MTT assay and designated as dose groups M1 and M2 respectively. M1 contained 32.4 mg of wet gel homogenate (corresponding to 125 µl aqueous homogenate) and M2 contained 64.8 mg of wet gel homogenate (corresponding to 250 µl aqueous homogenate), each prepared in 50 µl of dH_2O. The PROG group rats were tested for the protective activity of the gel when fed orally 125 µl of gel solution (corresponding to 1 gm/5 ml in dH_2O) continuously for 7 days prior to the experiment. No gel was further used in the culture medium for this group directly. A control group with untreated cells (C) was included to compare the data of the test and protection groups.

The doses of *A. vera* gel was calculated on the basis of the optimum dose taken by human, which is 50 grams per day for 60kg body weight.

Carrageenan induced paw edema

The anti-inflammatory activities of the aqueous extracts were determined using the methods described by others [10, 11, 12]. In 125D, 250D and STDG dose groups, the extracts were injected peritoneally 30 min prior to induction of oedema by administering 0.1 ml of 1% w/v carrageenan in the sub-plantar region of rat left hind paw. Protection group (PROG) received carrageenan injection 30 minutes after the oral feeding of *Aloe vera* on the experimental day. The degree of paw circumference of all the groups was measured (in millimeters) using a vernier calliper after 30, 90, 150, 210 minutes (0.5h, 1.5h, 2.5h, 3.5 h) of carrageenan injection. The Percentage of Inhibition (PI) was calculated using the following equation: PI (%) = [(V_t – V_0) Negative Control - (V_t – V_0) Treatment Group / (V_t – V_0) Negative Control] X 100, where V_t = final reading of paw circumference, V_0 = Initial reading of paw circumference [13].

MTT cytotoxicity assay

Rats were sacrificed under proper anesthesia and spleens were collected. Macrophages were collected from the peritoneal exudates by flushing the region with cold RPMI-1640. Cell suspensions were prepared at a concentration of 2 X 10^6 cells/ml following kit manufacturer's instructions. One hundred microlitre (100 µl) cell suspension was added to 12 µl of nutrient supplement containing 50 U/ml penicillin, 50 U/ml streptomycin, 50U/ml nystatin and 10% FBS. In 96 well plates, all four groups were columned with 6 replicates. Fifty microlitre (50 µl) of *Aloe* extract was added in each well along with 112 µl of such suspension and 10 µl of MTT, and then incubated for 4 hours. Absorbance was measured at 570 nm using BioRad I Mark microplate reader, BioRad, USA [14], [15].

Statistical Analysis

All statistical analyses were done using the softwares MS-Excel and Kyplot ver 2.0 beta. In Kyplot analysis, the data represented Mean ± S. E. M which was analyzed by one way ANOVA. The results were considered significant when $p <= 0.05$.

RESULTS

It is evident from the graphical presentation that indomethacin showed the best PI of 92.7% after 3.5 hours (210 min) of carrageenan injection,. Treatment groups 125D and 250D also effectively repressed paw swellings by 58.69% and 74.09% respectively. The PROG group also showed a substantial effect by reducing paw swelling by 82.6% which was even better than the 125D and 250D dose groups (Table 1 and Figure 1).

Table 1: Table showing paw circumference (mm) of different groups at different time intervals (h). All data represent Mean ± S. D Percentage of inhibition (PI) is mentioned in the brackets.

Groups	0 h	0.5 h	1.5 h	2.5 h	3.5 h
Positive Control (PC)	32.35±0.114	32.73±0.275	32.34±0.232	32.33±0.270	32.33±0.173
Negative Control (NC)	32.36± 0.709	37.94±0.344	38.82±0.553	38.22±0.448	38.15±0.287
Standard Group (STDG)	32.06±0.697	36.37±1.00	35.03±0.239	33.19±0.681	32.46±0.540 (92.7%)
Protection Group (PROG)	32.15±0.430	34.85±0.718	33.62±0.534	33.52±0.726	33.11±0.558 (82.6 %)
125 µL dose Group (125D)	31.77±0.923	35.36±0.779	35.38±0.637	34.51±0.785	34.05±0.711 (58.69%)
250 µL dose Group (250D)	31.93±0.776	37.74±0.315	36.48±0.369	34.79±0.645	33.36±0.774 (74.09%)

In the MTT assay, both peritoneal macrophage and spleenocyte cultures of M2 groups showed higher cell cytotoxicity than that of the M1 groups. Here again, the PROG group showed the best result with no cytotoxicity or some protective properties (Figure 2, A and B respectively).

DISCUSSION

Carrageenan-induced paw edema is an established model for *in vivo* study of anti-inflammatory activities. The early phase of carrageenan induction is mediated by the histamines and serotonins; the surrounding damaged tissues also showed an increased prostaglandin synthesis [9]. The later stage is mediated by bradykinins, polymorphonuclear cells and macrophage-secreted prostaglandins [9]. Prostaglandins elevate the temperature of paw and causes inflammation and pain. NSAID drugs like aspirin, ibuprofen or indomethacin inhibits the enzyme prostaglandin H_2 synthase (also known as cycloxygenase or COX) which catalyzes an early step of prostaglandin synthesis. There are evidences that high polysaccharides and anthroquinones present in *A. vera* can act as a pro-oxidant and pro-inflammatory product.

Polysaccharides between 5- and 400-kDa were found to exhibit the most potent macrophage-activating activity, as determined by increased cytokine production, nitric oxide (NO) release, expression of surface markers, and phagocytic activities. Talmadge *et al* (2004) purified a high-molecular-weight fraction of *A. vera* and showed the increased haematological and the hematopoietic activity compared to the gel starting material [16].

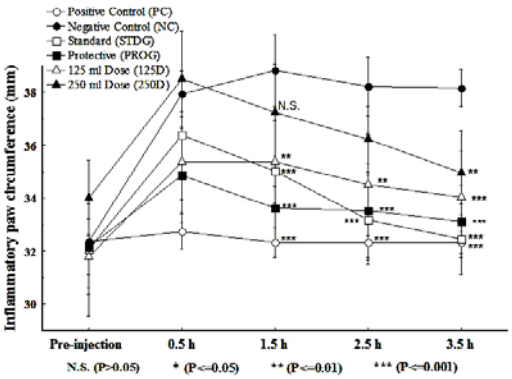

Fig. 1: Graphical presentation of paw circumference (mm) with respect to time in different rat groups. * showing the level of significance. * refers to most significant value.**

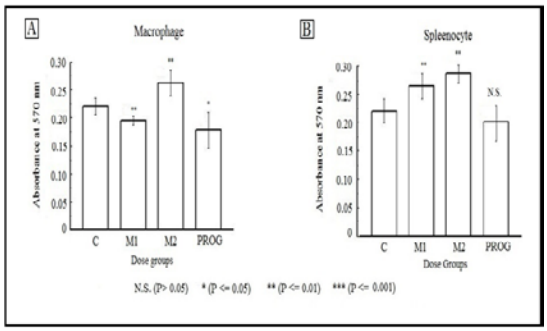

Fig. 2: Peritoneal macrophage (A) and spleenocyte (B) MTT assay of different dose groups. M1 and M2 represent 125 and 250 µl of Aloe dose equivalents.

We have recorded an increased level of inflammatory responses at the beginning of anti-inflammatory test (0.5 h interval) in the high dose group (250D). The high concentration of anthroquinones, like aloe-emodin and high amount of polysaccharides present in the crude *Aloe*-gel may be responsible for the flaring up of immune system in case of 250D groups [3] (see Figure 1). It appears that a synergistic role is played by the polysaccharides and anthroquinone derivatives that may be instrumental in the initial trigger of the immune response which then subsequently inhibits prostaglandin synthesis as the concentration goes down. A sharp decline of paw circumference during 0.5 to 3.5 h period in the 250D group supports the hypothesis. On the other hand, a lower amount of injected polysaccharides and anthroquinone in the 125D group do not induce such flaring up of immune system but protects the initial swelling and subsequently act significantly in decreasing the inflammatory symptoms in the experimental rats.

MTT cell viability tests were performed to investigate the cytotoxic effects of *Aloe* crude gel on peritoneal macrophages and spleenocytes collected from the experimental groups. In the MTT assay, it is evident that addition of higher dose of plant extract increased the death rate of the cells. The exact reason behind it is to be studied in detail to elucidate the molecular pathways of such cytotoxicity. However interestingly, we have observed that the animals (PROG group) that were fed orally before the experiment showed no toxic effects in the culture, rather the Aloe-gel showed some protective property on spleenocytes (Figure 2B). This finding confirms that the gel in low dose may act in a synergistic way by maintaining the normal immune status in one hand and by suppressing the inflammatory activity on the other hand. Therefore, our results suggest that the initial immunostimulatory activity of the gel is probably due to the presence of polysaccharides like acemannan, which can initiate macrophage activation and subsequently cytokine production when the gel is in high amount [17]. The later inhibitory effect of the aqueous extracts of *A. vera* on scarrageenan-induced paw edema may occur due to the suppression of the release of mediators including histamine, serotonin, bradykinin and prostaglandins that are responsible for the first and the second phase of acute inflammation by other bioactive compounds present in the gel, especially when the gel is present in low dose. The inhibitory effect of *Aloe vera* extract on carrageenan-induced inflammation could also be mediated via inhibition of cyclooxygenases (COX) [18].

Phytochemical analysis of *Aloe vera* has revealed the presence of flavonoids, anthraquinones, saponins. Saponins and flavonoids have previously been reported to have anti-inflammatory activities [3]. Such compounds may be responsible in part for the described anti-inflammatory activity of *A. vera* extract. However, a high dose of *A. vera* may elicit a different role in organism's body by inducing some serious inflammatory and pro-oxidant response as evidenced in the MTT assay. Low dose of the plant gel is suggested to be better for consumption; however the dosage has to be standardized accordingly for application in human.

CONCLUSIONS

Our data documents that *Aloe vera* crude unprocessed gel can reduce the inflammatory pain very efficiently if consumed daily. However, high dose may have some cytotoxic activity. It is also suggested that the consumption of low dose of *A. vera* orally in a regular fashion is better in case of human as repeated peritoneal injection may become inhuman or unethical.

CONFLICT OF INTERESTS

The authors declare no conflict of interest in the outcome of the study.

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Authors want to acknowledge Prof. A. P. Das, Department of Botany, University of North Bengal for the identification of the plant. The authors also thank the Department of Zoology for providing the animal house and instrument facilities for the research purpose.

REFERENCES

1. Valiollah H, Seyed ES, Mojtaba H. Anti-inflammatory and analgesic properties of *Heracleum persicum* essential oil and hydroalcoholic extract in animal models. J Ethnopharmacol 2009;124:475-80.
2. Surjushe A, Vasani R, Saple DG. *Aloe vera*: a short review. Ind J Dermatol 2008;53:163-66.
3. Shelton RM. *Aloe vera*: its chemical and therapeutic properties. Int J Dermatol 1991;30(10): 679–83.
4. Eshun K, He Q. *Aloe Vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review. Crit Rev Food Sci 2004;44(2): 91-6.
5. Tanaka M, Misawa E, Ito Y, Habara N, Nomaguchi K, Yamada M, *et al*. Identification of five phytosterols from *Aloe vera* gel as anti-diabetic compounds. Biol Pharm Bull 2006;29(7): 1418-22.
6. Hu Y, Xu J, Hu Q. Evaluation of Antioxidant Potential of *Aloe vera* (*Aloe barbadensis* Miller) Extracts. J Agr Food Chem 2003;51:7788-91.
7. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World J Agri Sci 2009;5(5): 572-6.
8. Davis RH, Leitner MG, Russo JM, Byrne ME. Wound healing. Oral and topical activity of *Aloe vera*. J Am Podiatr Med Assoc 1989;79(11): 559-62.
9. Gupta M, Mazumder UK, Gomathi P, Thamil Selvan V. Antiinflammatory evaluation of leaves of *Plumeria acuminate*. BMC Compl Altern Med 2006;6(36): 1-6.
10. Jain AK, Sharma P, Sikarwar MS. Evaluation of anti-inflammatory and anti-pyretic activity of total alcoholic extract of *Croton sparsiflorus* and its fractions. J Pharm Res 2010;3(5): 1149-51.
11. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in the hind paws of the rats as an assay for anti-inflammatory drugs. Exp Biol Med 1962;111:544-47.

12. Shrinivasan K, Muruganandan S, Lal J. Evaluation of anti-inflammatory activity of *Pongamia pinnata* leaves in rats. J Ethnapharmacol 2001;78:151-7.
13. Foyet HS, Abdou BA, Ponka R, Asongalem AE, Kamtchouing P, Nastasa V. Effects of *Hibiscus asper* leaves extracts on carrageenan induced oedema and complete Freund's adjuvant-induced arthritis in rats. J Cell Anim Biol 2011;5(5): 69-75.
14. Shang HF, Cheng HC, Liang HJ, Liu HY, Liu SY, Hou WC. Immunostimulatory activities of yam tuber mucilages. Bot Stud 2007;48:63-70.
15. Dey P, Dutta S, Chaudhuri TK. Evaluation of erythrocytic membrane stabilizing activity and cytotoxic effect of the areal tubers of *Dioscorea alata* L of North-Eastern region of India. J Pharm Sci Innov 2013;2(3): 1-4.
16. Talmadge J, Chavez J, Jacobs L, Munger C, Chinnah T, Chow JT, *et al.* Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity. Int Immunopharmacol 2004;4:1757–1773.
17. Boudreau DM, Beland FA. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe Vera*. J Env Sci Heal 2006;24:103–54.
18. Selvam C, Jachak SM. A cyclooxygenase (COX) inhibitory flavonoids from the seeds of *Semecarpus anacardium*. J Ethnopharmacol 2004;95:209-212.

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Review Article

RHEUMATOID ARTHRITIS: MOLECULAR BASIS AND CURES FROM NATURE

[1]SUBHASHIS PAUL, [2]ABHAYA PRASAD DAS, [3]SOUMEN BHATTACHARJEE*

[1,3]Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, P. O. North Bengal University, Raja Rammohunpur, Siliguri, District-Darjeeling, West Bengal, 734 013, India, [2]Plant Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal, Raja Rammohunpur, Siliguri, West Bengal 734013 India
*Email: soumenb123@rediffmail.com

Received: 22 Apr 2015 Revised and Accepted: 22 May 2015

ABSTRACT

Incidences of arthritic diseases in human have seen recent increases which are thought to have resulted from a complex interplay of several factors, such as changes in lifestyle, nutritional insufficiencies, aging and genetic factors. These putative factors possibly lead to different arthritic diseases in humans affecting 2-5% of the total population in India. This group of diseases results in serious malfunction and structural abnormalities in the patient body leading to permanent and substantial immovability of joints. Conventional medicinal systems usually elicit various side effects in which the defence mechanism of the body i.e. the immune system is compromised. In the last few decades many alternative medicinal systems have been developed that show promising effects on treating such diseases. Many purified compounds from natural origin, both from plants and animal sources have shown promise and many new compounds are continuingly being identified which have no marked side effects. In the light of modern science and technology, different natural products and ethnic practices that ensure health, seem to be the best weapon to combat these diseases. Endemic as well as naturalized plants from India have been screened by several groups for their anti-arthritic activities. The review summarizes our current knowledge on the molecular basis of Rheumatoid Arthritis and discusses the efficacious roles of those natural products, especially of plant origin, in arthritic conditions.

Keywords: Rheumatoid arthritis, Medicinal plant.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder affecting principally the flexible (synovial) joints and sometimes adjacent tissues and organs. In severe condition of the disease, if kept untreated, it may result in a disabling and painful condition, which can lead to substantial loss of function and mobility. This debilitating disease is prevalent in all over the world including in Indian population. Considering common symptoms, there is no strong difference between symptoms of RA and other joint arthritic diseases like gout, osteoarthritis or ankylosing spondylytis, so there lays a great difficulty in treatment. Early theories on the RA pathogenesis focused on autoantibodies and immune complexes [1]. But recent reviews focus on autoantibodies as well as T-cell mediated antibody responses and T-cell independent cytokine networks [1]. Cytokines are now regarded as the very important regulating factors in rheumatoid arthritis. Studies from cytokine network interruption by anti-TNF-α antibody have shown positive results in collagen-induced arthritic rat models [2]. Cytokines are also responsible for the bone destruction near the synovial joint where Cytokines like IL-1, IL-17 have been shown to be responsible for matrix destruction [3]. Till now, no specific drug is available for treating RA. Presently few immunosuppressive drugs like glucocorticoids are found to be effective to suppress the disease expression and inflammation of the synovial joint, which are disadvantageous due to their side effects [4].

Ayurveda or ayurvedic medicinal system, a system of traditional medicine native to India, is a form of complementary alternative medicine that generally depends on different plants and plant-derived products. However, the science underlying the working principle of such herbal products are not revealed in most cases and they face a problem of improper clinical trials. Many common Indian plants are being studied in different laboratories for their anti-arthritic and anti-inflammatory properties, which include Green tea (*Camellia sinensis*), Ashoka (*Saraca asoca*), Tulsi (*Ocimum sanctum*), Devdaru (*Cedrus deodara*) and many others. Few plants have been found to be effective in reducing inflammation and arthritic swelling. Several other plants or plant products are also being explored for their efficacy. Cytokine gene regulation, cellular signalling mechanism and mechanism of bone destruction are being extensively studied for that purpose.

Classification criteria are needed in population studies to establish the epidemiology of the disease, to define entry criteria for clinical trials, to evaluate whether individuals have the specific disease, and to train medical students. The methodology of development of classification criteria has gradually been refined over the last 50 years or so. According to American College of Rheumatology (ACR) criteria (1987) a total of 41 rheumatologists from university and private practice were asked to provide details of patients aged 16 years and older whom they considered to have RA and of the next consecutive patients who did not have RA or a localized rheumatic condition [5]. Based on those details, the following table (table 1) presents the classification criteria for arthritic diseases:

Table 1: Classification criteria for arthritic diseases according to the American College of Rheumatology (ACR) criteria, proposed in 1987 [5]

Types		Description
1.	Morning stiffness	Morning stiffness in and around the joints, lasting at least for 1 hour before maximal improvement.
2.	Arthritis in three or more joint areas	Soft tissue swelling or fluid (not bony overgrowth) observed by a physician, present simultaneously for at least 6 weeks.
3.	Arthritis of hand joints	Swelling of wrist MCP or PIP for at least 6 weeks.
4.	Symmetrical arthritis	Simultaneous involvement of the same joint areas (defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry for at least 6 weeks.
5.	Rheumatoid nodules	Subcutaneous nodules over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician.
6.	Rheumatoid factor	Detected by a method positive in less than 5% normal controls.
7.	Radiographic changes	Typical of RA on postero-anterior hand and wrist radiographs which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

Rheumatoid arthritis is defined by the presence of four out of the seven criteria (table 1) that were developed by the American College of Rheumatology (ACR) in 1987 [5]. Symptoms can vary greatly depending on the type of arthritis and the individual concerned. In the most common forms, joints of the knees, fingers, wrists, ankles, hips, and/or elbows become stiff, swollen, tender, and painful. This pain can be greater in the morning, or get worse as the day goes on. Often fatigue occurs and is sometimes accompanied by a sleep disorder. Painless lumps under the skin, called rheumatoid nodules, can be felt in RA. In juvenile rheumatoid arthritis, fever, rash, and anaemia occur. The symptoms can also be cyclical that appear, disappear and reappear with time, where reappearance is often termed "flare up" phase. This chronic pain ranges from mild to severe and can last for the lifetime.

As it is an autoimmune disease, genetic factors are predisposed in person to initiate the disease. For instance, in some people with RA, some specific alleles of HLA genes that affect the immune system are found in high frequency. Along with that, lifestyle also plays an important role in developing RA. The onset of symptoms can occur at a wide range of ages spanning from youth to the elderly although most forms become more common with ages. Older people can get arthritis because of general wear and tear on joints, as well as a direct effect of previous injuries like injuries related to sports. Likewise, obesity can also increase stress to joints and can, therefore, contribute to arthritis. RA is more prevalent in women, while other types of arthritic diseases, like gout, occur more frequently in men, therefore there seems to exist a role of hormones of the affected individual in eliciting the disease.

Epidemiology of rheumatoid arthritis

The rheumatoid arthritic disease affects 0.5–1% of the population in the industrialized world and commonly leads to significant disability and quality of life is consequently reduced [6]. It is 2 to 3 times more frequent in women than in men and can start at any age, with a peak incidence between the fourth and sixth decade of life. Its prevalence in India ranges from 0.28% in the urban population to 0.55% in the rural population as per the survey done [7] but the actual incidence range may vary from 2-5% in the country.

Pathogenesis of rheumatoid arthritis

RA is regarded as an autoimmune disease. The disease has some strong association with several types of auto-antibodies like rheumatoid factor (RF), anti-perinuclear factor (APF) and anti-keratin antibodies (AKA), anti-collagen antibodies, antibodies to nuclear antigens such as Epstein-Barr nuclear antigen and RA33, anti-Sa, and anti-p68 antibodies [4]. Most of these antibodies react/interact with citrullinated proteins [8]. But it is still unknown whether such autoantigens initiate the T-cell activation cascade from the very beginning by any signalling pathway to form inflammatory changes, or contribute to the disease at a later stage to boost and/or perpetuate the disease.

RA has a polygenic basis and 31 risk loci have been identified. The presence of some of those loci in different combinations increases the chance of the disease by several folds [9]. In such genetically predisposed individuals the innate immune response is possibly activated by events such as the triggering of dendritic cells (DCs) through TLRs (several of which are known to be expressed on synovial cells) by exogenous material or by a combination of foreign stimuli together with autologous antigens [1, 10, 11]. Synovial dendritic cells activated by TLR ligands can migrate to lymph nodes where primed T-cells can be biased towards the T_H1 phenotype. The synovial membrane is infiltrated by T-cells, which produce IL-2 and IFN-γ. So the T-cell response attains a T_H1 bias. Further, T_H17 cells may be an important effect or T-cell subset in RA [12]. These T-cells activate monocytes, macrophages and synovial fibroblasts through cell–cell contact and activation by different cytokines, such as IFN-γ, TNF-α and IL-17 [13, 14]. These immune cells then overproduce pro-inflammatory cytokines — mainly TNF-α, IL-1 and IL-6 — which seem to constitute the pivotal event leading to chronic inflammation.

Many other cytokines and chemokines are involved in RA progression, including IL-15, IL-18 and angiogenic factors. These molecules, after binding to their specific receptors, can regulate various signal transduction cascades, such as the MAPK, nuclear factor-κB (NF-κB) or Jak/STAT pathways, which ultimately lead to the activation/inhibition of transcription factors or subsequent induction of genes responsible for mediation of inflammation and tissue degradation. Among these products are various cytokines, chemokines and tissue-degrading enzymes, such as the matrix metalloproteinases (MMPs), cell-surface molecules that enhance cell activation and cell–cell interactions, such as co-stimulatory and adhesion molecules like selectins, integrins, that are involved in inflammatory pathways creating important intercellular interactions pivotal in the inflammatory responses. Apart from the inflammatory infiltration in the peri-vascular and sublining regions of joints, the lining layer consisting of synoviocytes become hyperplastic, and transforms into an aggressive tissue at the cartilage–bone junction, the 'pannus', which contains osteoclasts which is responsible for major bone degradation. While degradation of cartilage is mainly mediated by a plethora of metalloproteinases present in the joint, bone destruction is mediated by the generation and activation of osteoclasts, an event that apparently does not occur in non-destructive arthritis [15, 16]. The role of B-cells and autoantibodies, and/or immune complexes may be important in the propagation and enhancement of the inflammatory process. B-cells function as effective antigen presenting cells and also produce the autoantibodies against RF citrullinated proteins [17]. A schematic presentation of the events taking place during RA is shown in the fig. 1.

A growing body of evidence indicates the possible role of highly reactive products of oxygen and nitrogen, termed as free radicals, in the pathogenesis of RA as well as other degenerative diseases [18]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced endogenously during aerobic metabolism at the sites of chronic inflammation. ROS such as superoxide radical, hydroxyl radical and hypochlorous acid contribute significantly to tissue injury in RA. In addition, activated leukocytes also produce ROS. Superoxide radicals and hydrogen peroxide do not directly damage the majority of biomolecules, but they are converted into the highly reactive hydroxyl radicals, which react with almost all molecules in living cells. ROS can directly or indirectly damage basic articular constituents and lead to the clinical expression of the inflammatory arthritis. Synovial cavity damage correlates with fluctuating oxygen pressure in the joint, over production of ROS, lack of oxygen-processing enzymes and free radical-scavenging molecules has been reported in RA. Oxidative stress exacerbates inflammation and worsens joint tissue.

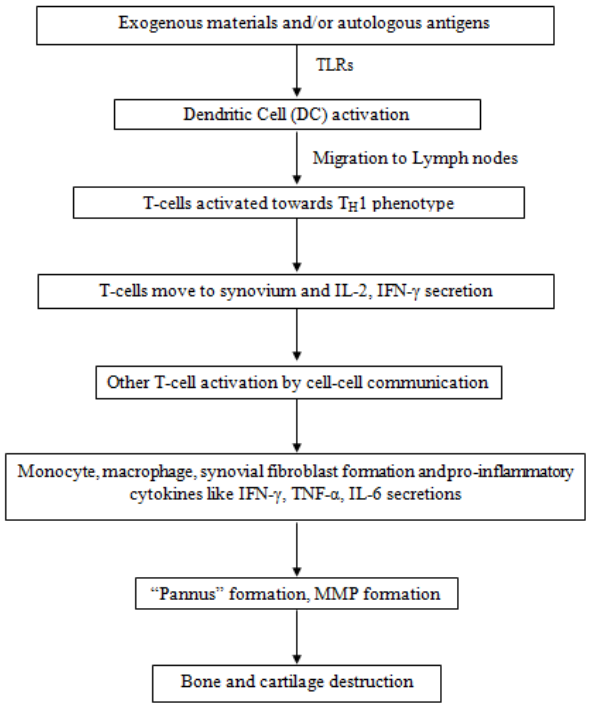

Fig. 1: Diagrammatic representation of the events of disease progression in RA

The normal equilibrium between ROS production and anti-oxidant system of the cell is disturbed due to oxidative stress, thus resulting in the damage to vital cell components such as proteins, DNA and membrane lipids. There are several studies demonstrating increased levels of malondialdehyde and decrease in the activities of catalase in RA patients. Similarly, glutathione reductase activities also get disturbed in the synovial fluid of patients. Moreover, the levels of thioredoxine, which is a marker of oxidative stress, are significantly higher in the synovial fluid of RA patients. Production of nitric oxide (NO) is also up regulated in arthritic tissue [18].

Genetic basis of rheumatoid arthritis

MHC and Rheumatoid arthritis

An extensive study of human genetics has been done to identify regions of the genome that are reproducibly associated with RA risk. Based on genetic information, from a large number of patients, the *HLA-DRB1* "shared epitope" alleles were first to be recognized in the late 1970's, followed by the discovery of *PTPN22* in 2004. In 2007, other three new loci have been identified (*STAT4*, *TRAF1-C5*, and *6q23*). Several other loci are showing promise, but the involvement of these loci with RA is not confirmed yet (*CD40*, *CTLA4*, and *PADI4*) [19].

The human leukocyte antigen (HLA) region remains one of the most powerful disease risk genes in different autoimmune diseases including RA. Several allelic variants of HLA-DRB1 genes have been associated with RA which supports a role for T-cell receptor-HLA-antigen to interact in the process. Disease-associated HLA-DRB1 alleles are polymorphic and certain allelic variants are preferentially high in the diseased population. Based on studies on the patients with the severe diseased condition a gene dose effect of HLA-DRB1 alleles has been suggested [20, 21]. Therefore, polymorphisms in HLA genes are being explored to find diagnostic tools for a rheumatoid syndrome. Besides HLA polymorphisms, other risk genes will be helpful in defining genotypic profiles correlating with disease phenotypes.

HLA genes in conjunction with other genetic determinants may lead the patient body to a certain pathway of synovial inflammation. Patients may or may not develop extra-articular manifestations, which are critical in determining morbidity and reducing healthy lifespan. HLA genes, complemented by other RA risk genes, are likely involved in shaping the T-cell repertoire. On the other hand, production of an unusual T-cell population characterized by the potential of vascular injury, as seen in extra-articular RA, is also accelerated by these genes [22].

Cytokines and rheumatoid arthritis

Cytokines are small polypeptide mediators of the immune and inflammatory responses. These molecules are secreted by almost all cell types at different titre in different time. Their action is generally at a paracrine or autocrine level. Cytokines function in a complex process with multiple relationships, making it difficult to predict the role of a single cytokine in any disease. The role of cytokines in the pathogenesis of RA has been the subject of multiple studies that generally agree on the overproduction of pro-inflammatory cytokines in the rheumatoid joint. There are several studies to monitor the roles of cytokines in different diseases. Most of these studies are based on immunohistochemistry or molecular biology techniques, which have detected the expression of cytokines (IL-α, IL-1b, IL-6, TNF-α, TGF-β) originating predominantly in the macrophage-fibroblast cells [23-26]. But it has been difficult to detect T-cell derived cytokines (mainly IL-2 and IL-4). However, these studies analyzed the synovial samples of patients with a long-standing disease treated with anti-rheumatic drugs which may alter the original profile of cytokine expression [27-29].

At present the mRNA expression of a broad spectrum of cytokines (IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, TNF-α, TGF-β) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been detected in the synovial tissues of RA patients. In both early and advanced stages of RA, it helps to know the cellular response and expression profiles of different cytokine molecules. The studies of the expression of HLA molecules during the progression of disease have been investigated to define precise cytokine–HLA network [27-29].

Role of cytokines in bone destruction

Studies using anti-TNF agents clearly show that they can slow down or prevent the progression of bone and cartilage damage in RA [30]. This activity probably involves suppression of osteoclasts-like cells in the joints [15]. Other cytokines like IL-1 and IL-17 are notable in regulating matrix degradation in animal models of arthritis [3]. The most exciting development in the pathogenesis of bone destruction in RA was the discovery of osteoclast-mediated bone resorption that is regulated by the RANK (receptor activator of nuclear factor (NF-kB) ligand or RANKL. RANKL is expressed by a variety of cell types involved in RA, including T-cells and synoviocytes. These cells, in the presence of cytokines like TNF-α and M-CSF, contribute to osteoclast maturation and activation. The soluble decoy receptor to RANKL, Osteoprotegerin (OPG), and RANKL are up regulated in RA, but normalize after treatment with TNF inhibitors [31]. The role of RANKL in inflammatory joint disease has been confirmed in several animal models. For instance, T-cell activation leads to an RANKL-mediated increase in osteoclasts and bone loss in rat adjuvant arthritis [32]. Osteoprotegerin administration to the arthritic animals blocks bone destruction but has a very little effect on inflammation. RANKL-knockout mice also have diminished bone erosion in arthritis models [33]. Cytokine pathways are involved in blocking the RANK/RANKL system that in the other hand blocks bone decay.

Mechanisms of cytokine gene expression and new therapeutic targets

As cytokines play a crucial role in any disease as a medium of cell-cell communication and cellular activation, cytokine regulation has been a key factor to regulate diseases like RA. For instance, NF-κB is activated in the synovium of patients with RA [34] which then regulates several genes, including TNF-α, IL-6, IL-8, inducible nitric oxidase synthase (iNOS) and cyclooxygenase-2 (COX-2), that contribute to inflammation. After stimulation of innate immunity or exposure to pro-inflammatory cytokines, the IκB kinase (IKK) signal complex is activated in synoviocytes, leading to phosphorylation of IκB [35]. IKKβ is both necessary and sufficient for induction of IL-6, IL-8 and intercellular adhesion molecule-1 (ICAM-1) gene expression. Targeting NF-κB is an effective therapeutic strategy in many animal models of arthritis. For instance, rat adjuvant-induced arthritis is suppressed by intra-articular gene therapy with dominant negative IKKβ adenoviral construct [36], while decoy oligonucleotides block streptococcal cell-wall arthritis [37].

The mitogen-activated protein kinases (MAPs) are also key regulators of cytokine and metalloproteinase production and could also be a target in RA. All three kinase families — extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 are expressed in rheumatoid synovial tissue [38]. All are constitutively expressed by cultured synoviocytes, and exposure to pro-inflammatory cytokines induces rapid phosphorylation. Upstream kinases that activate the MAP kinases, such as MKK3, MKK4, MKK6 and MKK7, are also activated in RA synovium and can form signalling complexes that integrate external environmental stresses to generate an appropriate cellular response. The MAP kinases have attracted considerable attention as potential therapeutic targets in RA. Pre-clinical studies show that p38 inhibitors are effective in murine collagen-induced arthritis, rat adjuvant-induced arthritis, and many other RA models [39]. A selective JNK inhibitor, SP600125, is mildly anti-inflammatory in the rat adjuvant-induced arthritis model but provides striking protection against bone and cartilage destruction [40]. Of the JNK isoforms, JNK2 is particularly important in arthritis because it is the dominant isoform expressed in synoviocytes. In JNK2-knockout mice, passive collagen-induced arthritis causes less cartilage damage compared to wild-type animals although clinical arthritis is still severe [41]. The transcription factor activator protein-1 (AP-1) also regulates many genes that participate in RA, including TNF-α and metalloproteinases.

High levels of AP-1 binding activity are detected in nuclear extracts of RA synovial tissue compared to osteoarthritis. Its components c-Jun and c-Fos are highly expressed in RA synovium, especially in the nuclei of cells in the intimal lining layer [42]. Pro-inflammatory cytokines can activate AP-1 activity in synoviocytes and lead to a

massive release of metalloproteinases. AP-1 molecules suppress collagen-induced arthritis and inhibit IL-1, IL-6, TNF-α, matrix metalloproteinase (MMP)-3 and MMP-9 production in synovial tissues [43]. One of the concerns with therapy directed at any of these key regulatory pathways is that they also participate in many normal cellular functions. The risks of toxicity or impaired host defence are significant potential problems because alterations in innate immunity or adaptive responses can be possible.

The advent of TNF inhibitors [44, 45] illustrates the success of applied translational research in RA, based on characterization of cytokine networks and studies suggesting that TNF-α production might serve as an autologous stimulus for other cytokines in RA synovium [46]. Although 40% of the patients have dramatic responses, the remainder have some evidence of persistent synovitis or minimal clinical benefit. IL-1Rα, a natural IL-1 antagonist, has also been approved for use in the United States of America. The response rates of IL-1Rα are less than that of TNF inhibitors, perhaps because IL-1Rα is a competitive antagonist that must be present in large excess. Additional cytokine-directed agents, such as anti-IL-6 receptor antibody, are also in clinical development, preliminary response rates being similar to TNF antagonists. Inhibition of IL-18 and IL-15 represents additional attractive approaches that could block T_H1 differentiation, production of inflammatory mediators, or TNF-α expression. Based on the T_H1 bias of T-cells in the synovium, treatment with T_H2 cytokines (IL-4, IL-10 and IL-13) was tested in many animal models of arthritis and has showed considerable promise [47]. But so far, administration of IL-10, which serves as a prototypic T_H2 cytokine, has met with only limited success [48].

Synthetic drug/plant extract & their efficacy in rheumatoid arthritis

There are two principal approaches to drug therapy for RA [4]:

- Symptomatic treatment with analgesics such as Acetaminophen and opioids, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), and intra-articular therapies such as glucocorticoids. These drugs only interfere with a small segment of the inflammatory cascade (e. g prostaglandin generation by cyclo-oxygenases [COXs]) but do not interfere with the underlying immuno-inflammatory events or retard joint destruction, and

- Disease-Modifying Anti-rheumatic Drugs (DMARDs) which 'modify' the disease process in all these respects, and once DMARDs are effective, no further symptomatic therapies are needed. Examples include methotrexate (MTX), sulfasalazine, leflunomide, hydroxychloroquine and few newer therapies such as anti-tumour necrosis factor (TNF)-α therapy (etanercept, infliximab and adalimumab), anti-CD20 therapy (rituximab) and abatacept.

All these NSAID and DMARD drugs are limited by low (<70%) response rate and induction (>30%) of severe adverse events. Some of the newer drugs are able to perform a little better in the remission rate and adverse events, but none of them have achieved ideal targets. This has been often cited as one of the reasons for increased use of complementary and alternative medicine (CAM) by patients suffering from RA. About 60% to 90% of patient populations have been reported to be using CAM from different countries [49].

According to the National Centre for Complementary and Alternative Medicine (NCCAM), "Complementary and alternative medicine is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine" [50].

The therapies and practices, included within the scope of complementary medicine field, can be broadly categorized into:

- Treatments in which people can administer themselves (e. g., botanical herbs, nutritional supplements, health food, meditation, and magnetic therapy),

- Treatments were administered by providers (e. g., acupuncture, chiropractic, massage, reflexology, and osteopathic manipulations), and

- Treatments people can administer under the periodic supervision of a provider (e. g., Tai Chi, yoga, homeopathy, and Ayurveda).

The world health organization (WHO) estimates that as many as 80% of the world population depends primarily on animal and plant based medicines. Of the 252 essential chemicals that have been selected by the WHO, 11.1% come from plants and 8.7% from the animals [50]. So there is an increasing need for research in this field.

Herbal medicine is the root of various traditional medicine systems around the world. Various traditional medicine systems around the world, including ancient Chinese and Indian medicinal system (consisting of two major branches—Unani and Ayurveda), rely heavily on herbs for health preservation and healing. Herbal medicines have been described in traditional texts and been used as anti-microbial, anti-inflammatory and anti-viral medicine for the cure of allergies, RA, infections, wound healing and fever. While many herbal extracts and formulations have undergone trials at different levels and have established their anti-arthritic potential, many need more research or remain partially explored.

At present, the current modalities for treating arthritis have not been shown to block, reverse or cure the disease. In these cases, disease flares up when the treatment is discontinued because the treatment is symptomatic. This has increased the interest of use of CAM therapies for the treatment of arthritis. However, much of the current research is focused on the identification, isolation and characterization of active principle(s) from crude extracts of known medicinal plants/herbs and animals/animal products, often overlooking the fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound and this may also help to nullify the toxic effects of individual constituents. The lack of sufficient clinical investigations too does not permit definitive conclusions to be drawn regarding the efficacy of plants and animals as CAM modalities in RA. However, several animal model based studies have helped in elucidating the potential mechanism of action of various CAM modalities. This encourages further studies but demands rigorous experimentation. There is a need for screening and scientifically evaluating number of known traditional medicinal plants and animals for providing newer and safer treatment options with minimum side effects. Once the underlying molecular mechanism for the observed anti-inflammatory and chondroprotective effects of nutraceuticals are elucidated, their health benefits may be fully exploited to develop new and better modalities for treating degenerative and inflammatory joint diseases.

Current knowledge on promising plant based medicines

***Camellia sinensis var. assamica* (J. W. Hart.) Kitam (Assam tea)**

Camellia sinensis (Family Theaceae) is native to east, south and south-east Asia, but it is today cultivated across the world in tropical and subtropical regions. The pharmacological properties of green tea are attributed to its high content of polyphenols/catechins, mainly epigallocatechin-3-gallate (EGCG). EGCG inhibits the transcription factor nuclear factor kappa-B (NF-κB), IL-1 induced phosphorylation of c-Jun-N-terminal kinase (JNK), expression and activities of matrix metalloproteinases MMP1 and MMP13 *in vitro* [51]. The catechin constituent of green tea was shown to inhibit the degradation of human cartilage proteoglycan and type II collagen. The effects of green tea were demonstrated in an animal model of inflammatory polyarthritis, wherein the collagen-induced arthritis (CIA) was ameliorated by prophylactic administration of green tea polyphenols (GTPs) in drinking water. In addition, the total immunoglobulins (IgG) and type II collagen levels were found to be lower in the serum and arthritic joints of GTP fed mice [18]. Immunostimulatory activity [52] and anti-microbial activity [53] of the plant is also promising.

***Curcuma longa* L. (Turmeric)**

Turmeric (Family-Zingiberaceae) is a commonly used colouring/flavoring agent with a long history of its use in Ayurveda for various medicinal conditions. It is native in south-east India and needs temperatures between 20-30 °C and a considerable amount of annual rainfall to thrive. The major component of turmeric is curcumin (diferuloylmethane) which constitutes approximately 90% of total curcuminoid content. Curcumin is a potent inhibitor of the common transcription factor NF-κB [54, 55]. Studies have also shown the inhibitory effect of curcumin on the arachidonic acid cascade (COX-2 and LOX) by inhibiting the catalytic activities of

phospholipases A2, Cγ1, and D. Curcumin also blocks the catabolic effects of IL-1 β induced upregulation of MMP-3, and IL-1β-induced decrease in type II collagen synthesis, that are known contributors in the pathogenesis of RA [56].

***Zingiber offcinale* roscoe (Ginger)**

Ginger (Family-Zingiberaceae) is indigenous to southern China, but now it is distributed worldwide and cultivated for different purposes. Ginger is a very commonly used dietary constituent worldwide, and is known to possess antioxidant, anti-inflammatory, antiseptic, and carminative properties. Ginger has a history of its use in Ayurveda for treating inflammatory and rheumatic diseases. The anti-inflammatory effects of ginger in treating arthritis are believed to be due to 6-gingerol, which is a pungent phenolic constituent of ginger. The 6-gingerol inhibits the LPS-induced NO production and effectively protects against peroxynitrite-mediated damage. Studies have shown that gingerols are excellent inhibitors of LPS-induced PGE2 production [57]. In some studies, RA patients experienced the marked reduction in pain after consumption of ginger [58, 59].

***Semecarpus anacardium* L. f. (Nut milk extract)**

Semecarpus anacardium (Family-Anacardiaceae) is a deciduous tree distributed in the sub-Himalayan tract and in tropical parts of India. In traditional medicine, it is highly valued for the treatment of gout, rheumatic pains, and cancer (60). The chemical constituents of the milk extract of *Semecarpus anacardium* include flavonoids, phenols, and carbohydrates. Studies have shown that *Semecarpus anacardium* or nut milk extract is effective against adjuvant arthritis (61**,** 62). The protective anti-oxidant role of flavonoids, is shown to be due to their inhibitory effects on the production of reactive oxygen species (ROS) by their free-radical quenching activities, and their potential to improve the levels of antioxidants in the body. Flavonoids have also been reported to exhibit anti-inflammatory activity by inhibition of phospholipase A2, thereby reducing the production of pro-inflammatory PGE2, and also by reducing the elevated levels of TNF-α and NO. Another protective effect of the plant is by enhancing the stability of the lysosomal membrane, thus preventing the rupture and release of lysosomal enzymes, which play a major role in erosive synovitis in RA (56). Furthermore, this plant has been shown to modulate both the humoral and cell-mediated immune responses along with its anti-inflammatory effects in adjuvant arthritic models. The humoral immunomodulatory response was explained by reversion of the elevated levels of IgG and IgA, and the cell-mediated immunomodulatory response by inhibition of T-lymphocyte migration to the inflamed joints.

***Saraca asoca* (Roxb.) Willd. (Ashoka)**

The original distribution of ***Saraca asoca*** (Family-Fabaceae) was in the central areas of the Deccan plateau of India, but now the plant is distributed throughout the country. Methanolic extracts of *Saraca asoca* has anti-inflammatory activity in rat arthritic models [63]. Treatment with *S. asoca* has also shown a significant reduction in the levels of both plasma and liver lysosomal enzymes. The protein bound carbohydrates and urinary collagen contents were also decreased at a significant level by the treatment of *S. asoca* methanol extract. Furthermore, treatment of *S. asoca* reduced the levels of pro-inflammatory cytokines in adjuvant-induced arthritic rats [64].

***Ananas comosus* (L.) Merr. (Pineapple)**

Ananas comosus (Family-Bromeliaceae), commonly known as Pineapple, is native to Central and South America and is grown in several tropical and subtropical countries including the Indian subcontinent. Chloroform and methanolic extracts of *A. comosus* leaf have shown activity against acute anti-inflammation in carrageenan-induced paw oedema in Wistar albino rats. The methanolic extract was found to be the most potent followed by the chloroform extract [65]. Bromelain, a protease extracted from the stem of pineapple reduces mild acute knee pain [66].

***Cannabis sativa* L. (Ganja)**

This species belonging to Family Cannabaceae is indigenous to Central and South Asia. Cannabis is one of the oldest known medicinal plants and produces pharmacologically important substances. Among them, most important are the cannabinoids that are unique components in the cannabis plant. The 9-Tetrahydrocannabinol (9-THC) and cannabidiol (CBD) are known to be major cannabinoids in the plant. Cannabidiol (CBD), a non-psychoactive marijuana constituent, was recently shown to act as an oral anti-hyperalgesic compound in a rat model of acute inflammation [67]. Cannabidiolic acid was shown to be a selective cyclo-oxygenase-2 (Cox-2) inhibitory component in cannabis [68].

Cannabinoids mediate their physiological and behavioral effects by activating specific cannabinoid receptors. With the recent discovery of the cannabinoid receptors (CB1 and CB2) and the endocannabinoid system, research in this field has expanded exponentially. Cannabinoids have been shown to act as potent immunosuppressive and anti-inflammatory agents and have been shown to mediate the beneficial effects in a wide range of immune-mediated diseases such as multiple sclerosis, diabetes, septic shock, rheumatoid arthritis, and allergic asthma. Cannabinoid receptor 1 (CB1) is mainly expressed on the cells of the central nervous system as well as in the periphery. In contrast, the cannabinoid receptor 2 (CB2) is predominantly expressed on immune cells [69].

***Ocimum tenuiflorum* L. Syn. *Ocimum sanctum* L. (Tulsi)**

Ocimum sanctum Linn. (Family-Lamiaceae), a small herb seen throughout warmer parts of India, have been recommended by Ayurveda for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. The fixed oil (non-volatile part of the plant, typically obtained from the seed or nut) of *O. sanctum* was shown to have anti-arthritic activity in Freund's adjuvant-induced arthritis, formaldehyde-induced arthritis and also in turpentine oil-induced joint edema in rats. The fixed oil showed significant anti-arthritic activities in both models and anti-edema activity against turpentine oil-induced joint edema [70]. The oil shows anti-inflammatory potential due to inhibition of arachidonate metabolism and anti-histaminic activity. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum* L., has been found to be largely responsible for its therapeutic potential.

***Withania somnifera* (L.) Dunal *(*Ashwagandha)**

Withania somnifera (Family-Solanaceae) is a small herb found in different parts of India and Nepal. It has promising anti-arthritic activity, ascribed to its stabilizing action on lysosomal enzyme activities [71]. The aqueous suspension of *Withania somnifera* root powder showed potent inhibitory activity towards the complement system, mitogen-induced lymphocyte proliferation and delayed-type hypersensitivity reaction. Immunosuppressive effect of *W. somnifera* root powder indicates that it could be a candidate for an immunosuppressive drug for inflammatory diseases [72]. Oral administration of *W. somnifera* root powder showed a significant increase in the level of lipid peroxides, glycoproteins, and urinary constituents with the depletion of antioxidant status and bone collagen in arthritic animals [73]. The effect of *W. somnifera* crude ethanol extract was studied on peripheral blood mononuclear cells of normal individuals and RA patients and synovial fluid mononuclear cells of RA patients *in vitro.* The study demonstrated that crude ethanol extracts of *W. somniferra* suppressed the production of pro-inflammatory molecules *in vitro*. This activity is partly through the inhibition of transcription factors NF-κB and AP-1 by the constituent with anolide [74]. Withaferin-A, a steroidal lactone from *W. somnifera* shows potent anti-arthritic and anti-inflammatory activity in arthritic animals [75].

***Moringa oleifera* Lam. (Moringa)**

Moringa oleifera (Family-Moringaceae), commonly known as moringa, drumstick, and horseradish, is a small, fast-growing tree that originates in India. Ethanolic seed extracts of *Moringa oliefera* is shown to have immunosuppressive activity in experimental immune inflammation [76]. A rare aurantiamide acetate and 1,3-dibenzyl urea has been isolated from the roots of M. oleifera [77]. In a study, the anti-arthritic activity of a hydro-alcoholic extract of Moringa flowers was investigated in adjuvant-induced arthritis in Wistar rats. Body weight, paw edema volume (primary lesion), inflammation at non-injected sites, and arthritic index (secondary lesion) in diseased

animals was reduced by treatment with the extract as compared to untreated control animals. The protective effects of Moringa were also noted in serum levels of Rheumatoid Factor (RF) and levels of the cytokines, TNF-α and IL-1 in treated diseased animals as compared with untreated control animals. Test animals showed decreased RF level, TNF-α and IL-2 levels in the serum when treated with Moringa. Histopathological sections from animals in the drug treatment group showed a protective effect that was evidenced by less infiltration of lymphocytes and less angiogenesis as compared with sections from arthritic animals [76].

***Cyanthillium cineveum* (L.) H. Rob. Syn. *Vernonia cinerea* (L.) Less (Sahadevi)**

This plant is very common in West and Central Africa but well distributed in India as well. It belongs to the Family Asteraceae. Latha *et al.* (1998) [78] tested the anti-inflammatory effect of an alcoholic extract from the flower of *Cyanthillium cineveum* (Syn. *Vernonia cinerea*) (Family-Asteraceae) in adjuvant arthritic rats. Changes in paw volume, body and tissue weights and serum and tissue enzyme activities of ALT, AST, ACP and cathepsin-D in adjuvant rats were reversed by oral administration of 100 mg/kg body weight of the flower extract. The extract also reversed the major histopathological changes in the hind paws of the arthritic rats. Phytochemical studies revealed the presence of alkaloids, saponins, steroids and flavonoids [78].

Methanolic extract of *Cyanthillium cineveum* was found to scavenge the hydroxyl radical generated by Fenton reaction, superoxides generated by photo- reduction of riboflavin and to inhibit lipid peroxidation significantly. The drug also scavenged nitric oxide. Intra-peritoneal administration of *Cyanthillium cineveum* was found to inhibit the PMA induced superoxide generation in mice peritoneal macrophages. The administration of *Cyanthillium cineveum* to mice significantly increased the levels of catalase, superoxide dismutase, glutathione, glutathione peroxidase and glutathione-S transferase in blood and liver, whereas lipid peroxidation activity was significantly decreased [78]. It was also found that *Cyanthillium cineveum* extract significantly inhibited carrageenan-induced inflammation, compared to control models. Down regulation of pro-inflammatory cytokine level and gene expression data also supported the above results [79].

***Justicia gendarussa* Burm f. (Water willow)**

Justicia gendarussa (Family-Acanthaceae) is a shade-loving, quick-growing, evergreen plant mostly found in moist areas. It is believed to be native to China and is distributed widely across India, Sri Lanka, and Malaysia. In Indian and Chinese traditional medicine, the leaf of the plant is recommended to treat ailments such as fever, hemiplegia, rheumatism, arthritis, headache, ear ache, muscle pain, respiratory and digestive disorders. The paw volume and lipid peroxide level of hemolysate and liver in arthritic rats were significantly reduced to near normal level by administration of ethanolic leaf extract of *Justicia gendarussa* [80]. The decreased level of enzymatic antioxidants activities and non-enzymatic antioxidants levels was reverted to normal levels when methanolic extract given to arthritic rats. The preliminary phytochemical analysis of methanolic extract showed the presence of many biologically active phytochemicals such as flavonoids, alkaloids, phenolic compounds, saponins, glycosides, and tannins and these compounds might be responsible for the anti-inflammatory properties. The possible anti-inflammatory mechanism of the *Justicia gendarussa* leaf extracts may be through its free radical scavenging activity, its stabilizing action on lipid peroxide and increased antioxidants levels [81].

Another study focused on the anti-inflammatory activity of *Justicia gendarussa* in a carrageenan-induced paw edema assay. Methanolic extract of *J. gendarussa* (JRM) roots significantly inhibited oedema formation 5 hours after carrageenan induction. JRM inhibited carrageenan-induced change in total cycloxygenase activity; 5-lipoxygenase and 15-lipoxygenase activities in blood mononuclear cells of rats, decreased neutrophil infiltration in paw tissue as shown by low myeloperoxidase activity [82]. It also caused an inhibition in" with "inhibited cyclooxygenase-2 activity in paw tissue [82]. Purification of JRM by liquid–liquid partitioning yielded an ethyl acetate fraction of JRM that showed interleukin-6 downregulation potential and the ability to inhibit prostaglandin E2 production *in vivo* [82].

***Premna serratifolia* L. (Agnimantha)**

Premna serratifolia (Family-Lamiaceae) is a large shrub widespread in the deciduous forests of India. The whole plant possesses medicinal properties, useful in the treatment of cardiovascular, skin, inflammatory diseases, arthritis, gonorrhoea, rheumatism, anorexia and jaundice. The anti-arthritic activity of ethanolic extract of *Premna serratifolia* wood was tested in Freund's adjuvant-induced rat arthritis model. The loss in body weight during arthritis condition was corrected on treatment with ethanol extract and standard drug indomethacin [82]. Biochemical parameters such as hemoglobin content, total WBC, RBC, erythrocyte and sedimentation rate were also estimated. The ethanolic extract at the dose of 300 mg/kg body weight inhibited the rat paw edema by 68.32% which is comparable to standard drug indomethacin which inhibited 74.87% paw edema after 21 days. The observed anti-arthritic activity may be due to the presence of phytoconstituents such as irridiod glycosides, alkaloids, phenolic compounds and flavonoids [83].

***Cissampelos pareira* L. (Abuta)**

Cissampelos pareira (Family-Menispermaceae) is a common plant seen in the subcontinent. In a study, 50% ethanolic extract of C. the roots exhibited significant anti-inflammatory activity in acute, subacute and chronic models of inflammation in rats [84]. In the same study, 50% aqueous ethanolic extract of *C. pariera* exhibited resistance against pain in mice. Further, it also showed resistance against adjuvant-induced arthritis in mice [84].

***Nyctanthes arbor-tristis* L. (Harshringar; Shiuli)**

Harshringar or *Nyctanthes arbor-tristis* (Family-Oleaceae) has been used widely as a decoction for the treatment of arthritis and sciatica in the Indian ayurvedic system of medicine for centuries. The plant has its origin from the Bengal region of India while it is distributed all over sub-tropical regions of the country. Arbortristosides, nyctanthic acid, and crocetin are the main active principles of the plant. Water soluble ethanolic leaf extract has been reported to reduce significantly the levels of inflammatory cytokines (IL-1, TNF-α) in experimental arthritis [85].

***Swertia chirayita* (Roxb.) Buch-Ham. Ex C. B. Clarke (Chirata)**

Swertia chirayita, (Family-Gentianaceae) a herb found abundantly in the temperate regions of Himalaya, is commonly used for chronic fever, anemia and asthma. The plant inhabits the pastures and slopes of the Himalayas and ranges between 2000 to 3,000 metres. Chirayita comprises of swerchirin, swertanone and swertianin as active components responsible for the anti-inflammatory activity. Chirayita is reported to reduce the elevated levels of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in experimental arthritis as well as in asthmatic conditions [86].

***Crocus sativus* L. (Saffron)**

Saffron (Family-Iridaceae) is native to Greece and south-west Asia and it was brought to this country and naturalized later. The plant is commonly used as folk medicine for various purposes such as aphrodisiac, anti-spasmodic and expectorant. It is a perennial flowering plant with very less growing height up to 40 cm. Commonly cultivated in Kashmir, the distribution of the plant is seen in North America, Greece and Spain as well.

Saffron stigma possesses anti-inflammatory action due to presence of the crocetin and carotenoids. Aqueous and ethanolic extracts of saffron petals exhibit radical scavenging as well as anti-inflammatory effects in xylene and formalin-induced inflammation [87].

***Strobilanthus callosus* Nees. (Karvi)**

Karvi (Family-Acanthaceae) is another Indian medicinal herb, commonly found in the Maharastra state and has been used by the local tribals for the treatment of inflammatory disorders. The Lupeol and 19α-H Lupeol isolated from the roots of *Strobilanthus callosus* have demonstrated the anti-inflammatory as well as anti-rheumatic activity in carrageenan-induced oedema [88].

***Aloe vera* (L.) Burm. f. (Ghritokumari)**

Dermatological aspects of *Aloe vera* (Family-Xanthorrhoeaceae) are very well known in the traditional medicinal systems. In this regard, studies on compound isolation are also done revealing the presence of potent anti-inflammatory biomolecules in the plant [89]. The plant is well distributed in India and neighbouring countries. Anti-inflammatory activity has been investigated in the *Aloe vera* crude extract in carrageenan-induced arthritic rat models [90]. Preliminary studies on anti-arthritic activity have also been done [91]. Experiments prove that the plant has some strong protective role against inflammation and rheumatism. However, some more precise experiments are needed to establish its role in such disease.

Other important plants with anti-inflammatory activities

Acacia farnesiana (L.) Willd. [92], *Aegle marmelos* (L.) Correa (Bel) [93], *Anacardium occidentale* L. (Cashew) [94], *Azadirachta indica* A. Juss. (Neem) [95], *Cedrus deodara* (Roxb. ex D. Don) G. Don (Deodar) [96], *Morus indica* L. (Mulberry) [97], *Emilia sonchifolia* (L.) DC. Ex DC.[98] are some other promising plants having potential anti-inflammatory activities. These plants can be further used to investigate their role in rheumatoid arthritis.

***Saraca asoka* (Ashok)** | ***Cannabis sativa* (Ganja)** | ***Withania somnifera* (Ashwagandha)**

***Cissampelos pereira* (Abuta)** | ***Justcia gendarussa* (Water willow)** | ***Aloe vera* (Ghritokumari)**

Fig. 2: Promising medicinal plants of the North-Eastern Himalayan region having anti-arthritic and anti-inflammatory properties. Leaf, fruiting bodies and flowers are shown in insets. (Photo courtesy: Garden of Medicinal Plants, University of North Bengal, India)

CONCLUSION

At present, the current modalities for treating arthritis are symptomatic and have not been shown to either block or reverse the cartilage degradation and joint destruction. This has resulted in heightened interest in the use of CAM therapies for the treatment of arthritis. However, much of the current research is focused on the identification, isolation and characterization of active principle(s) from crude extracts of known medicinal plants/herbs and animals/animal products, often overlooking the fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound and this may also help to nullify the toxic effects of individual constituents. Also, the lack of sufficient clinical investigations does not permit definitive conclusions to be drawn regarding the efficacy of plants and animals as CAM modalities in RA. However, several animal studies have helped elucidate the potential mechanism of action of various CAM modalities. This encourages further studies but demands rigorous experimentation. There is a need for screening and scientifically evaluating number of known traditional medicinal plants and animals for providing newer and safer treatment options with minimum side effects. Once the underlying molecular mechanism for the observed anti-inflammatory and chondro-protective effects of nutraceuticals are elucidated, their health benefits may be exploited to develop new and better modalities for treating degenerative and inflammatory joint diseases.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Firestein GS. Evolving concepts of rheumatoid arthritis**.** Nature 2003;423:356-61.
2. Williams RO, Mason LJ, Feldmann M, Maini RN. Synergy between anti-CD4 and antitumor necrosis factor in the amelioration of established collagen-induced arthritis. Proc Natl Acad Sci USA 1994;91:2762–6.
3. Lubberts E, Van Der Bersselaar L, Oppers-Walgreen B, Schwarzenberger P, Conen-de Roo CJJ, Kolls JK, *et al.* IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kB ligand/osteoprotegerin balance. J Immunol 2003;170:2655–62.
4. Smolen JS, Steiner G. Rheumatoid arthritis is more than cytokines: autoimmunity and rheumatoid arthritis. Arthritis Rheum 2001;44:2218–20.
5. Arnett FC, Edworthy SM, Bloch DA. The american rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
6. Gabriel SE. The epidemiology of rheumatoid arthritis. Rheum Dis Clin North Am 2001;27:269–82.
7. Chopra A, Ahmed AN. Epidemiology of rheumatic musculoskeletal disorders in the developing world. Best Pract Res Clin Rheumatol 2008;22(4):583–604.
8. Schellekens GA, DeJong W, Van den Hoogen FHJ, Van de Putte LBA, Van Venrooj W. Citrulline is anessential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998;10:273–81.

9. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, *et al.* Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Gen 2010;42(6):508-14.
10. Thomas R, MacDonald KPA, Pettit AR, Cavanagh LL, Padmanabha J, Zehntner S. Dendritic cells and the pathogenesis of rheumatoid arthritis. J Leukocyte Biol 1999;66**:**286-92**.**
11. Cavanagh LL, Boyce A, Smith L, Padmanabha J, Filgueira L, Pietschmann P, *et al.* Rheumatoid arthritis synovium contains plasmacytoid dendritic cells**.** Arthritis Res Ther 2005;7:R230-R40.
12. Cope AP. T-cells in rheumatoid arthritis**.** Arthritis Res Ther 2008;10(Suppl 1):S1.
13. Kinne RW, Bräuer R, Stuhlmüller B, Palombo-Kinne E, Burmester GR. Macrophages in rheumatoid arthritis**.** Arthritis Res 2000;2:189–202.
14. Kinne RW, Stuhlmüller B, Burmester GR. Cells of the synovium in rheumatoid arthritis Macrophages. Arthritis Res Ther 2007;9:224.
15. Redlich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias J, *et al.* Osteoclasts are essential for TNF-a-mediated joint destruction. J Clin Invest 2002;110:1419–27.
16. Goldring R. Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. Rheum 2003;42(Suppl 2):11–6.
17. Silverman GJ, Carson DA. Roles of B-cells in rheumatoid arthritis. Arthritis Res Ther 2003;5(4):S1-6.
18. Rathore B, Mahdi AA, Paul BN, Saxena PN, Das SK. Indian herbal medicines: possible potent therapeutic agents for rheumatoid arthritis. J Clin Biochem 2007;41(1):17-9.
19. Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, *et al.* A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330-7.
20. Kallberg H, Padyukov L, Plenge RM, Ronnelid J, Gregersen PK, van der Helm-van Mil AHM, *et al.* Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. Am J Hum Genet 2007;80:867-75.
21. Weyand CM, Hicock KC, Conn DL, Goronzy JJ. The influence of HLA-DRB1 gene on disease severity in rheumatoid arthritis. Ann Inter Med 1992;117(10):801-6.
22. Yang H, Rittner H, Weyand CM, Goronzy JJ. Aberrations in the primary T-cell receptor repertoire as a predisposition for synovial inflammation in rheumatoid arthritis. J Invest Med 1999;47:236–5.
23. Nabil M, Yanni G, Poston R, Panayi GS. Cytokine expression in synovial membranes of patients with rheumatoid arthritis. Ann Rheum Dis 1994;52:870-5.
24. Ulfgren AK, Lindblad S, Klareskog L, Anderson J, Anderson U. Detection of cytokine producing cells in the synovial membrane from patients with rheumatoid arthritis. Ann Rheum Dis 1995;54:654-61.
25. Buchan G, Barret K, Turner M, Chantry D, Maini RN, Feldmann M. Interleukin-1 and tumor necrosis factor-α mRNA expression in rheumatoid arthritis, prolonged production of IL-1 α. Clin Exp Immunol 1988;3:449-55.
26. Firestein GS, Alvaro-Gracia JM, Maki R. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol 1990a;144:3347–53.
27. Klimiuk PA, Goronzy JJ, Bjornsson J, Beckenbaugh RD, Weyand CM. Tissue cytokine patterns distinguish variants of rheumatoid synovitis. Am J Pathol 1997;151:1311–9.
28. Reveille JD. The genetic contribution to the pathogenesis of rheumatoid arthritis. Curr Opin Rheumatol 1998;10:187–200.
29. Auger I, Roudier J. HLA-DR and the development of rheumatoid arthritis. Autoimmunity 1997;26:123–8.
30. Lipsky PE, Van Der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. N Engl J Med 2000;343:1594–602.
31. Ziolkowska M, Kurowska M, Radzikowska A. High levels of osteoprotegerin and soluble receptor activator of nuclear factor kB ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor a treatment. Arthritis Rheum 2002;46:1744–53.
32. Kong YY, Feiqe U, Bolon B, Tafuri A, Morony S, Capparelli C, *et al.* Activated T-cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402(6759):304-9.
33. Pettit AR, Ji H, Stechow DV, Muller R, Goldring SR, Choi Y, *et al.* Trance/Rankl knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001;159:1689–99.
34. Han Z, Boyle DL, Manning AM, Firestein GS. AP-1 and NF-kB regulation in rheumatoid arthritis and murine collagen-induced arthritis. Autoimm 1998;28:197–208.
35. Aupperle K. NF-kB regulation by IkB kinase-2 in rheumatoid arthritis synoviocytes. J Immunol 2001;166:2705–11.
36. Tak PP, Gerlag DM, Aupperle KR, van de Geest DA, Overbeek M, Bennett BL, *et al.* Inhibitor of nuclear factor kB kinase b is a key regulator of synovial inflammation. Arthritis Rheum 2001;44:1897–907.
37. Miagkov AV, Kovalenko DV, Brown CE, Didsbury JR, Cogswell JP , Stimpson SA, *et al.* NF-kB activation provides the potential link between inflammation and hyperplasia in the arthritic joint. Proc Natl Acad Sci USA 1998;95:13859–64.
38. Schett G, Tohidast-Akrad M, Smolen JS, Schmid BJ, Steiner CL, Bitzan P, *et al.* Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum 2000;43:2501–12.
39. Badger AM, Bradbeer JN, Votta B, Lee JC, Adams JL, Griswold DE. Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function. J Pharmacol Exp Ther 1996;279:1453–61.
40. Han Z, Boyle DL, Chang L, Bennet B, Karin M, Yang L, *et al.* c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. J Clin Invest 2001;108:73–81.
41. Han Z, Chang L, Yamanishi Y, Karin M, Firestein GS. Joint damage and inflammation in c-Jun N-terminal kinase 2 knockout mice with passive murine collagen-induced arthritis. Arthritis Rheum 2002;46:818–23.
42. Kinne RW, Boehm S, Iftner T, Aigner T, Vornehm S, Weseloh G, *et al.* Synovial fibroblast-like cells strongly express jun-B and C-fos proto-oncogenes in rheumatoid-and osteoarthritis. Scand J Rheumatol 1995;101:121–5.
43. Shiozawa S, Shimizu K, Tanaka K, Hino K. Studies on the contribution of c-fos/AP-1 to arthritic joint destruction. J Clin Invest 1997;99:1210–6.
44. Moreland LW, Baumqartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, *et al.* Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997;337:141–7.
45. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, *et al.* Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 1994;344:1105–10.
46. Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M. Inhibitory effect of TNFα antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet 2001;2:244–7.
47. Lubberts E, Joosten LA, Van Der Bersselaar L, Oppers B, Conen-de Roo CJJ, Richards CD, *et al.* IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. J Clin Invest 2000;105:1697–710.
48. Smeets TJ, Kraan MC, Versendaal J, Breedveld FC, Tak PP. Analysis of serial synovial biopsies in patients with rheumatoid arthritis: description of a control group without clinical improvement after treatment with interleukin 10 or placebo. J Rheumatol 1999;26:2089–93.
49. Efthimiou P, Kukar M, Mackenzie CR. Complementary and alternative medicine in rheumatoid arthritis: no longer the last resort! HSSJ 2010;6:108-11.
50. World Health Organization. General guidelines for methodologies on research and evaluation of traditional medicine. WHO/EDM/TRM 2000;1:1-74.

51. Ahmed S, Anuntiyo J, Malemud CJ, Haqqi TM. Biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis. eCAM 2005;2(3):301–8.
52. Manami M**,** Kaori E**,** Fumiko K**,** Mari Maeda Y. Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) Extract. *J Agric Food Chem* 2008;56(4):1423–7.
53. Hamilton-Miller JM. Antimicrobial properties of tea (Camellia sinensis L.). Antimicrob Agents Chemother 1995;39(11):2375–7.
54. Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa β (NF-kappa β) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. Matrix Biol 2002;21(3):251–62.
55. Schulze-tanzilG, Mobasheri A, Sendzik J, John T, Shakibaei M. Effects of curcumin (diferuloylmethane) on nuclear factor kappaβ signaling in interleukin-1betastimulated chondrocytes. Ann N Y Acad Sci 2004;1030:578–86.
56. Kukar M. Complementary and alternative medicine use in rheumatoid arthritis: proposed mechanism of action and efficacy of commonly used modalities. Rheumatol Int 2010;30:571–86.
57. Lee TY, Lee KC, Chen SY, Chang HH. 6-Gingerol inhibits ROS and iNOS through the suppression of PKC-α and NF-κβ pathways in lipopolysaccharide-stimulated mouse macrophages**.** Biochem Biophys Res Commun 2009;382(1):134–9.
58. Srivastava KC, Mustafa T. Ginger (Zingiber officinale) and rheumatic disorders. Med Hypotheses 1989;29(1):25–28.
59. Srivastava KC, Mustafa T. Ginger (Zingiber officinale) in rheumatism and musculoskeletal disorders. Med Hypotheses 1992;39(4):342–8.
60. Ramprasath VR, Shanthi P, Sachdanandam P. Immunomodulatory and anti-inflammatory effects of Semecarpus anacardium LINN. Nut milk extract in experimental inflammatory conditions. Biol Pharm Bull 2006;29(4):693–700.
61. Vijayalakshmi T, Muthulakshmi V, Sachdanandam P. Effect of the milk extract of Semecarpus anacardium nut on adjuvant arthritis—a dose-dependent study in Wistar albino rat. Gen Pharmacol 1996;27(7):1223–6.
62. Ramprasath VR, Shanthi P, Sachdanandam P. Effect of Semecarpus anacardium Linn. nut milk extract on rat neutrophil functions in adjuvant arthritis. Cell Biochem Funct 2006;24(4):333–40.
63. Gupta M, Sasmal S, Mukherjee A. Therapeutic effects of acetone extract of saraca asoca seeds on rats with adjuvant-induced arthritis via attenuating inflammatory responses. Int Scho Res Notices ISRN Rheum 2014;4:1-12.
64. Saravanan S, Prakash B, Pandikumar P, Ignacimuthu S. Therapeutic effect of Saraca asoca (Roxb.)Wilde on lysosomal enzymes and collagen metabolism in adjuvant induced arthritis. Inflammopharmacology 2011;19:317–25.
65. Mondal S, Bhattacharya S, Pandey JN, Biswas M. Evaluation of acute anti-inflammatory effect of Ananus comosus leaf extracts in rats. Pharmacologyonline 2011;3:1312-5.
66. Walker AF, Bundy R, Hicks SM, Middleton RW. Bromelain reduces mild acute knee pain and improves well being in a dose dependent fashion in an open study of otherwise healthy adults. Phytomed 2002;9(8):681-6.
67. Costa B, Colleoni M, Conti S, Parolaro D, Franke C, Trovato AE, *et al.* Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. Naunyn-Schmiedeberg's Arch Pharmacol 2004;369:294-9.
68. Takeda S, Misawqa K, Yamamoto I, Watanabe K. Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. Drug Metab Dispos 2008;36(9):1917-21.
69. Rieder SA, Chauhan A, Singh U, Nagarkatti M, Nagarkatti P. Cannabinoid-induced apoptosis in immune cells as a pathway to immunosuppression. Immunobiol 2010;215(8):598–605.
70. Singh S, Majumder DK. Effect of fixed oil of ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. Pharm Biol 1996;34(3):218-22.
71. Rasool M, Latha LM, Varalakshmi P. Effect of Withania somnifera on lysosomal acid hydrolases in adjuvant-induced arthritis in rats. Pharm Pharmacol Commun 2000;6:187–90.
72. Rasool M, Latha LM, Varalakshmi P. Immunomodulatory role of Withania somnifera root powder on experimental induced inflammation: An *in vivo* and *in vitro* study. Vascu Pharmacol 2006;44(6):406–10.
73. Rasool M, Varalakshmi P. Protective effect of Withania somnifera root powder in relation to lipid peroxidation, antioxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats. Fundam Clin Pharmacol 2007;21:157–64**.**
74. Singh D, Aggarwal A, Maurya R, Naik S. Withania somnifera inhibits NF-κB and AP-1 transcription factors in human peripheral blood and synovial fluid mononuclear cell. Phytoth Res 2007;21:905–13.
75. Khade CP. Indian medicinal plants-An illustrated dictionary. First Indian Reprint: Springer (India) Pvt. Ltd. New Delhi; 2007. p. 717-8.
76. Mahajan SG, Mehta AA. Immunosuppressive activity of ethanolic extract of seeds of Moringa oleifera Lam. in experimental immune inflammation. J Ethnopharmacol 2010;130(1):183–6.
77. Sashidhara KV, Rosaiah JN, Tyagi E, Shukla R, Raghubir R, Rajendran SM. Rare dipeptide and urea derivatives from roots of Moringa Oleifera as potential anti-inflammatory and antinociceptive Agents. Eur J Med Chem 2009;44(1):432-6.
78. Latha RM, Geetha T, Varalakshmi P. Effect of Vernonia cinerea less flower extract in adjuvant-induced arthritis general pharmacology. Vasc Syst 1998;31(4):601–6.
79. Kumar PP, Kuttan G. Vernonia cinerea L. scavenges free radicals and regulates nitric oxide and proinflammatory cytokines profile in carrageenan induced paw edema model. Immunopharmacol Immunotoxicol 2009;31(1):94-102.
80. Sikha P, Latha PG, Suja SR, Anuja GI, Shyamal S, Shine VJ, *et al.* Anti-inflammatory and antinociceptive activity of *Justicia gendarussa* Burm. F. leaves. Indian J Nat Prod Resour 2010;1(4):456-61.
81. Chinna M, Renuka P, Fazaludeen MF. Evaluation of anti-inflammatory activity on justicia genadarussa burm. f. Leaf extract in adjuvant induced arthritic rats. Int J Medicobiol Res 2011;1(3):163-9.
82. Kavitha SK, Viji V, Kripa K, Helen A. Protective effect of Justicia gendarussa Burm. f. on carrageenan-induced inflammation. J Nat Med 2011;65(3-4):471-9.
83. Rajendran R, Krishnakumar E. Anti-arthritic activity of premna serratifolia Linn. Wood against adjuvant induced arthritis. Avicenna J Med Biotechnol 2010;2(2):101-6.
84. Amaresh G, Reddy GD. Evaluation of anti inflammatory properties of Cissampelos pareira roots in rats. J Ethnopharmacol 2007;110(3):526-31.
85. Paul BN, Saxena AK. Depletion of TNF-α in mice by Nyctanthes arbortristis. J Ethnopharmacol 1997;56:153.
86. Kumar IV, Paul BN, Asthana A, Saxena A, Mehrotra S, Rajan G. Swertia chirayita mediated modulation of IL-1β, IL-6, IL-10, IFN-γ and TNF-α in arthritic mice. Immunopharmacol Immunotoxicol 2003;25:573–83.
87. Hossein H, Younesi H. Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. BMC Pharmacol 2002;2;7.
88. Agarwal R, Rangari V. Anti-inflammatory and anti-arthritic activities of lupeol and 19α-H lupeol isolated from Strobilanthus callosus and Strobilanthus ixiocephala roots. Indian J Pharmacol 2003;35:384–7.
89. Grindley D, Reynolds T. The aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchymal gel. J Ethnopharmacol 1986;16:117-51.
90. Paul S, Dutta S, Chaudhuri TK, Bhattacharjee S. Anti-Inflammatory and Protective properties of *Aloe vera* leaf crude gel in carrageenan induced acute inflammatory Rat Models. Int J Pharm Pharm Sci 2014:6(9);368-71.
91. Guha P, Paul S, Das A, Halder B, Bhattacharjee S, Chaudhuri TK. Analyses of human and rat clinical parameters in rheumatoid arthritis raise the possibility of use of crude Aloe vera gel in disease amelioration. Immunome Res 2014;10(2). DOI: 10.4172/17457580.1000081 [Article in Press].

92. Hukkeri VI, Savadi RV, Tippimath CD, Karadi RV, Jaiprakash B. Anti-inflammatory activity of leaves of Acacia farnesiana Willd. Indian Drugs 2002;49(13):664-6.
93. Gurulingappa S, Halur MS. Anti–inflammatory assays of extracts of medicinal plants. Indian J Pharm Sci 2002;64(5):498-500.
94. Mota ML, Thomas RG. Anti inflammatory actions of tannins isolated from the bark of Anancardium occidentale L. J Ethnopharmacol 2002;13:289-300.
95. Chttpadhyay RR. Possible biochemical mode of anti-inflammatory action of Azadirachta indica A. Juss in rats. Indian J Exp Biol 1998;36(4):418-20.
96. Shinde UA, Phadke AM. Studies on the anti inflammatory and analgesic activity of Cedrus deodara (Roxb.) Loud wood oil. J Ethnopharmacol 1999;65(1):21-7.
97. Balasubramanian A, Ramalingam K. Anti-inflammatory activity of Morus indica. Linn. Iranian J Pharmacol Ther 2005;4(1):13-5.
98. Shylesh BS, Padikkala J. Antioxidant and anti inflammatory activity of Emilia sonchifolia. Phytother 1999;70(3):L275-8.

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Research Article Open Access

Analyses of Human and Rat Clinical Parameters in Rheumatoid Arthritis Raise the Possibility of Use of Crude *Aloe vera* Gel in Disease Amelioration

Pokhraj Guha[1], Subhashis Paul[2], Avishek Das[1], Biswajit Halder[3], Soumen Bhattacharjee[2] and Tapas Kumar Chaudhuri[1]*

[1]*Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, Siliguri, West Bengal, India*
[2]*Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, Siliguri, West Bengal, India*
[3]*Department of Pathology, North Bengal Medical College and Hospital, Shusrutnagar, Siliguri, West Bengal, India*

Abstract

Rheumatoid Arthritis (RA) is a chronic inflammatory condition affecting the joints causing swelling, stiffness and pain which finally leads to substantial loss of functioning and mobility in its advanced stages. In the present study we have monitored important serological parameters of fifty RA patients and also have discussed the justification of using rat as a model for human RA researches by comparing their respective serological parameters. We have also evaluated the anti-arthritic roles of raw *Aloe vera* gel and its effects in rat model where arthritis was induced by using Freund's Complete Adjuvant (FCA). Three essential conclusive statements were derived from the study. Firstly, the six clinical parameters that we have selected for the study namely, RA factor, CRP, ASO, ESR, ceruloplasmin and serum creatinine were all essential for the differential diagnosis of Rheumatoid Arthritis during its early and later stages, RA factor being the most sensitive of all parameters (92% sensitivity). Secondly, this study has supported the use of the rat as a model for designing therapeutic strategies against RA. Lastly, as evident from our study, *Aloe vera* extracts can be beneficial for the reduction of inflammatory edema and also for the reduction of ceruloplasmin in RA condition in rat model. However, further investigations are necessary for more refined therapeutic usage of *Aloe vera* for the treatment of RA in human.

Keywords: Rheumatoid arthritis; RA factor; North Bengal; *Aloe vera*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory condition affecting the bone joints causing swelling, stiffness and pain that gradually leads to the substantial loss of functioning and mobility in the advanced stages [1]. Effects of RA are not only limited to extreme physical distress but also cause mental distress. As the etiology behind RA prognosis is not well understood confirmed curative measures are not discovered till date. All treatment regimes and therapies presently applied, such as cytokine therapy (anti-TNFα therapy) or Disease Modifying Anti-Rheumatic Drugs (DMARDs) are only limited to the reduction of symptoms of the disease and delay of pathogenesis [2]. Thus RA has become an undeniable threat to human life and therefore preventive measures must be developed to cure this disorder.

In the recent times, a large body of research has been directed towards finding herbal solutions to the treatment of the diseases. In Indian Ayurveda, one such promising herbal candidate having anti-arthritic effect is *Aloe vera* (Family Xanthorrhoeaceae), a perennial succulent xerophytic plant. In this plant water is held in the form of viscous mucilage within the thin walled parenchymatous cells in the innermost part of the leaves. *Aloe vera* has been used for many centuries for its curative and therapeutic properties [3]. It has been traditionally used in various skin ailments [4] and has well known wound healing and anti-inflammatory activities [5,6]. *Aloe vera* gel has been traditionally consumed or applied dermally to reduce joint pains. Phytochemical screening of *Aloe vera* confirms the presence of flavonoids, alkaloids, resins, tannins, steroids and other chemical substances [7]. The anti-inflammatory activity of the crude unprocessed gel has also been documented [8]. It has been documented that *Aloe vera* contains anthroquinone that may play a key role in anti-arthritic activity [9]. Many of the medicinal effects of Aloe leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue [5,6], but it is believed that these biological activities should be assigned to a synergistic action of the compounds contained therein rather than a single chemical substance [7].

In the present study we have estimated the levels of certain clinical parameters in Rheumatoid arthritic patients of northern West North Bengal, India compared to the control subjects and also have discussed the possibility of using *Aloe vera* crude gel in the treatment of RA by evaluating the effects of crude leaf gel in experimental arthritic conditions in rat groups induced by Freund's complete adjuvant.

Materials and Methods

This section has been compartmentalized into separate categories for better understanding as follows:

Human based studies

Sample collection: Blood samples of 50 RA patients and 50 controls subjects were collected from an authorized diagnostic laboratory of Siliguri and also from North Bengal Medical College and Hospital (NBMCH, Sushrutnagar, West Bengal, India) under the guidance of a medical practitioner. Both patients and control subjects have provided their written consent after knowing the purpose of the study. The patients were diagnosed on the basis of physical examinations, clinical symptoms,, disease progression studies and were confirmed of having RA based on the reports of Anti-CCP assays.

Estimation of RA clinical parameters: Each blood sample was

***Corresponding author:** Tapas Kumar Chaudhuri, Professor, Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, Siliguri, West Bengal, India, Tel: +91-9434377127; Fax: +91-353-2699001; E-mail: tapas.chaudhuri@gmail.com

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divided into two parts, the anti-coagulated (EDTA added) part for ESR estimation and the clotted part for serum extraction for the estimation of RA factor, CRP assay, ASO titre estimation, Ceruloplasmin and creatinine assays.

Erythrocyte Sedimentation Rate (ESR) of each blood sample was immediately estimated by Westergren method by measuring the rate of gravitational settling of anti-coagulated erythrocytes in 1 hour from a fixed point in an upright calibrated tube of predefined dimensions [10]. Its normal upper limit for males is 15 mm/hr, and for females is 20 mm/hr [11]. ESR is an indirect measure of the acute phase reaction, it being a simple and inexpensive laboratory test for assessing inflammation.

Rheumatoid (RA) factor, C-Reactive Protein (CRP) and Anti-streptolysin O (ASO) were estimated by quantitative turbidometric assay and their normal range reference values in the serum were considered to be up to 20 IU/ml, 6 mg/dl, and 200 IU/ml respectively. RA factor is a very potent marker of RA as majority of the patients have this abnormal antibody in their serum at a range higher than normal.

Furthermore, we analyzed ceruloplasmin and creatinine concentration in the serum samples of the RA patients and the control subjects. Ceruloplasmin estimation was done spectrophotometrically by using p-phenylenediamine oxidase activity [12]. The levels of serum creatinine were measured spectrophotometrically by studying reactions between creatinine and alkaline picrate [13].

Rat model based study

Experimental setup: Swiss Albino male rats weighing 60 ± 10 gm each were used for all the experiments under this section and were procured from an authorized animal dealer (Ghosh Enterprise, Kolkata, India). Animals were maintained under standard laboratory conditions. The study was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) of the University of North Bengal, West Bengal, India.

The animals were divided into 6 groups of 4 male rats in each. The first group was considered as Non-treated or control group (NT) as arthritis was not induced in the rats of this group. The second group was considered as Arthritic group or Negative Control (AG) as arthritis was induced in the members of this group as per the method proposed by Bendele et al. [14]. All the animals of AG were administered a dose of 0.1 ml of Freund's Complete Adjuvant (FCA) in the left hind paw and a booster dose of 0.1 ml was given on the 15th day of the experiments. The animals of three other groups were also induced with FCA following the methods as mentioned above. These three groups were designated as the Experimental Groups (EGs) and were fed raw *Aloe vera* gel as mentioned below.

Wild *Aloe vera* plants collected from the sub-Himalayan Terai regions of Northern West Bengal were used for the experiments. The plants were identified by the Department of Botany, University of North Bengal [accession no. 09884 (NBU)]. They belong to class Magnoliopsida under order Asparagales and family Xanthorrhoeaceae. The crude gel was obtained by peeling out the outer cuticle layer and cutting the gel aseptically into small pieces. The sample homogenate was freshly prepared with distilled water (1:5 w/v) every time before use. Each piece of gel was weighed and then dried separately in an air oven at 37°C for 48 hours to know the dry weight of the gel doses.

Three experimental groups (EGs) were treated with different doses of *Aloe vera* doses viz., 125 µl (EG-125), 250 µl (EG-250) and 500 µl (EG-500) respectively corresponding to 25 gm wet gel/kg body weight (20 mg dry weight/kg body weight), 50 gm wet gel/kg body weight (40 mg dry weight/kg body weight), and 100 gm wet gel/kg body weight (80 mg dry weight/kg body weight). The above mentioned doses were selected on the basis of the quantity of *Aloe vera* gel that should be taken by a person per day for therapeutic use. However, the absorption rate may be different in case of rat and human systems.

The sixth group was considered as the Protective group (PG) where animals were fed with 250 µl *Aloe vera* (50 gm wet gel/kg body weight or 40 mg dry weight/kg body weight) 7 days prior to the FCA injection (arthritis induction).

Parametric studies: Two rats from each of the six groups were sacrificed on 21st day of the experiment and the rest were sacrificed on the 28th day to know the levels of modulatory activities of crude *Aloe vera* after the booster doses of FCA. The left hind paws of all the six rat groups were amputated from the body after their sacrifice and were subjected to radiological analysis. The measurements of paw circumference was done at regular intervals of 2 or 3 days with the help of a vernier caliper following methods of Paquet et al. and Rathore et al. [15,16]. Body weight was also recorded at regular intervals. Blood samples were collected using insulin syringes from the tail vein for serum isolation for biochemical tests. Serum ceruloplasmin and creatinine estimation were done by applying the procedures followed

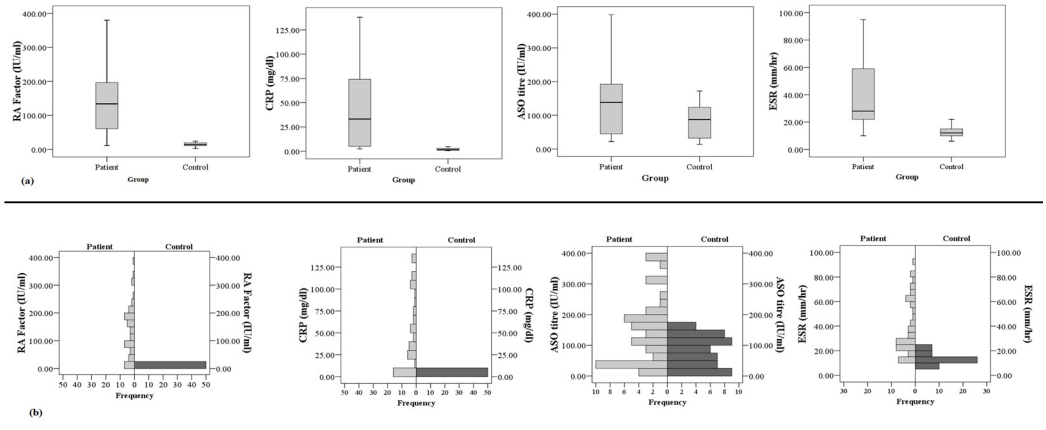

Figure 1: (a) Box plot analyses show the differences in the distribution of the different clinical parameters in the Rheumatoid Arthritic patients and the control samples. **(b)** Pyramid charts compared the distribution of the levels of different clinical parameters in the RA patients and the control samples.

in case of the human samples for reducing complications during further analyses.

Statistical analysis

All the statistical calculations were performed using the softwares MS-Excel and Kyplot ver. 2.0 β and SPSS ver. 16.0. In the Kyplot analysis the data represented mean ± SD which was analyzed by T-test for finding the significance. The results were considered significant when $p>0.05$. Box plots and the frequency plots were constructed using SPSS ver. 16.0.

Results

Out of 50 selected anti-CCP positive RA patients 92 % were found to be positive for the Rheumatoid (RA) factor test having RA factor values above the normal range of 0-20 IU/ml. The RA factor values fluctuated within a very wide range of 11-380 IU/ml having mean ± SD value of 135.9 ± 91.4 IU/ml (Table 1). In contrast, 88% of the control patients (anti-CCP negative) were found to be RA factor negative with a mean value of 14.0 ± 5.5 IU/ml. The calculated t value of patients versus control for the RA factor was found to be 9.45 ($p \leq 0.001$) indicating significant differences in the distribution of the RA factors in the RA patients and control samples. The sensitivity and specificity of the RA factor estimation were found to be 92% and 88% respectively. The mean CRP value in the RA patients was found to be 46.4 ± 42.9 mg/dl whereas in the control samples it was found to be 2.2 ± 1.6 mg/dl which is well within the normal clinical range (Table 1). Thus considerable differences also exist in case of CRP levels among the patients and the control samples, which is supported by the t-test. The estimated ASO titre also showed considerable differences among the RA patients and the controls with mean ± SD values of 147.5± 107.8 IU/ml and 83.1 ± 49.7 IU/ml respectively (Table 1). The estimated ESR values in the RA and the control subjects were found to be 37.6 ± 23.0 and 12.7 ± 4.1 mm/hr respectively.

The box plot analyses were also performed based on the quantitative estimation of the four essential clinical parameters of Rheumatoid Arthritis namely RA factor, CRP, ASO titre and ESR (Figure 1a). The box plots revealed that there exist considerable differences in the distribution of each of the four clinical parameters among the patients and the control samples. It can also be observed that each of the four clinical parameters have shown considerable fluctuations in 50% of the RA patients, whereas such wide range distribution could not be observed for the control samples except for the moderate fluctuations in the ASO titre. From Figure 1b it can be seen that the frequencies of the distribution of the above mentioned four parameters varied over a very wide range of values compared to that in the control samples. However, as evident from the Figure 1b, minor variations in the frequency distribution of two parameters namely ASO titre and ESR were also observed in case of the controls.

The two other parameters i.e. plasma ceruloplasmin and serum creatinine, which are generally not considered as conventional parameters for RA diagnosis, were then compared with RA factor level in the RA patients and the control samples with the help of scatterplot (Figure 2). Surprisingly in both the cases the scatterplots showed positive correlation with an upwardly directed linear trendline.

Correlation analyses were also performed among the different parameters for the RA patients (Table 2). From the correlation table it was evident that the correlation coefficients between RA factor and other five parameters were all greater than 0.5 with RA versus CRP value

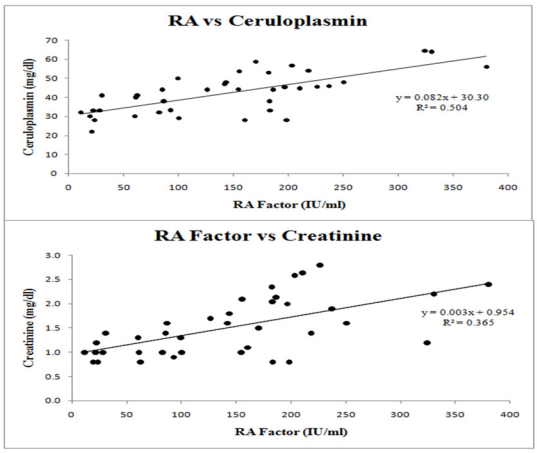

Figure 2: Scatterplot analyses to show RA factor vs Ceruloplasmin and RA factor vs. creatinine levels respectively in RA patients.

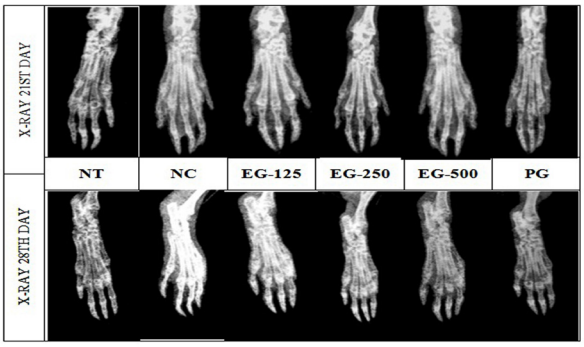

Figure 3: X ray photographs of the rat paw on the 21st and the 28th days of the experiment in different rat groups.

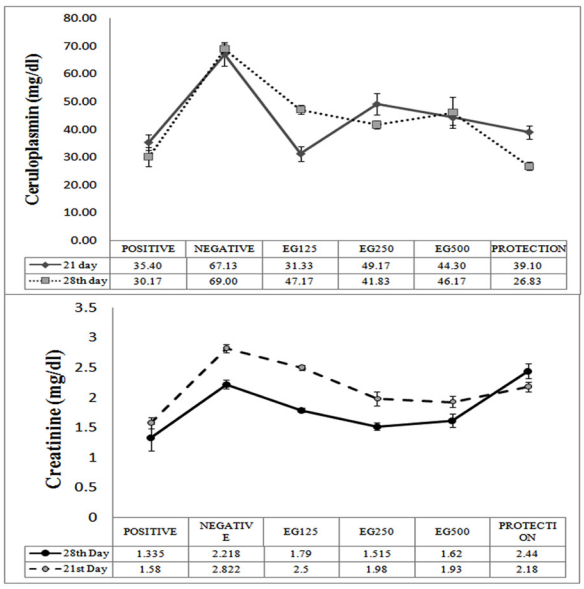

Figure 4: Line diagram shows (a) the ceruloplasmin level on the 21st and 28th day respectively in the different rat groups and also (b) creatinine level on the 28th day.

leading the chart. One interesting observation was that no negative correlation was evident among the six clinical parameters. However, it was also found that among the RA patients, the lowest correlation coefficient was evident between ASO titre and creatinine level.

In rat model based analyses, it was observed that with the exception of non-arthritic positive control (NT) group, there was significant increase in paw circumferences in the FCA-induced arthritic group. The paw circumference in all the groups was presented in Table 3. Interestingly the paw circumference in treatment groups showed significant reduction after treatment with *Aloe vera* crude gel homogenate. Among the three experimental groups, 125 µl dose, corresponding to 20 mg dry gel/kg body weight, showed the maximum reduction rate of paw edema. Whereas 250 µl and 500 µl doses, corresponding to 50 gm and 100 gm wet weight/kg body weights respectively, showed greater protection rates after the booster dose of FCA. However in the protective group (PG), 250 µl doses showed very minor paw swelling even after FCA injection in both the initial and booster doses. Similar results were also evident from the x-ray photographs where it was observed that in the protection group, the paw swelling was significantly lower compared to the other experimental groups (Figure 3).

The levels of ceruloplasmin and serum creatinine in the control and the experimental rat groups have also been presented in Figure 4. It was clearly observed that the ceruloplasmin level was much more elevated in the arthritic group in comparison to the positive control group. However the administrations of *Aloe vera* plant extract in different doses have shown considerable reduction in the ceruloplasmin levels in varying degrees. In the EG125 group, it was found that the ceruloplasmin level was almost reduced to normal level when measured on the 21st day whereas the ceruloplasmin level went high on the 28th day. In case of EG250 and EG500 groups, we found that the ceruloplasmin level was not only reduced from that of the AG but was also maintained at a steady state both on the 21st and 28th day. Interestingly, it was found that the Protection Group (PG) did not show any elevations in their plasma ceruloplasmin during both the 21st and 28th day of the experiments. In serum creatinine assay, it was found that all the experimental groups showed lowered levels compared to the Arthritic Group (AG). However, unlike the ceruloplasmin results, we found no significant reduction in creatinine level in the PG group compared to the negative control group.

Discussion

A good number of observations were derived from the results of the estimation of the different clinical parameters that we have conducted on the blood samples of 50 RA patients and 50 control subjects. Interestingly it was observed that none of the four clinical parameters were 100% positive (above the threshold value) in all the affected individuals as were also evident from their sensitivity and specificity measures. These discrepancies have been explained below. In case of RA factor, it was found that 92% of the patients had values above the threshold level while 8% had lower values. When compared to data of other populations, it was found that in case of the Korean population, 80.56% of the RA patients were found to be RF positive while 19.44 were RF negative [17]. In a similar study conducted on the Iranian population, it was found that 66.5% of the RA patients were RF positive while 33.5 were negative [18]. In another study based on Turkish patients, it was found that 53.335 were RF positive while 46.67 were RF negative [19]. These variations may be the outcome of some peculiar fundamental facts. RA factor is the antibody against the FC portion of the IgG and it may belong to different isotypes e.g. IgM, IgE, IgG, IgA and IgD and thus their detection may vary based on the proportion of the different isotypes present in the serum. Moreover, diagnosis of the RA factors in the patients suffering for not more than 6 months may result as seronegative, who may become positive during the progression of the disease. In fact three patients in our study population, who were diagnosed as seronegative, had a history of only 4 month suffering. However the sensitivity and specificity of the RA factor test as calculated in our study are 92% and 88% respectively. Therefore it could be said that the test of RA factor may prove beneficial and is an essential clinical parameter for evaluating the disease prognosis provided it is accompanied by other parametric evaluations for confirmation of the results. Moreover from Table 1 it is observed that the odds of exposure to RA factor were greater by 84.33% among the RA patients compared to the control. The CRP and ESR are the two primary parameters which are frequently used for the clinical detection of acute phase reactions like inflammations. The concentration of these two parameters become relatively high during inflammation compared to normal level. Moreover they have a relatively short time lag from the moment of stimulus, and are cost-effective. CRP is a very sensitive parameter in detection of inflammation and therefore was included in our study. In a study conducted in a Pakistani population it was found that the CRP values ranged between 11.2 to 108 mg/dl with a mean value of 39.1mg/dl in severe RA condition, while the ESR values ranged between 12 to 146 mm/hr with a mean value of 62.5 mm/hr [20]. In another study conducted in Chandigarh, the mean CRP and ESR values were found to be 22.8 mg/dl and 51.3 mm/hr respectively [21]. Thus these already published results are very much comparable to our reports. CRP is produced in the liver induced by monocytes and macrophages derived pro-inflammatory responses. These proinflammatory responses triggers increased secretion of interleukin –1β (IL-1β) and tumor necrosis factor – α(TNF-α), which via the release of interleukin 6 (IL-6) stimulate the liver to secrete CRP. Recent studies have suggested the direct contribution of the CRP in the inflammatory activities, where it stimulates secretion of inflammatory cytokines such as IL-1β, IL-6 and TNF-α from the monocytes and also directly provides pro-inflammatory stimulus to phagocytic cells [22,23]. CRP is also the only independent determinant of microvascular endothelial dysfunction in patients with RA [24]. On the other hand, the basic principle underlying the estimation of ESR is that the erythrocytes normally repel each other due to the net negative charges. However, at the time of acute phase reactions, positively charged high molecular weight proteins such as fibrinogens, present in the blood, increase in amount and promote rouleaux formation which further increases the ESR. Inflammatory processes play a pivotal role in the pathogenesis of RA and therefore these two parameters are also considered important measures of RA prognosis. However, sometimes severe inflammation does not corroborate the prognosis of RA and clinicians have to depend on other diagnostic features for determining RA. As evident from our results, both CRP and ESR are not always present above the threshold value in case of the RA patients though they are positive for 74% and 78% respectively. This may happen due to the lack of prominent acute phase reactions in some of the patients. This may also occur because of the tendency of these parameters to return to lower values if measured during early phases of the disease. However the sensitivities and specificities of both these tests were quite high thereby assuring us to keep our faith on these tests in RA diagnostic purposes. The ASO titre is another essential parameter for the diagnosis of RA. This test was employed in our experiments to detect the rheumatic fever caused by environmental triggers like streptococci infection. It measures the plasma levels of anti-streptolysin O antibodies produced against

Parameters	Normal Range	RA patients (n=50)	Control Subjects (n=50)	T-value	Odds Ratio	Relative Risk	Sensitivity (%)	Specificity (%)
Age		47.2 ± 14.0	35.1 ± 11.4					
RA factor (IU/ml)	0-20.0 IU/ml	135.9 ± 91.4	14.0 ± 5.5	9.45***	84.33	7.67	92	88
CRP (mg/dl)	0-6 mg/dl	46.4 ± 42.9	2.2 ± 1.6	7.32***	44.59	12.33	74	94
ASO (IU/ml)	0-200 IU/ml	147.5 ± 107.8	83.1 ± 49.7	3.66***	29.41	23.00	22	100
ESR (mm/hour)	0-20 mm/hour	37.6 ± 23.0	12.7 ± 4.5	8.01***	31.91	7.80	78	90
Ceruloplasmin (mg/dl)	20-35 mg/dl	42.1 ± 11	23.8 ± 6.1	8.96***	34.62	12.50	65.79	94.74
Creatinine (mg/dl)	0.6-1.2 mg/dl	1.5 ± 0.6	0.9 ± 0.2	5.79***	11.69	5.50	57.89	89.47

*p<0.05, **p<0.01, ***p<0.001.

Table 1: Statistical analyses of the different clinical parameters of the Rheumatoid Arthritic patients and control subjects.

	RA	CRP	ASO	ESR	Cerulo plasmin	Creatinine
RA	1.00					
CRP	0.73	1.00				
ASO	0.65	0.48	1.00			
ESR	0.71	0.72	0.63	1.00		
Ceruloplasmin	0.57	0.52	0.38	0.40	1.00	
Creatinine	0.60	0.65	0.22	0.48	0.62	1.00

Table 2: Correlation coefficient of the different clinical parameters in the Rheumatoid Arthritis patients.

	Days								
Groups	**1**	**4**	**7**	**10**	**13**	**16**	**19**	**23**	**27**
+ Control(NT)	22.57	23.76	22.46	23.24	22.84	22.56	22.29	22.14	21.99
- Control(AG)	22.38	32.00	28.28	26.84	26.02	25.14	32.85	30.80	26.98
EG-125	21.63	27.17	25.54	23.63	23.21	23.27	29.21	27.55	25.04
EG-250	22.75	27.67	25.84	24.75	24.00	23.44	26.53	26.42	24.36
EG-500	22.86	26.00	25.47	25.17	23.94	22.92	26.43	26.25	24.69
Protective (PG)	22.21	22.39	22.14	22.24	21.98	21.78	24.18	22.92	22.95

Table 3: Measurement of paw circumference (mm) of different rat groups in different days.

streptolysin of different streptococcal strains. It has been observed that out of 50 samples only 11 samples had higher than 200 IU/ml ASO titre values. However it is clearly evident that the mean ASO titre value was higher in case of the RA patients compared to the control samples (Table 1) which may have resulted due to the presence of ASO positive patients having ASO titre above threshold value (200 IU/ml). Francois (1965) [25] has shown significantly higher prevalence of 3-haemolytic streptococci of Groups A, C, and G at any point of the study duration in the RA patients compared to control samples which he assumed to be due in part to a slightly higher acquisition rate, but mainly to a reduced elimination rate. Thus due to lower elimination rate, prolonged infection may lead to higher antibody response and this statement may justify the facts that although raised anti-streptolysin-O titres (>150 IU/ml) were found in both the groups, their numbers were higher in RA patients compared to the controls and also more higher titres (>300 U) were significantly found in the RA patients but such high values of ASO titre were not observed in the control samples (Figure 1b). One interesting observation was that the sensitivity of the test was as low as 22% which may result due to the presence of only 11 patients having positive titre result i.e. ASO>200 IU/ml, but on the contrary, the specificity was 100% indicating the negative outcome of the test result in absence of acute phase reactions which is an well-established pathophysiological condition in case of rheumatoid arthritis. Thus ASO titre may also prove useful in the diagnosis of RA.

It is interesting to note that the plasma ceruloplasmin and creatinine showed significant differences in their levels in the RA patient and the control blood samples though they are not generally considered as essential diagnostic tool for RA diagnosis. Serum ceruloplasmin was selected for the study because of its high correlation with serum antioxidant property which have already been reported elsewhere [26] and may have considerable protective role [27,28] especially in presence of tissue damage or destruction. In a study conducted in Poland, mean ceruloplasmin level was found to be 0.3 g/L which is very much comparable to our data [29]. Serum creatinine estimation has been carried out in this study as it is an important metabolic by-product of creatine which in turn is a very crucial amino acid for building and repairing of muscular tissues.

Increased level of plasma ceruloplasmin level during inflammation may occur due to an increased production of interleukins such as IL1 and IL6 in the RA patients which stimulate the hepatocytes to release and increase the amount of ceruloplasmin into the blood. As evident from our study, such rise of ceruloplasmin level in case of RA patients was also evident from other previously published reports [26]. Previous reports have also shown highly significant correlation between the serum ceruloplasmin and serum antioxidant activity [26] and thereby supporting the concept that ceruloplasmin may be responsible for actively governing the serum antioxidant activity [30] which when increased may be an important component of the systemic inflammatory response. On the other hand the creatinine level showed moderate differences in between the RA and the control blood samples. Blood creatinine generally shows a rise only after marked damage of nephrons which is observed in patients having long term RA and inflammatory responses. Thus it may not serve as a very potent tool for early diagnosis of RA but can be useful for detection and treatment of nephropathy during late Rheumatoid Arthritis. Moreover these two tests have considerable sensitivity and specificity indicating that their results may prove affirmative with that of the other above mentioned tests for RA diagnosis.

In case of the rat model it was found that considerable foot swelling

and increased paw circumference induced by FCA injection in the Arthritic rat group were also accompanied by significant increase in serum ceruloplasmin and creatinine levels in this group compared to that of the non-treated or the positive control groups. In both the 21st and 28th days, there was significant increase of ceruloplasmin levels in arthritic group. Thus the above mentioned similarity between human and the rat models in the distribution of ceruloplasmin and creatinine in the arthritic and the normal population strongly suggest the efficacy of the rat model for various pharmaceutical and clinical experiments targeted towards the Rheumatoid Arthritis therapy in the humans.

It was found that the paw circumference was decreased towards the normal state on applying *Aloe vera* gel in the experimental rat groups. It clearly indicated the rapid regression of the foot swelling in the experimental groups compared to the negative control or the arthritic groups (Figure 3 and Table 3). The differences in the mean paw circumference between the members of the experimental and the negative control (AG) groups started to increase from the 3rd day. Paw swelling reverted back almost to the normal condition within 10th day in all the experimental groups while the swelling was retained in the negative control (Arthritic group) rats throughout the experiment. The same feature was also observed after the booster injection of FCA. Moreover, X-ray photographs (Figure 3) have clearly indicated that in case of the arthritis induced rats, the hind paw joints appeared loosened and the spaces increased in between the joints, confirming the diseased state in the arthritic rats [31]. Thus, it can be clearly said that these two parameters can be used as effective visual tool for understanding the disease severity. The treatment groups showed a tendency to restore the joint structure towards the normal state (Figure 3). Moreover, the decreased levels of ceruloplasmin in the experimental groups were evident compared to the negative controls which confirm the efficacious role of *Aloe vera* gel in lowering the ceruloplasmin level. Interestingly, the protective group showed no significant difference in the ceruloplasmin levels when compared with the Non-Treated (NT) group rats, both on 21st and 28th day, indicating the strong protective role of *Aloe vera* in the amelioration of rheumatoid arthritis.

Serum creatinine concentration increased in the negative control arthritic group rats when compared to the normal rats ($p>0.05$) but rats of all the other experimental groups showed no significant change when compared to the positive control rats. This observation indicated that the level of creatinine is normal ($p>0.05$). However the protective group did not show any effect on the creatinine level probably because this factor increases due to increasing nephropathy in case of long term RA. In correlation with other biochemical parameters examined in this part of the study, it can be said that *Aloe vera* plays a significant role in amelioration of rheumatoid arthritis induced paw edema.

In conclusion, the work can be summarized with three principal sentences. Firstly, six clinical parameters that we have selected for the study like, RA factor, CRP, ASO, ESR, ceruloplasmin and serum creatinine, are all essential for the differential diagnosis of RA during its early and later stages. Secondly, this study has supported the justification of the experimentation on rat model for designing the therapeutic strategies against Rheumatoid Arthritis in humans. Lastly, as evident from our study, *Aloe vera* extracts can be beneficial for the reduction of inflammatory edema and also for the reduction of ceruloplasmin in RA condition in rat model. However, further investigations are necessary for more refined therapeutic usage of *Aloe vera* for the treatment of RA in humans.

References

1. Firestein GS (2003) Evolving concepts of rheumatoid arthritis. Nature 423: 356-361.
2. Newman SP, Fitzpatrick R (1995) Understanding rheumatoid arthritis, Burns & Oates.
3. Nejatzadeh-Barandozi F (2013) Antibacterial activities and antioxidant capacity of *Aloe vera*. Org Med Chem Lett 3: 5.
4. Surjushe A, Vasani R, Saple DG (2008) *Aloe vera*: a short review. Indian J Dermatol 53: 163-166.
5. Davis RH, Leitner MG, Russo JM, Byrne ME (1989) Wound healing. Oral and topical activity of *Aloe vera*. J Am Podiatr Med Assoc 79: 559-562.
6. Davis RH, Donato JJ, Hartman GM, Haas RC (1994) Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. J Am Podiatr Med Assoc 84: 77-81.
7. Domkat LC, Habibu T (2013) Comparative studies of the aqueous extracts of Ocimun gratissmum, *Aloe vera*, Brassica oleracea and Ipopoea batatas on some biochemical parameters in diabetic rats. J Pharm Biol Sci 6: 23-29.
8. Paul S, Dutta S, Chaudhuri TK, Bhattacharjee S (2014) Anti-Inflammatory and Protective Properties of *Aloe vera* Leaf Crude Gel in Carrageenan Induced Acute Inflammatory Rat Models. Int J Ph Pharma Biol 6: 368-371.
9. Tan ZJ, Li FF, Xu XL (2013) Extraction and purification of anthraquinones derivatives from *Aloe vera* L. using alcohol/salt aqueous two-phase system. Bioprocess Biosyst Eng 36: 1105-1113.
10. Ng T (1997) Erythrocyte sedimentation rate, plasma viscosity and C-reactive protein in clinical practice. Br J Hosp Med 58: 521-523.
11. Caswell M, Pike LA, Bull BS, Stuart J (1993) Effect of patient age on tests of the acute-phase response. Arch Pathol Lab Med 117: 906-910.
12. Sunderman FW Jr, Nomoto S (1970) Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clin Chem 16: 903-910.
13. Lustgarten JA, Wenk RE (1972) Simple, rapid, kinetic method for serum creatinine measurement. Clin Chem 18: 1419-1422.
14. Bendele A, McComb J, Gould T, McAbee T, Sennello G, et al. (1999) Animal models of arthritis: relevance to human disease. Toxicol Pathol 27: 134-142.
15. Paquet J, Goebel JC, Delaunay C, Pinzano A, Grossin L, et al. (2012) Cytokines profiling by multiplex analysis in experimental arthritis: which pathophysiological relevance for articular versus systemic mediators? Arthritis Res Ther 14: R60.
16. Rathore B, Paul B, Chaudhury BP, Saxena AK, Sahu AP, et al. (2007) Comparative studies of different organs of Nyctanthes arbortristis in modulation of cytokines in murine model of arthritis. Biomed Environ Sci 20: 154-159.
17. Choi SW, Lim MK, Shin DH, Park JJ, Shim SC (2005) Diagnostic performances of anti-cyclic citrullinated peptides antibody and antifilaggrin antibody in Korean patients with rheumatoid arthritis. J Korean Med Sci 20: 473-478.
18. Binesh F, Salehabadi HS, Behniafard N, Ranginkaman K, Behniafard N (2014) A Comparative Assessment of the Diagnostic Value of Anti-cyclic Citrullinated Peptide Antibodies and Rheumatoid Factor in Rheumatoid Arthritis. J Clin Exp Pathol 4: 158.
19. Kuru O, Bilgici A, Birinci A, Ulusoy H, Durupinar B (2009) Prognostic value of anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis. Bratisl Lek Listy 110: 650-654.
20. Naqi N, Ahmed TA, Malik JM, Ahmed M, Bashir MM (2012) Identification Of Laboratory Markers Of Disease Activity In Rheumatoid Arthritis. Pak Arm F Med J 1: 1-6.
21. Taneja SK, Mandal R (2009) Assessment of mineral status (Zn, Cu, Mg and Mn) in rheumatoid arthritis patients in Chandigarh, India. Rheum Rep 1: e5.
22. Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW (1999) The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. J Exp Med 190: 585-590.
23. Stancíková M, Rovenský J (1993) Effect of cyclosporin on the activity of cytidine deaminase and adenosine deaminase in the serum and polymorphonuclear leukocytes of patients with rheumatoid arthritis. Int J Tissue React 15: 169-174.
24. Galarraga B, Khan F, Kumar P, Pullar T, Belch JJ (2008) C-reactive protein: the underlying cause of microvascular dysfunction in rheumatoid arthritis. Rheumatology (Oxford) 47: 1780-1784.

25. François RJ (1965) Beta-haemolytic streptococci and antistreptolysin-O titres in patients with rheumatoid arthritis and a matched control group. Ann Rheum Dis 24: 369-377.

26. Scudder PR, Al-Timimi D, McMurray W, White AG, Zoob BC, et al. (1978) Serum copper and related variables in rheumatoid arthritis. Ann Rheum Dis 37: 67-70.

27. Al-Timimi DJ (1977) Serum antioxidant activity. MPhill thesis, University of London.

28. Al-Timimi DJ, Dormandy TL (1977) The inhibition of lipid autoxidation by human caeruloplasmin. Biochem J 168: 283-288.

29. Strecker D, Mierzecki A, Radomska K (2013) Copper levels in patients with rheumatoid arthritis. Ann Agric Environ Med 20: 312-316.

30. Stocks J, Gutteridge JM, Sharp RJ, Dormandy TL (1974) The inhibition of lipid autoxidation by human serum and its relation to serum proteins and alpha-tocopherol. Clin Sci Mol Med 47: 223-233.

31. Ekambaram S, Perumal SS, Subramanian V (2010) Evaluation of antiarthritic activity of Strychnos potatorum Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complement Altern Med 10: 56.

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Curative and protective properties of crude gel of *Aloe vera* from sub-Himalayan West Bengal in chronic and acute inflammatory rat models

[1]Subhashis Paul, [2]Tanmoy Dutta, [3]Tapas Kumar Chaudhuri & [4]Soumen Bhattacharjee*

[1,2,4]Cell & Molecular Biology Laboratory, [3]Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, PO North Bengal University, Raja Rammohunpur, Siliguri-734 013, West Bengal

E-mails: psubhashis21@gmail.com; tanmoy_zool@rediffmail.com; dr_tkc_nbu@rediffmail.com; soumenb123@rediffmail.com / sbhnbu@gmail.com

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Aloe vera (L.) Burm. f. (Xanthorrhoeaceae) has been used traditionally as a wound healer and an anti-inflammatory medication. The *Aloe* gel has natural combination of different bio-active compounds and is commonly consumed or applied in crude condition. We have used unprocessed *Aloe* gel homogenate in acute and chronic inflammatory Wistar albino male rat models to investigate its properties in a systematic way. Chronic inflammatory arthritic model was established by injecting 0.1 ml Freund's Complete Adjuvant (FCA) in the hind paw of rat. Radiograph, histology, hematological and biochemical properties of the serum were used to determine the ameliorative role of crude *Aloe* gel homogenate in the rat experimental groups *in vivo*. Acute inflammatory rat model was established using sub-cutaneous cotton pellet induction. The weight of dried cotton pellets were determined to estimate the extent of granuloma formation. *A. vera* crude gel inhibited paw swelling up to 65.59 % in experimental groups of arthritic rats and decreased the granuloma formation up to 25 % in cotton pellet induced inflammatory rats. It is well evidenced from the experiments that unprocessed *A. vera* crude gel possesses a good anti-inflammatory property for both chronic and acute types when fed at a dose of 0.40 gm/kg body weight.

Keywords: Paw edema, Rheumatoid arthritis, Freund's complete adjuvant, Cotton pellet

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Inflammation is the immediate manifestation of the triggering of immune system against any infiltrating pathogen in the host body, expressed by means of redness, swelling, pain and heat generation. The etiology of inflammation and inflammatory diseases such as rheumatoid arthritis (RA) is not well understood till date. The clinical symptoms of the inflammatory RA are inflammation and swelling of the joints. This leads to the damage of bone and cartilage and as a result finally disrupts the joint structure and function[1]. Recent treatment strategies like cytokine therapy (anti-TNFα therapy) or disease modifying anti-rheumatic drugs (DMARDs) regime can only suppress the disease. The disease flares up whenever the treatment is stopped. In past few decades researchers have shown interest in the herbal products which are reported to ameliorate disease in ancient *Ayurvedic* system. The intention of the present work was to reveal the synergistic effect of crude *Aloe* gel on structural, biochemical and hematological parameters in RA. *Aloe vera* (L.) Burm. f. (Xanthorrhoeaceae) is a stem-less or short-stemmed succulent plant growing up to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up. It is found all over the North-Eastern India and sub-Himalayan region and is being traditionally used in various skin ailments[2]. The gel is consumed or applied topically to reduce joints pain. Phytochemical screening of *A. vera* has confirmed the presence of flavonoids, alkaloids, resins, tannins, steroids and other chemical substances[3]. Anti-inflammatory properties of the crude *A. vera* gel in wound healing, carrageenan induced paw oedemal model of rats have also been documented[4,5,6,7]. Experiment-based chronic and acute anti-inflammatory properties of this agent are not well

*Corresponding author

documented though it is used traditionally as anti-inflammatory agent in *Ayurveda*. Therefore, we have tried to investigate the role of wild *A. vera* raw gel in both chronic and acute inflammation by studying appropriate parameters in appropriate rat model, which are established standard models for research in experimental inflammation. The gel was used in crude form as we hypothesize that the bioactive compounds present in *A. vera* in different proportions may act synergistically for better result. In other words, isolated bioactive chemical(s) may not act optimally. Moreover, ethnic people consume it in crude condition as medicine and therefore it is important to know the effect of this gel in a crude condition.

Therefore, the present study was designed to evaluate the anti-inflammatory activities of raw or unprocessed *Aloe* gel and its effects in FCA-induced arthritic rats and cotton pellet induced inflammatory rats through radiology, hematology, biochemical and biometric parameters.

Methodology

Collection of plant specimens

Naturalized *Aloe vera* (L.) Burm. f. (Family: Xanthorrhoeaceae) plants were collected from the sub-Himalayan Terai areas of northern West Bengal, India and were identified in the Department of Botany, University of North Bengal. The voucher specimen was deposited in the NBU herbarium (Accession No. NBU09884).

Preparation of extract

The leaf gel was collected by peeling out the outer cuticle and cutting out the gel aseptically into small pieces. The gel was mixed with distilled water (1:5 w/v) and then homogenized to create a crude homogenate. The sample was freshly prepared every time before use. The experimental animal groups were fed with 125 µl and 250 µl of the homogenates. To determine the dry weight, pieces of *A. vera* gel was weighed and then dried separately in an air oven at 37 ºC for 48 hrs.

Experimental animals

Wistar albino male rats (80-100 gm) used for all the experiments were procured from an authorized animal dealer (Ghosh Enterprise, Kolkata, India). Animals were kept in polypropylene cages (max. 4 rats per cage) and maintained at a room temperature of 25 ± 3°C. Rats were maintained in the laboratory with standard feed and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) of the University of North Bengal, West Bengal, India.

Induction of chronic and acute inflammation

Adjuvant induced Arthritis (AIA) model was produced as chronic inflammatory model as per the method described by Bendele *et al*. (1999)[8]. All the animals were administered a dose of 0.1ml of FCA in the left hind paw interplanetary except the positive control group. The animals were administered a dose of 0.1ml FCA again on the same region to boost up the immune response on the 14th day. The paw diameter was measured with the help of a vernier caliper and recorded at regular intervals of 2 or 3 days. Acute inflammation was induced by cotton pellet granuloma model. This method was adopted from Penn (1963)[9] with slight modifications, which was carried out by using sterilized cotton pellet implantation method in rats[10]. Under light ether anesthesia subcutaneous tunnel was made by using blunted forceps and the sterilized cotton pellets (25 ± 1 mg) were implanted in one side of the vertebra of each rat.

Experimental setup

Wister male rats were considered as the experimental animals in both the anti-arthritic and anti-inflammatory experiments. For chronic inflammatory test, in anti-arthritic rat models, animals were divided into 5 groups, each containing 10 rats. The first two groups were considered as the Control groups. Among them one group was considered as non-treated or positive control (PC) having neither FCA nor *A. vera* and the other group was considered as negative control (NC) in which only FCA injection was given but no treatment was administered. The third and the fourth groups were considered as experimental groups (EGs). These groups were treated with *A. vera* doses of 125µl (125D) and 250 µl (250D) respectively corresponding to 25 gm wet gel/60 kg body weight (20 mg dry weight/kg body weight) and 50 gm wet gel/60 kg body weight (40 mg dry weight/kg body weight). The doses were empirically calculated on the basis of daily consumption of 50 gm of *A. vera* in a man weighing 60 kg, although the absorption rates may differ considerably between rat and human systems. The fifth group was the Protective group (PG) in which

125 µl of *A. vera* (25gm wet gel /60 kg body weight or 20 mg dry weight/kg body weight) was given for 7 days prior to FCA injection. Five rats from all the groups were sacrificed on the 21st day of experiment, while the rest were sacrificed on the 28th day as per our experimental design to measure the different parameters in different times. The left hind paws of all the animals of 5 groups were separated out from the body after sacrifice and were subjected to radiological and histological analyses. Blood samples were collected using insulin syringes from the hearts and were subjected to different hematological and biochemical tests.

Acute inflammation model was established by cotton pellet induced granuloma formation in rat. Four groups of animals, including a control and a standard group, were selected for the experiments, each group comprising of 4 rats. The control group (Control) was left without any treatment. The standard group (Standard) animals were fed with indomethacin, as a control drug, at a concentration of 4 mg/kg body weight. Third and fourth groups were experimental groups namely AV1 and AV2, treated with 125 µl and 250 µl *A. vera* crude gel homogenates respectively as in anti-arthritic experiments. The experimental animals were sacrificed on the 8th day and the dry weight of each granuloma was measured[11].

Parameters for anti-arthritic tests

Biometric studies

Body weight was recorded at regular intervals. Rats from all the groups were subjected to radiographic study. After dissection, the joints were taken immediately for radiography. Measurement of paw circumference was done with the help of a vernier caliper and circumference was measured using the formula: 2π $[\sqrt{(A^2+B^2)}]$ /2, where A and B are measures of diameter at two different planes of paw taken with the help of Vernier Caliper)[12, 13] (Fig. 1).

Histological studies

Samples were preserved in 10% formaldehyde after radiographic analysis. Decalcification of the histological samples was done in 3% HCl for 4 days followed by paraffin block preparation. Histological sections were made along the longitudinal plane of joint axis and were stained in hematoxylin-eosin stain following standard protocols.

Hematological studies

Hematological parameters included RBC total count, WBC total count using hemocytometer and hemoglobin estimation by using Sahli's hemoglobinometer.

Biochemical studies

Total blood glucose estimation was done by ortho-toluidine method[14] to estimate the effects of *Aloe* gel on blood glucose level of rats. Total protein was estimated by Coral Total Protein Kit (Coral Clinical Systems, Goa, India) (Biuret method) according to the manufacturer's protocol. Total albumin estimation was done using the Coral Total Albumin Kit (Coral Clinical Systems, Goa, India) (BCG method). Serum ceruloplasmin estimation was done using p-phenylenediamine oxidase activity spectro-photometrically[15]. The levels of serum creatinine were measured spectrophotometrically by studying reactions between creatinine and alkaline picrate[16].

Parameters for anti-inflammatory tests

Animals of all groups were sacrificed on the 8th day by cervical dislocation and the pellets were removed, freed from extraneous tissue and dried at 60 ºC for 24 hrs. The percentage inhibitions of the dry weight of the granuloma were calculated and compared[11].

Statistical analysis

All statistical analyses were done using the MS-Excel 2007 and Kyplot ver 2.0 Beta. In Kyplot analysis, the data represented mean ± SD which was analyzed by one way ANOVA. The results were considered significant when $p \leq 0.05$.

Results

There was significant increase in paw circumference in every arthritic group except the non-arthritic positive control group (Fig. 1). But paw circumference in the experimental groups showed significant reduction after treatment with *A. vera* crude gel homogenate. 125 µl doses showed the maximum reduction rates, whereas 250 µl dose elicited greater protection rates after the booster dose of FCA. 125D, 250D and PG groups showed 23.30%, 65.59% and 83.79% less swelling after the 28th day of experiment commencement. In the PG, 125 µl doses showed very less paw swelling after FCA injection in both initial and booster dose injections signifying the protective property of the plant against arthritis. Radiographs further confirmed the role of *A. vera* gel in arthritis (Fig. 2). Histological observations showed less affected cartilage and joints in all experimental groups when compared to negative control. Cellular

infiltration and cartilage damage was less in all the experimental groups (Fig. 3). The hematological parameters showed decrement in RBC and hemoglobin counts in arthritic condition, whereas WBC count increased significantly. The treatment with *A. vera* in experimental groups brought back the parameters toward their normal levels. However, protective group (PG) showed more or less normal levels of all the parameters in both experimental time points (day 21 and 28) (Table 1).

There was no significant change in the blood sugar level in both arthritic and non-arthritic rats (CG, EG and PGs) (data not shown). In all the groups, the blood glucose level remained <100 mg/dl. Arthritic rats showed a significant decrease in the total protein level that was restored within their normal ranges after *Aloe* gel treatment. In both 21st and 28th day of experiment, the protective group showed increased total protein compared to other EGs (Fig. 4 & Table 2). Confirming the role of *Aloe* gel treatment regime on serum protein level, the experimental groups showed elevated levels of serum albumin on 21st day than the negative control group. The protective group animals showed greater level of albumin that approached towards the normal value showing the protective property of the plant (Table 2). The experimental groups restored the elevated levels of serum ceruloplasmin after treating with *A. vera* gel. In all the cases the protective group showed normal ceruloplasmin level (Fig. 4). Arthritic rats showed an increase in serum creatinine level on the 21st day. However, the value of creatinine decreased significantly towards the normal value in experimental groups. Protective group also showed decreased level of serum creatinine compared to the negative controls (Table 2). Standard group had a mean weight of dry cotton pellet 0.051±0.006 gm. AV1 and AV2 measured for 0.053±0.007 and 0.057±0.005 gm. Control group had the mean weight of 0.062±0.007 gm (Fig. 5).

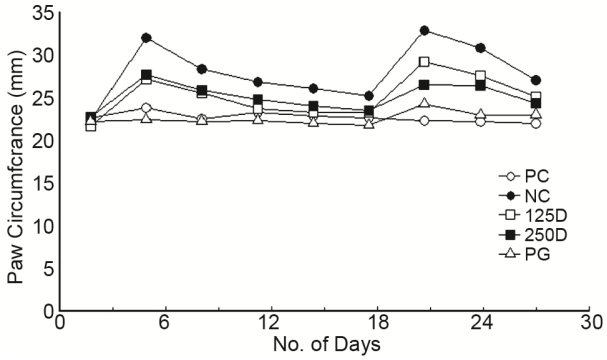

Fig. 1—Comparison of rat paw edema expressed in circumference (mm) in different experimental groups. PG shows maximum protection against the paw swelling than that of the NC.

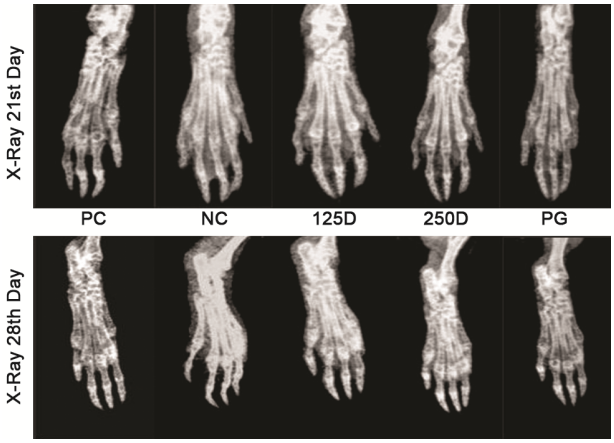

Fig. 2—Radiographs of control and experimental rats on the 21st (upper panel) and the 28th (lower panel) days of FCA injection comparing effects of *Aloe vera* treatment.

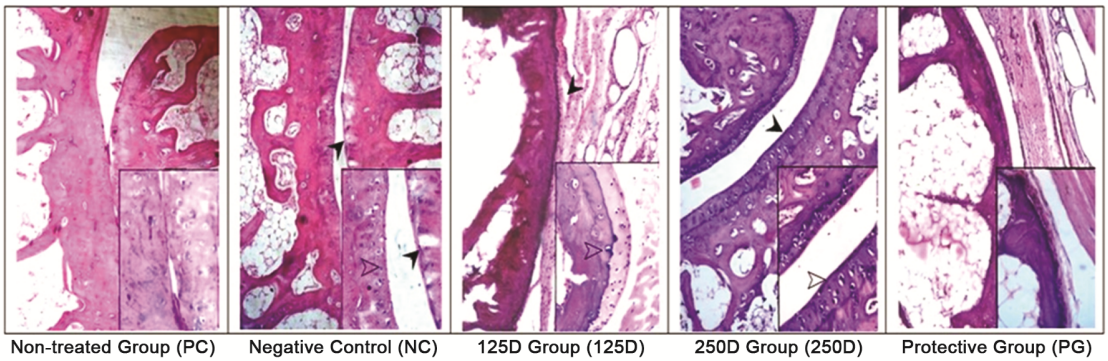

Fig. 3—Longitudinal sections of rat hind paw joints stained in hematoxylin-eosin on the 21st day of treatment in different groups of rats. Black arrows (➤) indicate cartilage breakdown and hollow arrows (▷) indicate immune cell infiltration in the joint. Inset indicates 40X magnification of a region within the 10X region.

Table 1—Hematological parameters of different experimental rat groups expressed as mean value of the group. Data represented in the form of mean ± standard deviation.

	RBC (7-10x10$^{6/}$mm^3)		WBC (6-17x10^4/mm^3)		Hemoglobin (11-18 gm/dl)	
Days	21st Day	28st Day	21st Day	28st Day	21st Day	28st Day
PC	9270000±490408	9270000 ±732802	5100± 503	5500 ±683	11.6±0.56	12.8±0.58
NC	5430000 ±485283 a***	6240000 ±693181 a***	7200± 489 a***	6900 ± 600 a*	7.20±0.52 a***	7.80±0.46 a***
125D	8440000 ±674907 b***	8880000 ±737054 b***	5700± 382 b**	5900±600 b	10.5±0.72b***	11.0±1.06 b***
250D	7920000 ±442436 b***	8840000 ±763544 b***	6200± 516 b*	6600±765 b	11.2 ±0.79 b***	10.2±1.08 b**
PG	8760000 ±571182 b***	8450000 ±325960 b***	5300± 382 b*	5400±516 b**	10.8±0.66 b***	10.0±1.24 b**

a = comparison between NT (+ control) and other groups, b = comparison between NC (- control) and other groups, *** = significant at P ≤ 0.001; **= significant at P≤0.01; * = significant at P ≤ 0.05; ( ) = normal ranges in rats according to CPCSEA, MoEF, India

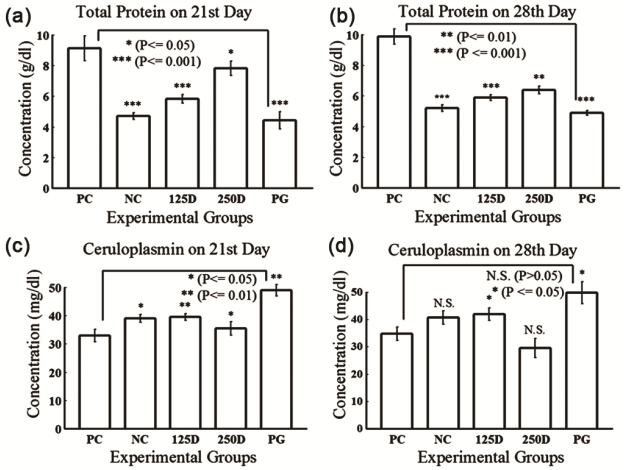

Fig. 4—Comparison of total protein on the 21st day (a) and the 28th day (b) in different groups or rats. Comparison of ceruloplasmin in different groups of rats is shown on 21st day (c) and 28th day (d). NS: Non significant (*** = significant at P ≤ 0.001; ** = significant at P≤ 0.01; * = significant at P ≤ 0.05).

Discussion

The measurements of rat paw circumference clearly indicated that the swelling was regressed rapidly in the experimental groups (Fig. 1). The difference in the mean paw circumference between the experimental and the negative control groups started to diverge from the 3rd day and swelling approached almost normal state within 10th day in all the experimental groups, while negative control rats retained the swellings. The same feature was also observed after the booster dose of FCA. At the end of the 28 day treatment schedule, all the experimental groups showed significant less swelling, respectively when compared with the NC group, which strongly supports the ameliorative property of the crude extract. Moreover, the paw swelling in the protective group (PG) was negligible compared to the negative control (NC) group. In the radiography data, the intact structures of the joint appeared loosened and the increment of space in between the joints (Fig. 2) confirmed the diseased state in the arthritic rats. The treated groups showed a tendency to restore the joint structure towards normalcy (Fig. 2). Histological observations clearly showed that the treated groups had less cellular infiltration in the cartilage compared to the negative controls (Fig. 3). The structure of the cartilage layer showed more breaks and uneven lining in the negative control group rat joints. These observations confirmed that the cartilage layer is protected when treated with *A. vera*. Interestingly, PG showed a very well formed joints structure with very little cellular infiltration with even and clear cartilage lining (Fig. 3). RBC count usually decreases in arthritic conditions. This is because the bone marrow loses its normal functioning and fails to respond to anemic condition[17]. The RBC count increased significantly in experimental groups after 21st and 28th days (Table 1). There was no significant difference between the experimental groups and the positive control group (PC). The results indicated that the RBC count reached almost the normal level when

Table 2—Measurement of serum total protein, albumin and creatinine in different experimental rat groups (mean ± S.D.)

	Total protein (gm/dl)	Total protein (gm/dl)	Albumin (gm/dl)	Creatinine (mg/dl)
	21st Day	28st Day	21st Day	21st Day
PC	9.12±0.50	9.871±0.52	6.958±0.02	2.922±0.22
NC	4.430±0. 48	4.89±0.15	3.312±0.06	4.856±0.07
125D	4.702±0.10	5.280±0.23	3.951±0.05	3.755±0.03
250D	5.821±0.16	5.891±0.12	3.730±0.02	2.970±0.06
PG	7.806±0.31	6.378±0.13	3.825±0.07	2.908±0.12

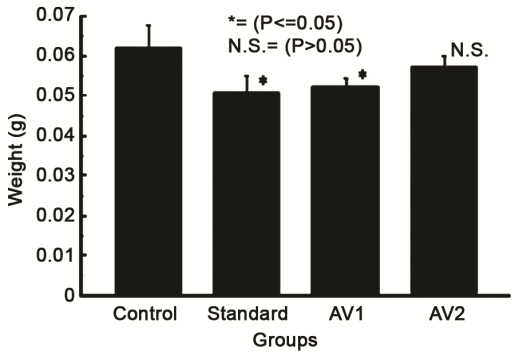

Fig. 5—Cotton pellet test showing the anti-inflammatory property of *Aloe vera*. AV1 shows better result compared to AV2. The standard group shows the best inhibition of granuloma formation.

treated with *A. vera*. In contrast, WBC count increased in arthritic condition. This is because of the boosting of the immune system with antigens[18] (here FCA). The levels of WBC in experimental groups reached the normal values and showed no significant difference with the normal rats (Table 1). The hemoglobin count also showed similarity with the value of RBC, as low RBC count results in low hemoglobin levels. The experimental groups showed significant increase in hemoglobin when compared with the negative control group (Table 1). Blood glucose level >100 mg/dl is known to be normal in rats. The blood glucose level (data not shown) remained normal in every group of rats confirming that the physiology of glucose metabolism in not affected by the development of RA. No significant change within the groups is seen, as also reported by other researchers[3]. The level of total protein usually decreases during arthritis[18]. Rats of the negative control group showed a significant decrease in the total protein level ($P \leq 0.05$) in comparison to the positive control rats in both the experimental time points (21^{st} and 28^{th} days). All the experimental groups showed the tendency to increase the protein level with statistical significance. The levels of total protein between positive control and the protective group reflected no (or very less on 28^{th} day) significance. It reflects the role of *A. vera* gel in maintaining total protein level of serum during RA (Fig. 4 & Table 2). However, there was an increase in the amount of total protein recorded between 21^{st} to 28^{th} days of treatment. When compared to the increment of the body weight in these treatment groups, the role of protein synthesis in gaining the body weight is apparent. We have observed that the total albumin of all the rat groups in 21^{st} day correlated with the total protein values. The total serum albumin level is reported to decrease in arthritic condition[17]. It can be postulated that the increased permeability of vascular cells during the arthritic inflammation may increase the diffusion of albumin into vascular tissue and thereby may decrease the level of serum albumin. The albumin level increased 1.15 folds in the protective group when compared to the negative controls, suggesting a role of Aloe crude gel homogenate in the restoration of serum albumin level ($p>0.05$) (Table 2). The experimental group rats also showed an increased albumin concentration when compared to the negative control group rats. However, the experimental results did not show much significant alteration in the albumin levels of arthritic group. This may be due to the small interval between the booster dose (day 14) and the day of the experiment (day 21), which was probably insufficient to restore the normal albumin levels in rats (Table 2). Longer study duration may establish a clearer picture. Ceruloplasmin is a major superoxide scavenger and increases during the rheumatoid arthritis condition[18]. The experimental rat groups showed a restoration of elevated serum ceruloplasmin levels after the treatment with *A. vera* gel (Fig. 4). In all the cases, the protective group (PG) showed normal ceruloplasmin levels (Fig. 4). In both the 21^{st} and 28^{th} days, there was significant increase of ceruloplasmin levels in negative control group (Fig. 4). For example, negative control showed 1.5 fold increase in ceruloplasmin level on 21^{st} day whereas 125D, 250D and PG showed 1.1 fold, 1.2 fold and 1.1 fold increase, respectively, confirming the efficacious role of *A. vera* gel in the decrement of ceruloplasmin level ($P \leq 0.05$). Interestingly, both on 21^{st} and 28^{th} day, the protective group rats showed no significant difference in the ceruloplasmin levels when compared with that of the normal (PC) group rats confirming the significant protective role of *A. vera* in the amelioration of RA. Arthritic rats showed an increase in the serum creatinine level on 21^{st} day (Table 2). However, serum creatinine concentration increased in the negative control rats when compared to the normal rats ($P \leq 0.05$) but all the other rats of the experimental group showed no significant change when compared to the positive control rats which indicated that the level of creatinine is normal ($P>0.05$). Protective group rats also showed normal creatinine level when compared to the positive control ($P>0.05$) (Table 2).

From the results, it is clearly seen that oral administration of *A. vera* crude leaf gel can reduce

granuloma formation (Fig. 5). It was found that the extract elicited significant anti-inflammatory activities in AV1 and AV2 experimental group rats by reducing the dry weight of granuloma and inhibiting the migration of WBC. AV1 showed a better result with 25% reduction in granuloma than the control followed by 15% reduction in AV2 (Fig. 5). Hematological and serum biochemical parameters determine the protective role of the plant in the animal body. However, restoration of ceruloplasmin and creatinine levels indicates the restoration of non-toxic environment of the body. In our earlier work, toxicity of *A. vera* unprocessed gel on rat spleenocytes and macrophages were shown to be absent, rather the gel showed protective properties on the cells in 4 hrs cell culture system[4].

It is well evidenced from the experiments that unprocessed *A. vera* crude gel possessed a good anti-inflammatory property for both chronic and acute types. Keeping in mind that all the physiological parameters need different time to restore, most of the critical parameters studied in the arthritic rats have shown significant changes during disease state and that were brought back to normal or near normal states during *Aloe* gel feeding. These biochemical experiments were strongly supported by the biometric, radiographic, hematological and histological parameters. Our results thus confirm that consumption of crude *Aloe* gel not only ameliorates RA conditions in the bone joints but also shows no notable side effects. The protective value of the gel was also demonstrated in animal model against subsequent development of RA. Changes in the immunological and cell biological parameters will be done in future to confirm the role of the plant in rheumatoid arthritis as well as inflammation.

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References

1 Firestein GS, Evolving concepts of rheumatoid arthritis, *Nature*, 423 (2003) 356-361.

2 Surjushe A, Vasani R & Saple DG, *Aloe vera*: a short review, *Ind J Dermatol*, 53 (2008) 163-166.

3 Luka CD & Tijjani H, Comparative studies of the aqueous extracts of *Ocimum gratissmum*, *Aloe vera*, *Brassica oleracea* and *Ipopoea batatas* on some biochemical parameters in diabetic rats, *J Pharm Biol Sci*, 6 (2013) 23-29.

4 Paul S, Dutta S, Chaudhuri TK & Bhattacharjee S, Anti-Inflammatory and protective properties of *Aloe vera* leaf crude gel in carrageenan induced acute inflammatory rat models, *Int J Pharm Pharma Sci*, 6 (2014) 368-371.

5 Guha P, Paul S, Das A, Halder B, Bhattacharjee S & Chaudhuri TK, Analyses of human and rat clinical parameters in rheumatoid arthritis raise the possibility of use of crude *Aloe vera* gel in disease amelioration, *Immunome Res*,10(2) (2014). Available at: http://dx.doi.org/10.4172/1745-7580.1000081.

6 Davis RH, Leitner MG, Russo JM & Byrne ME, Wound healing: oral and topical activity of *Aloe vera*, *J Am Podiatr Med Assoc*, 79 (1989) 559-562.

7 Davis RH, Donato JJ, Hartman GM & Haas RC, Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*, *J Am Podiatr Med Assoc*, 84 (1994)77-81.

8 Bendele A, McComb J, Gould T, McAbee T, Sennello G, *et al*., Animal models of arthritis: relevance to human disease, *Toxicol Pathol*, 27 (1999) 134-138.

9 Penn GB & Ashfold A, The inflammatory response to implantation of cotton pellets in the rats, *J Pharm Pharmacol*, 15 (1963) 798-803.

10 Balasubramanian A, Ramalingam K, Krishnan S & Christina AJM, Anti-inflammatory activity of *Morus indica* L., *Iranian J Pharmacol Ther*, 4 (2005) 13-15.

11 Mishra NK, Panda RK, Rajakumar V, Kumar S, Tejonidhi K, *et al*., Evaluation of anti-inflammatory activity and dose selection of *Mollugo pentaphylla* by using cotton pellet induced granuloma in rat, *Int J Pharm Health Sci*, 1 (2010) 155-162.

12 Paquet J, Goebel JC, Delaunay C, Pinzano A, Grossin L, *et al*., Cytokines profiling by multiplex analysis in experimental arthritis: which pathophysiological relevance for articular versus systemic mediators?, *Arthr Res Ther*, 14 (2012) R60. Available at: http://arthritis-research.com/content/14/2/R60.

13 Rathore B, Paul B, Chaudhury BP, Saxena AK, Sahu AP, *et al*., Comparative studies of different organs of *Nyctanthes arbor-tristis* in modulation of cytokines in murine model of arthritis, *Biomed Environ Sci*, 20 (2007) 154-159.

14 Arun N & Nalini N, Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats, *Plant Foods Hum Nutr*, 57 (2002) 41-52.

15 Sunderman FW & Namato S, Measurement of human serum ceruloplasmin by its p-phenyldiamine oxidase activity, *Clin Chem*, 11 (1970) 903-910.

16 Lustgarten JA & Wenk RE, Simple, rapid, kinetic method for serum creatinine measurement, *Clin Chem*, 18 (1972) 1419-1422.

17 Mowat AG, Hematologic abnormalities in rheumatoid arthritis, *Semin Arthritis Rheum*, 1 (1971) 195-219.

18 Ekambaram S, Perumal SS & Subramaniam V, Evaluation of antiarthritic activity of *Srtychnos potatorum* L. seeds on freund's adjuvant induced arthritic rat model, *BMC Compl Altern Med*, 10 (2010) 56-65.

Research Article

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IDENTIFICATION OF PHYTOCOMPONENTS FROM HEXANE SOLUBLE FRACTION OF WILD *ALOE VERA* (L.) BURM. F. GEL COLLECTED FROM UNIVERSITY OF NORTH BENGAL CAMPUS THROUGH GC-MS ANALYSIS

Subhashis Paul, Tanmoy Dutta and Soumen Bhattacharjee*
Cell and Molecular Biology Laboratory, University of North Bengal, Raja Rammohunpur, Darjeeling 734013, West Bengal, India.

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ABSTRACT
Gas Chromatography Mass Spectrometry is an advanced technique to recognize individual compounds from a mixture of compounds. Recently this technique has been extensively used in the identification of herbal constituents from potential medicinal plant materials. *Aloe vera* (L.) Burm. F. (Family Xanthorrhoeaceae) is a naturalized plant found in all over India. This plant is very commonly used by the ethnic people as food and medicinal resource for amelioration of various diseases. In this study, GC-MS of the selected wild plant from the University of North Bengal campus has revealed 61 major compounds with at least 16 compounds with biological activities. Aloe gel was collected and sample was prepared using hexane as solvent. Qualitative detection of phytochemical components were done in Thermo Scientific Trace 1300 GC and ISQ Mass spectrophotometer equipped with an AI/AS 1310 auto sampler in a 30 meter long TG5ms column. Some major compounds isolated are ethyl iso-allocholate, octadecane, geldaramycin, astaxanthin, picrotoxinin etc. All these compounds have documented biological activities in different diseases conditions. This study re-establishes the importance of *Aloe vera* as a potent medicinal plant by exploring the phytoactive compounds of the plant.

Key Words- *Aloe vera* gel, hexane, GC-MS.

INTRODUCTION

Aloe vera (L.) Burm. F. (Family Xanthorrhoeaceae) is a very well known plant found in the sub-Himalayan region. *Aloe vera* is a short-stemmed or stemless succulent plant which grows up to 100 cm in height and spreads by offsets. The leaves are thick, filled with parenchymal gel and are fleshy. Colour varies from green to grey-green. Some varieties have white spots and flecks on their upper and lower stem surfaces. This plant is largely used in Ayurveda and as a traditional dermal care product for its phytochemical properties. *Aloe vera* contains different phytochemical agents which are able to cure different disease symptoms (Sujushe *et al*., 2008). The plant is used widely in dermal diseases and is a good laxative agent (Eshun and He, 2004). It also represents compounds responsible for anti-diabetic (Tanaka *et al*., 2006), anti-oxidant (Hu *et al*., 2003), anti-microbial (Arunkumar and Muthuselvan, 2009), wound healing (Davis *et al*., 1989) and anti-inflammatory activities (Paul *et al*., 2014). Topical use of the gel in dermal care and in wound healing is also very accepted. This plant is reported to have more than 200 phytochemical compounds. In this study, the plant was collected from the adjacent regions of University of North Bengal and the phytochemicals were identified using Gas Chromatography Mass Spectrometry.

Materials and Methods

Collection of the sample: Naturaly growing wild

***Author for correspondence:** Dr. S. Bhattacharjee (E. Mail: soumenb123@rediffmail.com/sbhnbu@gmail.com)

Aloe vera (L.) Burm. F. (Class Magnoliopsida, Order Asparagales, Family Xanthorrhoeaceae) was collected from the adjacent regions of the University of North Bengal campus. The sample was identified by Prof. A. P. Das, plant taxonomist in the Department of Botany, University of North Bengal [Accession no. 09884 (NBU)].

Preparation of plant extract: Plant extract was prepared in HPLC grade n-hexane which is a non-polar solvent. The plant was collected, washed thoroughly and the leaf was cut open to collect the gel with the help of a sterilized scalpel. The gel was then properly homogenized to prepare a slimy end product. This slimy end product is then mixed with n-hexane in 1:5 (w: v) ratio. The mixture was kept in magnetic stirrer for 24 hours at room temperature. Next day, the supernatant was collected, filtered through Whatman No 1 filter paper and the filtrate was dried completely using nitrogen flow as it is an inert non-reactive gas. The dried end product was re-suspended in hexane and was used for GC-MS analysis.

Instrumentation and Chromatographic conditions: The GC-MS analysis of n-hexane extract of *Aloe vera* leaf gel was performed in Thermo Scientific Trace 1300 GC associated with ISQ Mass spectrophotometer. The machine was equipped with an AI/AS 1310 auto sampler. The experiment was done in TG 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 in film thickness. The column oven temperature was kept 80°C with of 5°C/min to 300°C gradual increase in steps; injection temperature was set at 250°C at a pressure of 5 kPa, with total flow and column flow of 10 ml/min and 1 ml/min, respectively. The purge flow rate was 3.0 ml/min. The GC program ion source and interface temperature were 220°C and 300°C, respectively, with solvent cut time of 5 min. The MS program starting time was 5 min which ended at 46.00 min with event time of 0.50 s, mass range 50 650. The sample volume of *Aloe vera* n-hexane fraction was 1 ìl (split ratio 10:1). The samples were repeatedly used to find the best result.

Data interpretation: The analysis of GC-MS fragments and data interpretation was done using Xcalibur software version 2.0.1.3 with the help of the NIST Mass Spectral Search Programme for the NIST/EPA/NIH Mass Spectral Library version 2.0g, built May 19, 2011.

RESULT

GC-MS analysis: The GC-MS analysis of n-hexane fraction of *Aloe vera* has shown the presence of 61 major peaks each corresponding to a single compound. We have found some principle compounds which are already documented as molecules with potent biological properties. The principle anti-inflammatory and/or anti-arthritic compounds from the plant are listed below in the Table 1. The total chromatogram is presented in the Figure 1.

DISCUSSION

Use of plant-derived drugs has got an increased acceptance in the recent years throughout the World. There is a continuous search of natural-products for bioactive phyto-constituents that could serve as drug leads for treatment of various human ailments world-wide (Kalimuthu and Prabakaran, 2013). In the GC-MS analysis, 61 compounds are identified by the NIST library search in which at least 9 molecules are documented as potent agents for different biological activities. Ethyl iso-allocholate is a steroid derivative which has important anti-bacterial, anti-oxidant, anti-tumor, cancer preventive, chemopreventive and pesticidal activity. Fatty acids like 9, 12, 15-Octadecatrienoic acid and Octadecane are documented to have potent anti-arthritic and anti-microbial activities. Antibiotic like geldaramycin is anti-tumor antibiotic used in medicinal biology. (5á)Pregnane-3,20á-diol which is a neuroactive steroid, is involved in the neuromodulatory activity in different model animal systems. Astaxanthin, a

Table 1: List of major phytocompounds isolated from Aloe vera gel and their biological activities with references.

Sl No	Name of the Compound	RT	Mol. Formula	Biological Activity	Reference
1.	Ethyl iso-allocholate	5.64	$C_{26}H_{44}O_5$	Antibacterial, Antioxidant, Anti-tumor, Cancer preventive, Chemo preventive and Pesticide.	Saravanan *et al.*, 2014
2.	9,12,15-Octadecatrienoic acid	5.87	$C_{21}H_{36}O_4$	Omega 3 fatty acid, Anti-RA activity, heart disease.	Online: University of Maryland Medical System online database.
3.	Octadecane	6.07	$C_{26}H_{54}$	Antioxidant and Antimicrobial.	Saravanan *et al.*, 2014
4.	Geldaramycin	9.18	$C_{29}H_{40}N_2O_9$	Anti-tumor antibiotic.	Jilani *et al.*, 2013
5.	Astaxanthin	10.15	$C_{40}H_{52}O_4$	Alzheimer's disease, Parkinson's disease, Brain stroke, AMD.	Nakao *et al.*, 2010
6.	9,10-Secocholesta-5,7,10 (19)-triene-3,24,25-triol	10.79	$C_{27}H_{44}O_3$	Calcitriol derivative (Chemspider), treat hyperparathyroidism, Vitamin D3.	Online: ChemSpider online database
7.	(5á)Pregnane-3,20á-diol	11.27	$C_{28}H_{43}NO_6$	Neuroactive steroid antagonist to the glycine receptor in the nervous system.	Lan and Gee, 1994
8.	Picrotoxinin	11.39	$C_{15}H_{16}O_6$	A non-competitive channel blocker for the $GABA_A$ receptor chloride channels.	Wang *et al.*, 2007
9.	1,2-dihydro-2,2,4-trimethyl-Quinoline	15.78	$C_{12}H_{15}N$	Preparation of hydroxyquinoline sulfate and niacin, used as dye.	Collin and Hoke, 2005

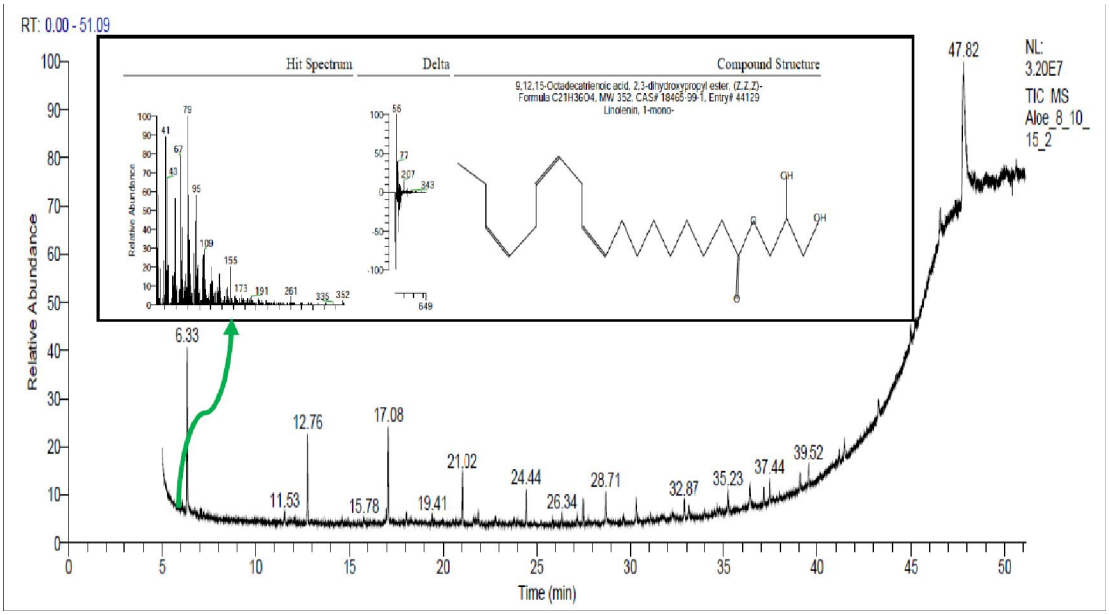

Figure 1: Chromatogram of *Aloe vera* gel hexane fraction showing major peaks and their relative abundance. 9,12,15-Octadecatrienoic acid, with reported anti-rheumatoid arthritis activity has been shown (inset).

remedy for diseases like Alzheimer's disease and Parkinson's disease is also found from our GC-MS analysis.

In Ayurveda, synergy is thought to be a principle way of action that involves a mixture of herbal products containing different bioactive principles in natural proportion. In this study, the data clearly states that the *Aloe vera* gel contains different bioactive compounds which can cure different diseased conditions and hence, it is very good as a dietary supplement. Many ethnic populations emphasize on limited regular consumption of such plants in their medicinal practices. Studies on *Aloe vera* phytocompouds by other workers have also revealed similar compounds from the plant. Similar fatty acids and steroidal compounds are found from the analysis of other workers as well (Lakshmi and Rajalakshmi, 2011).

CONCLUSION

The presence of various bioactive principles in *Aloe vera* crude gel extract is an indication that the plant could be used as a herbal drug of pharmacological significance. However, the isolation of individual constituents and their biological activities will be of advanced medical significance.

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REFERENCES

Arunkumar, S. & Muthuselvam, M. 2009. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World. J. Agri. Sci.* 5(5): 572-576.

Collin, G., Höke, H. 2005. *Quinoline and Isoquinoline*, Ullmann's Encyclopedia of Industrial Chemistry, Weinheim: Wiley-VCH.

Davis, R.H., Leitner, M.G., Russo, J.M. & Byrne, M.E. 1989. Wound healing. Oral and topical activity of *Aloe vera*. *J. Am. Podiatr. Med. Assoc*. 79(11): 559-62.

Eshun, K. & He, Q. 2004. *Aloe Vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review. *Crit. Rev. Food. Sci*. 44(2): 91-96.

Hu, Y., Xu, J. & Hu, Q. 2003. Evaluation of Antioxidant Potential of *Aloe vera* (*Aloe barbadensis* Miller) Extracts. *J. Agr. Food. Chem.* 51:7788-7791.

Jilani K, Qadri, S.M. & Lang, F. 2013. Geldanamycin-induced phosphatidylserine translocation in the erythrocyte membrane. *Cell. Physiol. Biochem*. 32:1600-1609.

Kalimuthu, K. & Prabakaran, R. 2013. Phytochemical screening and GC-MS analysis of methanol extract of *Ceropegia pusilla in vitro* Tuber. *Int. J. Inv. Pharm. Sci*. 4: 376-383.

Lakshmi, P.T.V. & Rajalakshmi P. 2011. Identification of phyto-components and its biological activities of Aloe vera through the gas chromatography-mass spectrometry. *Int. Res. J. Pharm.* 2(5): 247-249.

Lan, N.C. & Gee. K.W. 1994. Neuroactive steroid actons at the $GABA_A$ receptor. *Horm. Behav.* 28 (4): 537-544.

Nakao, R., Nelson, O.L., Park, J.S., Mathison, B.D., Thompson, P.A. & Chew, B.P. 2010. Effect of Astaxanthin supplementation on inflammation and cardiac function in BALB/c mice. *Anticancer. Res.* 30: 2721-2726.

Paul, S., Dutta, S., Chaudhuri, T.K. & Bhattacharjee, S. 2014. Anti-inflammatory and protective properties of *Aloe vera* leaf crude gel in carrageenan induced acute inflammatory rat models. *Int. J. Pharm. Pharm. Sci*. 6(9): 368-371.

Saravanan, P., Chandramohan, G., Mariajancyrani, J. & Shanmugasundaram, P. 2014. GC-MS analysis of phytochemical constituents in ethanolic bark extract of *Ficus religiosa* Linn. *Int. J. Pharm. Pharm. Sci*. 6(1): 457-460.

Surjushe, A., Vasani, R. & Saple,D.G. 2008. *Aloe vera*: a short review. *Ind. J. Dermatol*. 53:163-66.

Tanaka, M., Misawa, E., Ito, Y., Habara, N., Nomaguchi, K. & Yamada, M. 2006. Identification of five phytosterols from *Aloe vera* gel as anti-diabetic compounds. *Biol. Pharm. Bull.* 29 (7): 1418-1422.

Wang, D.S., Buckinx, R., Lecorronc, H., Mangin, J.M., Rigo, J.M. & Legendre, P. 2007. Mechanisms for picrotoxinin and picrotin blocks of alpha2 homomeric glycine receptors. *J. Biol. Chem.* 282 (22):16016-16035.

Web links:

University of Maryland Medical System online. University of Maryland Medical Centre. 2015. [Online Database] https://umm.edu/health/medical/altmed/supplement/alphalinolenic-acid

ChemSpider. Royal Society of Chemistry. 2015. [Online Database] http://www.chemspider.com/Chemical-Structure.4444108.html

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In vivo and *in silico* investigations of the toxicological and analgesic properties of unprocessed *Aloe vera* gel in experimental rat models

Subhashis Paul[1], Arnab K. Chakraborty[2], Debabrata Modak[1], Arnab Sen[2] and Soumen Bhattacharjee[1,*]

[1] *Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal*, Darjeeling 734013, West Bengal, India

[2] *Bioinformatics Facility, Department of Botany, University of North Bengal*, Darjeeling 734013, West Bengal, India

***Corresponding author**: soumenb123@rediffmail.com; sbhnbu@gmail.com

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Abstract: *Aloe vera* is a commonly used plant in both food and medicine industry. The potential toxicological side-effects of prolonged intake of *Aloe* extract have not been evaluated in detail. This work presents an in-depth toxicological study of the crude unprocessed *A. vera* gel in experimental rats. Acute and sub-chronic toxicity was evaluated in a 1 to 28-day long feeding schedule of the aqueous homogenized gel material. Hemoglobin, total protein, high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, triglyceride, serum creatinine, serum alanine transaminase (SGPT), aspartate transaminase (SGOT) and alkaline phosphatase were examined and kidney and liver histology was performed. In the acute toxicity test, the behavioral aspects were also considered. A molecular docking assay was performed to investigate the binding affinities of pure *A. vera* compounds with liver and kidney toxicological marker enzymes, in order to assess the probable mode of action of selected *Aloe* constituents. Solubility factors for the active constituents were also studied to determine their possible miscibility with body fluids. The results from *in vivo* tests provided no major toxicological indications. Crude *Aloe* gel consumption up to 4 g/kg body weight (b.w.) showed no toxicological side effects. From the structural standpoint, *Aloe*-based bioactive molecules, such as *Aloe*-emodin, acetophenone, β-sitosterol, cholestenol and squalene showed promising binding affinity to qualify as alternative and complementary medicines. The synergistic roles of all *A. vera* constituents remain to be validated in human disease models.

Keywords: *Aloe vera*; nociception; molecular docking; *Aloe*-emodin; hepatitis B viral protein

INTRODUCTION

The genus *Aloe* has a long history of providing an array of health benefits as a traditional medicine. With about 400 *Aloe* species, one of the most frequently used is *Aloe vera* (L.) Burm.f. (Xanthorrhoeaceae). *A. vera* has served as a remedy for different ailments, such as wounds and burns, constipations, external and internal ulcers, hyperlipidemia, diabetes and many more [1-6]. It is also documented as a wound healing and anti-inflammatory agent in the Indian ayurvedic system, a complementary medicinal system of ancient India used as protective or preventive medication against many ailments. Only 8% of the online databases on *Aloe*, including research articles, deal with the toxicological effects of this plant, and are with or without any scientific references [7]. Less than 3% of the herbal product-related websites cite scientific literature regarding the usage, adverse effects, drug interactions and safety precautions regarding *A. vera* consumption or application. These findings indicate that the data regarding the toxicological effects of *A. vera* following short- or long-term consumption is incomplete. Moreover, when toxicological parameters are considered, only a few reports present toxicological studies on *Aloe* gel use. Orally supplemented *A. vera* extract was shown to protect from oxidative stress and restore blood reduced glutathione (GSH) concentration in experimental rat models of exposure to Arsenic (As), however, it did not reduce the concentration of As in tissues [8]. Crude *A. vera* ethanolic extract was reported to be a potent anti-oxidant against azoxymethane-induced stress in rats [9]. The *A. vera*-derived phytocompound *Aloe*-emodin exhibited

hepatoprotective activity in the experimental model of carbon tetrachloride-induced hepatic injury [10]. Anilakumar *et al.* [9] pointed to a probable synergy between *A. vera* phytocomponents that showed antioxidant effects in experimental rats. *Aloe* latex and whole leaf extract have been implicated in some cases of carcinogenesis, genotoxicity and *in vivo* toxicity in animal models. But the *Aloe* gel, which is free from latex and the epidermal layer, has not been evaluated systematically with regard to its potential toxicological effects [7,11].

We have considered the human-consumable aqueous preparation of homogenized, latex-free *A. vera* gel for feeding to Wistar albino rats of both sexes, in order to determine its acute and sub-chronic toxicological effects *in vivo*. The doses used were based on daily consumption of 50 g of *Aloe* gel by a healthy person weighing 60 kg. Available information indicates that ethnic people consume *Aloe* gel in a crude, unprocessed and fresh state [4]. Hence, we simply homogenized *A. vera* gel in water and used the fresh homogenate daily in each feeding schedule. Acute and sub-chronic toxicities were measured following 1 to 28-day feeding schedules, respectively. Animal death, behavioral parameters resulting from skin irritation, dizziness, as well as liver and kidney enzyme profiles, were evaluated along with other toxic and physiological biomarkers, including serum creatinine, triglyceride and cholesterol. We had previously confirmed insignificant changes in hematological parameters in rat models following extended *Aloe* gel consumption [4].

Molecular docking provides a prediction of the affinity of one molecule to another in a preferred orientation. The stability of such an association on a potential 3-dimensional surface of two moieties can further indicate their pharmaceutical utilities. We can even predict the probable solubility of compounds by mimicking their physiological conditions. Therefore, we screened and studied all the available phytochemical constituents of *Aloe* and we have selected 8 potentially bioactive molecules for molecular docking assays [12]. This can provide us with an understanding of the drug-like properties of the phytochemicals under study. These are the basic steps of *in silico* drug design.

MATERIALS AND METHODS

Ethics statement

The experiments were approved for the period 2013-16 by the Institutional Animal Ethical Committee (IAEC) of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with registration number 840/ac/04/CPCSEA, dated 01/01/2004, of the University of North Bengal, West Bengal, India prior to commencement of the experiments.

Experimental animals

Wistar albino rats of both sexes, weighing 140-160 g, served as experimental animals for acute and sub-chronic toxicity and pain sensation. The animals were purchased from Ghosh Enterprise, Kolkata, India and were kept in the departmental animal house facility at 24±2°C, a 12h day-night cycle, and were fed with standard pellet obtained from Ghosh Enterprise and water *ad libitum*. All animals were acclimatized for a period of 10 days before initiation of the experiments. Four or fewer rats were kept per cage during the entire period.

Collection and processing of plant material

Naturally grown plants were collected in the post-monsoon seasons (September-November) from the Medicinal Plant Garden of the University of North Bengal, located at Siliguri (Darjeeling, West Bengal, India). The plant was identified and deposited in the Department of Botany, University of North Bengal. The voucher specimen was provided an accession number in the NBU herbarium (Accession No. NBU09884). The *Aloe* gel was collected after aseptically peeling off the outer green layer and latex part of the plant leaf. The gel was weighed and chopped into pieces before homogenization. Five g of *Aloe* gel was mixed with 3 mL of water during homogenization, to produce a final 7 mL mixture. Considering that 7 mL of the mixture contained 5 g of *Aloe* gel, the feeding doses contained the gel at concentrations of 1, 2, 4 and 5 g/kg body weight (b.w.). The oral administration of the plant material was done using an oral gavage. The process was repeated daily to ensure fresh consumption of unprocessed raw *Aloe* gel.

Chemicals

All the serum biochemical test kits for toxicological parameters were purchased from Coral Clinical Systems, Goa, India. Other chemicals, such as formalin and chloroform, were procured from Merck (USA) and SD Fine Chemicals (India). All chemicals were molecular biology grade.

Experimental design

Acute toxicity test

In the acute toxicity test, three groups of mature rats were selected, each contained 6 rats (3 males and 3 females). The first group (N) was not fed with *A. vera* gel; the other two were the experimental groups, TA1 and TA2 and were fed with 2 and 5 g of *Aloe* crude gel/kg b.w., respectively. The animals fasted overnight prior to feeding with a single dose of the gel homogenate. The mortality rate, salivation, fur irritation, sleep/dizziness, lethargy and diarrhea were observed for the next 24 h. This one-day long observation was further followed by a 7-day long screening for any toxicological effects. All the experiments were performed according to the OECD guideline 423 (Adopted on 17th December 2001) for acute oral toxicity study in rodents.

Sub-chronic toxicity

For the determination of sub-chronic toxicity, the animals were divided into four groups. All the groups contained 6 rats each (3 male and 3 females). The first group was considered as Normal (N), the second, third and fourth groups, T1, T2, and T3 respectively, were the treatment groups with a 28 day-long daily feeding schedule of *Aloe* gel at concentrations of 1, 2, and 4 g/kg b. w., respectively. After completion of the 28 day-long schedule, the animals were killed with anesthetic. Blood from each animal was collected separately by heart puncture. Kidney and liver tissues were collected and fixed in 4% formalin for histological processing. The clear serum was collected by allowing the blood samples to clot. Hemoglobin was measured immediately after blood collection. All the experiments were performed according to the standard guidelines with slight modifications [13,14].

In a previous study, we showed that the red and white blood cell (RBC and WBC, respectively) counts, total protein, total albumin remained normal in the experimental Wistar albino rats while evaluating the anti-arthritic properties of the same plant [4]. Next, the hemoglobin and the serum enzymatic parameters, including alkaline phosphatase (ALP), aspartate transaminase (SGOT), alanine transaminase (SGPT), and serum high density lipoprotein (HDL-D), low density lipoprotein (LDL-D), triglycerides, cholesterol, creatinine were measured using the appropriate assay kits, following the manufacturers' instructions provided with the Coral Biosystem's (Goa, India) product-specific user manual. All spectrophotometric assays were performed using in a Systronics VIS spectrophotometer (Model 105).

Formalin-induced paw licking test

Experimental animals were fed with the indicated doses of *Aloe* gel homogenate for 7 days (designated as T1, T2, and T3 respectively; doses as used in the sub-chronic toxicity tests i.e. 1, 2 and 4 g/kg body b.w.). Normal (N) group rats were fed with an equal amount of distilled water. On the 7th day, 50 μL of 2.5% formalin solution was injected into the right hind paw. The animals were kept under observation in a glass cage for the next 30 min. The duration(s) of paw licking by the animals during the first 5 min and then for the following 15-30 min after the formalin injection was recorded. The first five min was considered as neurogenic pain and the succeeding 15-30 min were considered as inflammatory pain [15-17]. The total paw licking time used by the animals during 30 min was an indicator of nociceptive pain. Morphine, used as the standard drug to compare the pain-ameliorating activity of the plant extract, was fed orally 30 min prior to formalin injection at a dose of 5 mg/kg b.w.

Molecular docking

Molecular docking analyses of selected toxicologic and analgesic marker proteins against the selected chemical constituents of *A. vera* were carried out as described [12,18]. Depending on the variations that may occur during sample preparation and variations of the phyto-constituents vis-a-vis seasonal and geographical changes, 10 major potent bioactive mol-

ecules were examined. All protein structures were retrieved from a protein data bank (http://www.rcsb.org/pdb/home). Docking was performed using Auto Dock Vina [18]. The X, Y and Z dimensions were chosen to be 100 for aspartate transaminase, glutamate dehydrogenase, hepatitis B viral protein (hepatitis Bx), and 80 for alanine transaminase, alkaline phosphatase. Center Grid Box was chosen to fit the protein. The exhaustiveness was set to 8. Each concerned protein was rendered ready by removing the water molecules and by adding polar. Gasteiger charges were added to the proteins on the basis of electronegativity equilibration and the non-polar hydrogens were merged. Gasteiger charges were also calculated for the respective ligands and all the torsions were allowed to rotate.

Solubility analysis

The basic solubility analysis of Log P and Log S values were determined using ALOGPS 2.1 [19] maintained by Virtual Computational Chemistry Laboratory (http://www.vcclab.org/lab/alogps/). Log P, the logarithm of the partial coefficient, is defined as the ratio of the concentration of a solute between two solvents specifically for unionized solutes. Log S value is a unit stripped logarithm (base 10) of the solubility measured in mol/L. Chemcalize.org beta (http://www.chemicalize.org/) by Chem Axon was used to determine the log D values at physiological blood plasma pH of 7.4. Log D is the distribution coefficient, log D, is the ratio of the sum of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two immiscible phases.

Statistical Analysis

All statistical analyses were performed using Kyplot ver. 5.0. In the Kyplot analysis, the data represented the mean±S.D., which was analyzed by one-way ANOVA. The results were considered significant when $p \leq 0.05$.

RESULTS

Acute toxicity test

No death of the animal was observed during the 1st day of the trial in the acute toxicity test. The animals were observed at regular 2 h intervals. All the animals were alive for the next 7 days and no death or change in the physiology and behavior was observed (Table 1). Normal weight gain was observed in all groups and no gross abnormal findings were documented in any of the groups. The *Aloe* gel was therefore considered to be non-toxic up to the dose of 5 g/kg b. w. and the lethality value was considered "unclassified" up to the feeding range.

Table 1. Behavioral changes in rat groups following a one-day single dose trial of *Aloe* gel for acute toxicity. The behavior was followed in each animal for 24 h after feeding with the plant extract.

Groups	N (Normal)	TA1 (2 g/kg b.w.)	TA2 (5 g/kg b.w.)
Number of animals	6	6	6
Number of deaths	None	None	None
Abnormal motor activity	-	-	-
Salivation	-	-	-
Fur irritation	-	-	-
Sleep/dizziness	-	-	-
Lethargy	-	-	-
Diarrhea	-	-	-
'-' denotes absent.			

Sub-chronic toxicity test

We observed that out of 6 animals per group, only one animal died in the T2 group (i.e. the medium dose group (2 g/kg b.w.)). This death was unrelated to the toxicological properties of the *A. vera* as no other toxicological signs were seen in other rats in the same nor in other dose groups. Following anatomical investigation, the dead animal was diagnosed with multiple cysts in the liver. Body weight changes were normal in all the groups (Fig. 1.). The changes observed in the histology of the livers and kidneys were also non-significant (Fig. 2.). The enzymatic parameters of the serum, ALP, SGPT and SGOT (Fig. 3A, B and C, respectively), showed no differences between the experimental and control animal groups. However, some non-significant variations were present in the serum profiles of creatinine, triglycerides, cholesterol, LDL-D and HDL-D (Fig. 3.D, E, F, G, and H, respectively). In addition, no change in the blood sugar level in the experimental groups was observed. The triglyceride level was normal in T1 and T3 but slightly decreased in T2. The serum cholesterol concentration was decreased in

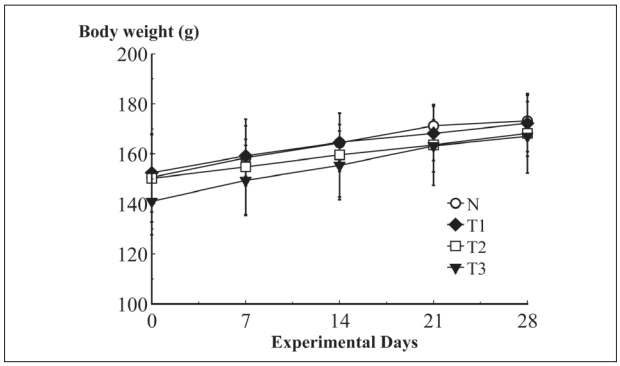

Fig.1. Diagram showing the body weight increment in experimental rat groups in the 28-day long sub-chronic toxicity assay. N refers to normal, T1, T2, and T3 refer to the second, third and fourth treatment groups, respectively, in a daily feeding schedule with *Aloe* gel (1, 2 and 4 g/kg b. w., respectively).

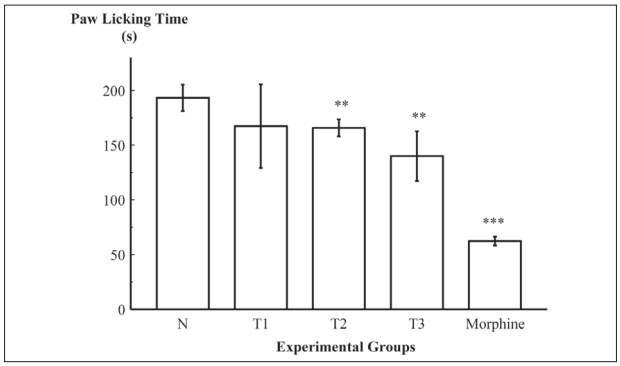

Fig.4. Bar diagram showing total paw licking time (s) in the formamide-induced paw licking test in different experimental groups. Dose groups T1, T2 and T3 showed decreased paw licking time, which revealed the analgesic property of the *Aloe* gel compared to the normal (N) group. Morphine was used as a standard analgesic drug. *** – significance at $P \leq 0.001$; * – significance at $P \leq 0.01$.

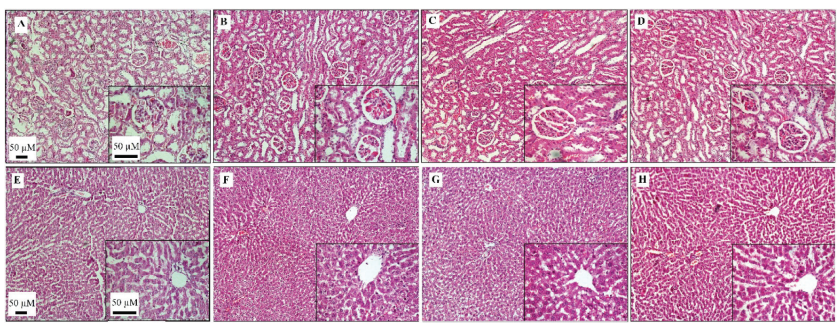

Fig.2. Histological study of kidney (upper panel) and liver (lower panel) of rats fed with unprocessed *Aloe* gel. **A** and **E** – normal, **B** and **F** – low dose (T1), **C** and **G** – medium dose group (T2); **D** and **H** – histological sections of the high dose group (T3). The larger photographs are 10X magnified, and the insets at the bottom right hand side of every photograph are 40X magnified. The size bar is in µM.

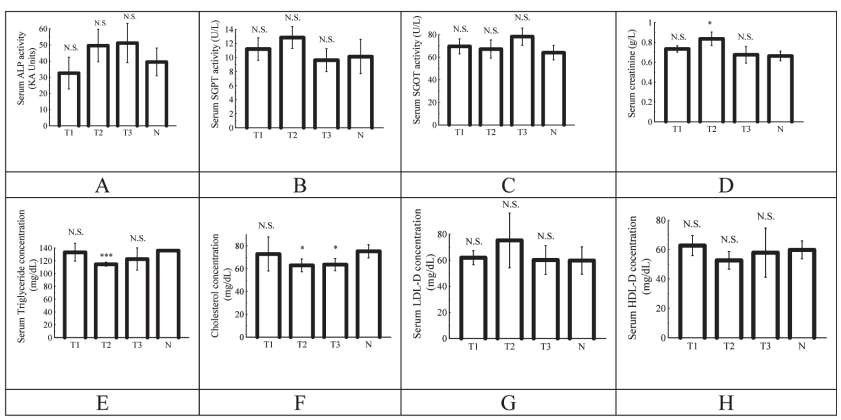

Fig.3. Different toxicological parameters in experimental rats fed with *Aloe* gel. N – normal animals; T1, T2 and T3 – low, medium and high dose *Aloe* gel-fed groups. *** – significance at $P \leq 0.001$; * – significance at $P \leq 0.05$.

T2 and T3, while the concentrations of HDL-D and LDL-D were at the physiological level in all experimental groups. SGPT and SGOT also exhibited insignificant differences in all the groups (Fig. 3).

Formalin-induced paw licking test

In the medium and high dose groups, the *Aloe* gel significantly reduced the nociception elicited by the injected formalin. The high dose group (T3) showed a better result than the low (T1) and medium (T2) groups (Fig. 4).

Molecular docking

The combination displaying the best binding affinity with the least root mean square deviation (RMSD) from zero was considered for our analysis. Glutamate dehydrogenase showed the best binding affinity on average with all the phytochemicals, followed by hepatitis Bx (Fig. 5A and B, Fig. 6). When we consider the interactions with respect to ligands, *Aloe*-emodin showed the best

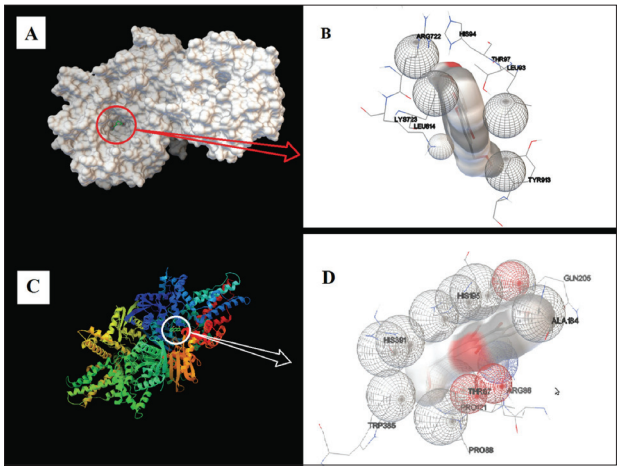

Fig. 5. A – Molecular docking of *Aloe*-emodin with hepatitis Bx protein. The encircled area shows the active binding site of *Aloe*-emodin. **B** – The binding site is enlarged in the right hand side indicating the participating amino acids. **C** – Docking image of glutamate dehydrogenase with *Aloe*-emodin. The encircled region indicates the best binding site, enlarged in **D**.

	Alanine transaminase	Alkaline phosphatase	Aspartate transaminase	Glutamate dehydrogenase	Hepatitis BX
Acetophenone	-5.2	-5	-5.3	-5.6	-6.1
Aloe-emodin	-6.9	-7.8	-7.5	-8.5	-8.6
Azelaic acid	-3.8	-5	-4.3	-4.8	-4.2
β-sitosterol	-7.3	-6.8	-6	-8.1	-8.3
Cholestanol	-6.8	-7.9	-5.8	-8.2	-7.1
Hydro-p-coumaric	-4.5	-5.7	-4.6	-5.9	-6
Hypoxanthine	-5	-5.5	-5.1	-5.9	-5.5
Oleic acid	-4.6	-4.8	-3.5	-4.9	-4.4
Salicylic	-5.5	-5.9	-6	-5.6	-5.8
Squalene	-5.6	-5.9	-4	-7.8	-7.1

High Low

Fig. 6. Heat map image of binding affinity strengths (Kcal/mol) of *Aloe* gel-derived pure compounds against toxicity marker enzymes. *Aloe*-emodin showed the best binding affinity with hepatitis Bx, followed by glutamate dehydrogenase.

result, followed by cholestanol. Parallel with the existing trend, *Aloe*-emodin had the best binding affinity of -8.6 kcal/mol with the standard control hepatitis Bx when individual interactions were considered. The 2nd best interaction was displayed by *Aloe*-emodin,

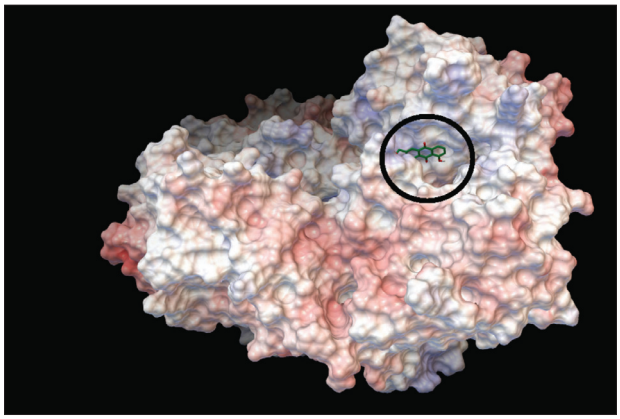

Fig. 7. Molecular docking of *Aloe*-emodin with Cox-2. The encircled region shows the best binding site of *Aloe*-emodin with Cox-2.

with a binding affinity of -8.5 kcal/mol with glutamate dehydrogenase (Fig. 5C and D, Fig. 6). When Cox-2 binding was examined, cholestanol had a binding affinity -6.5 kcal/mol, second to *Aloe*-emodin which had a binding affinity of -8.0 kcal/mol (Fig. 7).

Solubility analysis

According to the octanol-water partition coefficient, log P should be between -0.4 and +5.6. β-sitosterol, cholestanol, hypoxanthine, oleic acid, and squalene all lie outside the ideal range (marked red). The log S value is the solubility parameter of the phytochemicals. The ideal range of log S value lies around -5 mol/L. Compounds such as acetophenone, azelaic acid, hydro-p-coumaric, hypoxanthine and salicylic lie outside the range of good solubility. Another solubility parameter of importance is the log D value, with the ideal range between -3 and 3. Molecules with low log D values readily pass through cell membrane and have better solubility. Compounds that were out of range were β-sitosterol, cholestanol, oleic acid and squalene (Table 2).

DISCUSSION

The acute toxicity test revealed that unprocessed *A. vera* gel in Wistar albino rats was not toxic when the rats were fed a single dose of 5 g/kg b.w. (validated by a 7-day long observation), which also indicated that further testing for unprocessed *Aloe* gel is not nec-

Table 2. Solubility analysis of the *Aloe* gel-derived pure compounds. Log P is defined as the ratio of the concentration of a solute between two solvents specifically for un-ionized solutes. Log S value is a unit stripped logarithm (base 10) of the solubility measured in mol/L. Log D is the ratio of the sum of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two immiscible phases.

Chemicals	logP	logS	LogD
Acetophenone	1.65	-1.95	1.53
Aloe-emodin	1.27	-2.96	1.88
Azelaic acid	1.37	-1.92	-3.65
β-sitosterol	7.27	-7.35	7.84
Cholestenol	7.02	-7.41	7.52
Hydro-p-coumaric	1.15	-1.79	-1.28
Hypoxanthine	-0.74	-1.79	-0.42
Oleic acid	7.68	-6.37	4.40
Salicylic	1.96	-1.09	-1.52
Squalene	8.64	-5.91	10.42

essary [20]. Our findings can also serve as the basis for toxicological classification. Acute toxicity tests are not only performed to determine the precise lethality value but also to indicate the maximum dose for the survival of animals [21]. In traditional practice, the gel of this plant is consumed fresh after collection, without any processing. This was applied during preparation of the plant extract. In a previous study we showed that hematological parameters varied non-significantly following consumption of crude *Aloe* gel extract [4]. Now we determined that the highest dose of *Aloe* gel (5 g/kg b.w.) neither produced lethality nor elicited harmful effects in experimental rats after 7 days. No visually detectable behavioral abnormalities were observed (including body fur irritation, diarrhea, salivation).

A sub-chronic toxicity study was undertaken to assess the harmful effects associated with long-term repeated exposure of the animals to *Aloe* gel. It also provided information on organ-based toxicity [11]. The consumption of *Aloe* gel for 28 days did not initiate any deleterious changes or death, and all serum enzymatic and biochemical parameters remained unchanged. Generally, a loss in body weight relates to an immediate toxic effect of a plant, but no significant change was observed in any of the animal groups during the 28-day long feeding schedule [22]. Decreased food and water intake and loss of appetite are the early signs of physiological abnormalities. Our findings suggest that there were no interactions between the major metabolic pathways and *Aloe* gel constituents. This was further corroborated by the serum biochemical parameters in all animal groups. Blood glucose and hemoglobin were normal in all experimental groups. Serum creatinine was slightly elevated in the T2 group, which may indicate kidney malfunction; however, this data was not supported by the histological observation of the kidney. Creatinine is a by-product of muscle metabolism and is excreted by the kidneys. All other parameters, body weight and serum lipid, did not correlate with this finding. Moreover, the other experimental groups showed no such change in serum creatinine. Cholesterol decreased significantly in the T2 and T3 groups, indicating that *Aloe* gel consumption reduced the cholesterol content of serum, however, all groups had normal triglyceride content (100-140 mg/dL). Moreover, lowered levels of triglycerides in T2 and T3 groups indicated a potential positive effect of the *Aloe* gel in blood triglyceride regulation. LDL-D and HDL-D values showed no significant changes in the experimental groups. ALP, secreted from the bile duct and liver, is a bile duct malfunction marker when SGOT and SGPT levels are normal; on the other hand, SGPT, SGOT and ALP altogether are liver damage markers [23,24]. ALP, SGPT and SGOT remained at normal levels through the experimental feeding schedule. Histological analysis of kidney and liver tissues also supplemented the results of the biochemical experiments. There were no structural alterations in the hematoxylin-eosin stained kidney and liver sections. Kidneys possessed clear Bowman's capsule and vascularized glomerular region surrounded by proximal convoluted tubules. Both organs showed a normal arrangement of cells. Damage in parenchymal liver cells results in an elevation of SGOP and SGPT levels [23]. The obtained normal values of these two liver enzymes are supported by the normal appearance of the parenchymal region of the liver.

These parameters were supported by the *in silico* study of the active compounds of *A. vera* with the aforementioned enzymes through molecular docking analyses. GLDH, an important regulator of the urea cycle and an indicator of liver function was found to have the best interactions with the phyto-constituents, particularly with *Aloe*-emodin, cholestanol and β-sitosterol. The interaction profile of hepatitis Bx points to an overall good interaction with all exam-

ined ligands, with the best interaction observed between *Aloe*-emodin (binding affinity of -8.6 kcal/mol). Hepatitis Bx protein is a marker of liver dysfunction during hepatitis and upregulated expression of this protein increases the rate of hepatic carcinoma [24]. The strong interactions of *Aloe* gel components with hepatitis Bx protein indicated potential inactivation of this protein.

The other test proteins analyzed herein (SGPT, SGOT and ALP) are indicators of liver functioning, and all phytochemicals displayed moderate to average binding affinity with these proteins. Compounds with high binding affinities are supposed to alter the functioning of the protein(s). Thus, the strong binding observed between *Aloe*-emodin and Cox-2 could potentially downregulate the inflammatory pathway [25]. Our docking study revealed that there are numerous interaction sites for *Aloe*-emodin and other phyto-constituents with Cox-2. Compounds with high binding affinity should have a proper solubility index to reach the proper target protein. *Aloe*-emodin cleared all the logP, logS, LogD thresholds to qualify as a very good ligand. The logD value taken at a physiological pH of 7.4 ensures that *Aloe*-emodin can circulate through the plasma and reach the effector target to alter its functioning.

The formalin paw licking test is an established method to assess the anti-nociceptive activity of drugs in rodent models. We observed that the *Aloe* gel has potent pain-ameliorating activities. However, low doses decreased the paw licking time but variations between individual animals within the group resulted in high standard deviation, which is responsible for the non-significant change in T1 when compared to the control. Previous works reported by our group on the *Aloe* gel have established its efficacy as a potent anti-inflammatory and anti-arthritic agent [4,26,27]. The current work indicates that the consumption of unprocessed *Aloe* gel on a regular basis can ameliorate arthritic pain by its probable interaction with Cox-2.

To conclude, this work presents the first detailed examination of unprocessed crude *A. vera* gel-related toxicity after oral consumption, in an experimental rat model. *Aloe*-emodin, a unique constituent of the plant, has the best interaction, as per the molecular docking analyses, it has ideal solubility at physiological pH, and it can be considered for clinical trials. Our toxicological studies show no harmful toxicity in the rat when *A. vera* gel was consumed at doses up to 4 g/kg b.w for a month.

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Author contributions: S. Paul and D. Modak performed the *in vivo* experiments, analyzed the data and prepared the tables and figures; A. K. Chakraborty performed the *in silico* experiments, analyzed the data and prepared the related figures and tables. S. Paul and A. K. Chakraborty prepared and revised the manuscript. A. Sen and S. Bhattacharjee contributed to the concept and design of the experiments and data analysis, critical revision of the manuscript, and gave the final approval of the manuscript for publication.

Conflict of interest disclosure: Authors declare no conflict of interest.

REFERENCES

1. Atiba A, Nishimura M, Kakinuma S. *Aloe vera* oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor-2 and fibroblast growth factor production. Am J Surgery. 2011;201(6):809-18.
2. Foster M, Hunter D, Samman S. Evaluation of the nutritional and metabolic effects of *Aloe vera*. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 3..
3. Reynolds T, Dweck AC. *Aloe vera* leaf gel: a review update. J Ethnopharmacol. 1999;68(1):3-37.
4. Paul S, Dutta T, Chaudhuri TK, Bhattacharjee S. Curative and protective properties of crude gel of *Aloe vera* from sub-Himalayan West Bengal in chronic and acute inflammatory rat models. Ind J Trad Knowl. 2017;16(1):121-7.
5. Vogler BK, Ernst E. *Aloe vera*: a systematic review of its clinical effectiveness. Br J Gen Pract. 1999;49(447):823-8.
6. Nejatzadeh-Barandozi F. Antibacterial activities and antioxidant capacity of *Aloe vera*. Org Med Chem Letters. 2013;3(1):1-5.
7. Guo X, Mei N. *Aloe vera*: A review of toxicity and adverse clinical effects. J Environ Sci Health, Part C. 2016;34:77-96.
8. Gupta R, Flora SJS. Protective value of *Aloe vera* against some toxic effects of arsenic in rats. Phytother Res. 2005;1:23-8.
9. Anilakumar KR, Sudarshanakrishna KR, Chandramohan G, Ilaiyaraja N, Khanum F, Bawa AS. Effect of *Aloe vera* gel

extract on antioxidant enzymes and azoxymethane-induced oxidative stress in rats.Ind J Exp Biol. 2010;48(8):837-42.
10. Arosio B. *Aloe* emodinquinine pretreatment reduces acute liver injury induced by carbon tetrachloride. Basic Clin Pharmacol Toxicol. 2010;87:229-33.
11. National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of EMODIN (CAS NO. 518-82-1) Feed Studies in F344/N Rats and B6C3F1 Mice National Toxicology Program technical report series. Natl Toxicol Program Tech Rep Ser. 2001;493:1-278..
12. Saljooghianpour M, Javaran TA. Identification of phytochemical components of *Aloe* plantlets by gas chromatography-mass spectrometry. Afr J Biotech. 2013;12(49):6876-80.
13. Sharif HB, Mukhtar MD, Mustapha Y, Baba G, Lawal AO. Acute and subchronic toxicity profile of Euphorbia pulcherrima methanol extract on Wistar albino rats. Adv Pharm. 2015;2015:539646.
14. Yuet Ping K, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. BioMed Res Int. 2013;2013:182064.
15. Wheeler-Aceto H, Porreca F, Cowan A. The rat paw formalin test: comparison of noxious agents. Pain. 1990;40:229-38.
16. Wheeler-Aceto H, Cowan A. Standardization of the rat paw formalin test for the evaluation of analgesics. Psychopharmacology. 1991;104:35-44.
17. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30:103-14.
18. Trott O, Olson AJ. Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61.
19. Tetko IV, Tanchuk VY. Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program. J Chem Info Comp Sci. 2002;42(5):1136-45.
20. Roopashree TS, Raman D, Rani RHS, Narendra C. Acute oral toxicity studies of antipsoriatic herbal mixture comprising of aqueous extracts of Calendula officinalis, Momordica charantia, Cassia tora and Azadirachta indica seed oil. Thai J Pharm Sci. 2009;33:74-83.
21. Raza M, Al-Shabanah OA, El-Hadiyah TM, Al-Majed AA. Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Scientia Pharmaceutica. 2002;70:135-45.
22. Brautbar N, Williams J. Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. Int J Hygiene Eenviron Health. 2002;205:479-91.
23. Giboney PT. Mildly elevated liver transaminase levels in the asymptomatic patient. Am Fam Physician. 2005;71:1105-10.
24. Kew MC. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. J Gastroenterol Hepatol. 2011;26:144-52.
25. Abdalla SI, Sanderson IR, Fitzgerald RC. Effect of inflammation on cyclooxygenase (COX) -2 expression in benign and malignant oesophageal cells. Carcinogenesis. 2005;26:1627-33.
26. Paul S, Dutta S, Chaudhuri TK, Bhattacharjee S. Anti-inflammatory and protective properties of *Aloe vera* leaf crude gel in carrageenan induced acute inflammatory rat models. Int J Pharm Pharm Sci. 2014;6(9):368-71.
27. Guha P, Paul S, Das A, Halder B, Bhattacharjee S, Chaudhuri TK. Analyses of human and rat clinical parameters in rheumatoid arthritis raise the possibility of use of crude *Aloe vera* gel in disease amelioration. Immunome Res. 2014;10:1.

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Aloe vera gel homogenate shows anti-inflammatory activity through lysosomal membrane stabilization and downregulation of TNF-α and Cox-2 gene expressions in inflammatory arthritic animals

Subhashis Paul[1], Debabrata Modak[1], Sutanuka Chattaraj[1], Deblina Nandi[1], Aditi Sarkar[1], Joyjit Roy[1], Tapas Kumar Chaudhuri[2] and Soumen Bhattacharjee[1*]

Abstract

Background: *Aloe vera* leaf gel has proven efficacious roles in the amelioration of several human diseases and illness-conditions. Specific purified gel-derived bio-constituents as well as the naturally harvested unprocessed *A. vera* gel have shown promise in modifying systemic inflammation. However, the synergistic role of natural herbal remedies, a mainstay of traditional Indian Ayurveda, has not been evaluated rigorously in this plant. In this study, the prevention of membrane lysis and protein denaturation in the presence of *A. vera* gel homogenate up to the concentration of 1000 μg/ml of gel has been assessed in vitro. Also, regulation of expression of inflammation-mediator genes (TNF-α and Cox-2) has been investigated in vivo in Freund's complete adjuvant (FCA)-induced inflammatory arthritic Wistar albino rats in a 28-day long study following the daily oral supplementation of *Aloe vera* gel homogenate doses up to 0.40 and 0.80 g/kg body weight (low-dose and high-dose groups respectively).

Results: Our results indicated that *A. vera* gel homogenate inhibits hypotonicity-induced (74.89 ± 1.26%) and heat-induced (20.86 ± 0.77%) RBC membrane lyses respectively at a concentration of 1000 μg/ml, compared to indomethacin standard (80.52 ± 0.65% and 43.98 ± 1.52% respectively at 200 μg/ml concentration). The similar concentration of gel also showed 39.35 ± 4.25% inhibition of protein denaturation compared to standard diclofenac sodium (46.74 ± 1.84% at 100 μg/ml concentration) in vitro. When assessed in vivo, TNF-α expression was found to be decreased by 35.88% and 38.52%, and Cox-2 expression was found to be decreased by 31.65% and 34.96%, in low-dose and high-dose groups respectively, when compared to the arthritic controls.

Conclusions: Our findings justify the role of unprocessed *A. vera* gel homogenate in preventing tissue damage and in the downregulation of TNF-α and Cox-2 gene expressions for the immune-modulation of inflammatory arthritis condition.

Keywords: *Aloe vera* gel, Membrane stabilization assay, TNF-α, Cox-2, Relative expression, RTqPCR

* Correspondence: soumenb123@rediffmail.com; soumenb@nbu.ac.in
[1]Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, District Darjeeling, Siliguri, West Bengal 734013, India
Full list of author information is available at the end of the article

Background

Inflammation is a common complex biological cascade of events that occurs in response to any kind of injuries, mechanical damage, infection, toxin exposure, or chemical irritation in the tissue. Inflammatory pathways lead to the protection of the body. However, prolonged inflammation can cause chronic disorders and extensive tissue damage. Depending on the duration of the inflammatory responses in the body, the inflammatory processes are commonly grouped into acute (short-duration) and chronic (long-duration) inflammation. During inflammation, immune cells release lysosomal contents that act as mediators of inflammation. These enzymes work non-specifically on the nearby cells and cause further damage by breaking their membranes. This process significantly increases the degree of inflammation [1]. The erythrocyte (RBC) membranes are considered as a model of the lysosomal membrane for their structural similarity [2]. It is expected that the drugs or products capable of preventing the rupture of the erythrocyte membrane experimentally would also protect the lysosomal membrane of the affected tissue in vivo resulting in the inhibition of inflammation. On the other hand, an increase in protein denaturation has been a hallmark of inflammation [3]. Drugs exhibiting the protein denaturation-inhibition in vitro are expected to elicit similar results in biological systems and would stabilize inflammation [4].

Rheumatoid arthritis (RA) is a complex systemic inflammatory disease of the bone joints which leads to disabilities of joint movements. Presently, about 0.5–2% of the world population is affected by the disease. The current treatment of RA focuses on the pain reduction and inhibition of disease manifestation through blockage of prime mediator molecules related to the disease [5]. Cytokines and Cox-2 are the prime regulators of inflammatory diseases. TNF-α is one of the important cytokines which regulates the progression of inflammatory RA by inducing different signaling pathways. These signaling pathways are instrumental in immune cell infiltration and increased production of other cytokines [6, 7]. Elevated COX-2 expression in synovial tissues of the arthritic joint is mediated chiefly by the pro-inflammatory cytokines TNF-α and IL-1. The effector product of Cox-2 is prostaglandin E2, which contributes extensively to the degree of inflammation [8, 9].

To treat the consequences of extensive inflammation and inflammatory diseases like RA, non-steroidal anti-inflammatory drugs (NSAIDs), or steroids are commonly used. But due to the different side effects of conventional NSAIDs and steroids, the use of herbal remedies is increasingly becoming a popular choice. However, many of these herbal remedies lack scientific exploration and thus experimental validation [10]. These products are generally classified as complementary and alternative medicines (CAMs). CAMs are generally inexpensive and with no or low side effects. In the Indian CAM system, Ayurveda emphasizes the use of plant and animal products, dietary supplements, minerals, and salts, largely in crude and unprocessed forms, in amelioration of disease conditions. World Health Organization (WHO) has documented that about 80% of the world population directly or indirectly depends on ethnic medications [10, 11].

Aloe vera (Family Xanthorrhoeaceae) is one of the pioneer plants which have been used historically to reduce inflammatory symptoms by different ethnic populations. Researchers have found potent wound healing, anti-inflammatory, anti-arthritic, and anti-nociceptive properties of the *Aloe vera* gel in appropriate model systems [5, 12–14]. The interaction of the plant gel constituents with inflammation modulators is a key area of interest at the experimental level [15]. There is no detailed work reported on such naturally occurring unprocessed *A. vera* gel in animal models and thus the presented data is expected to contribute to the existing body of knowledge on the medicinal properties of *A. vera* gel. In the present work, assessment of in vitro anti-inflammatory potentials of unprocessed naturally harvested *A. vera* plant gel homogenate is done and in vivo assessment of the expression of two apex biomolecules of inflammation namely TNF-α and Cox-2 has been done in FCA-induced inflammatory arthritic rat model following *A. vera* gel homogenate oral consumption.

Methods

Collection of plant materials and authentication

Naturally grown *A. vera* plants were collected from Siliguri and adjacent regions which is located in the sub-Himalayan Terai region of West Bengal, India. Collection of the plant material and experiments were done during the year 2016–2018. The plant was identified by competent authority, and a voucher specimen was deposited [Accession No. 09884].

Preparations of plant extract and dose determination

Crude *Aloe* gel was collected by peeling out the green outer dermal layer and by taking out the gel aseptically with the help of a scalpel. For the in vitro anti-inflammatory tests, the gel was weighed and properly homogenized with isotonic buffer solution or with distilled water (for hypotonicity-induced membrane stabilization test) to obtain final concentrations of 600, 800, and 1000 μg of gel/ml. The sample was freshly prepared every time before use. Authors postulate that the process of simple homogenization preserves all the ingredients of the crude gel in the same proportion as it is obtained naturally from the leaves. The use of homogenization as an extraction process has been supported by other works [16, 17]. For the in vivo experiments on arthritic rat models, doses of 0.40 and 0.80 g of *Aloe vera* gel/kg body weight (b.w.)

(low dose or LD and high dose or HD groups respectively) were prepared by homogenizing *Aloe vera* gel with distilled water at a ratio of 1:3 (w/v). These doses were prepared based on our previous works [5, 18] considering the possible amount of daily consumption of the *Aloe vera* gel to be 25–50 g for a human weighing 60 kg. No post-harvesting extraction or processing was done to preserve the naturally available bioactive components of the gel.

Drugs and chemicals

All the chemicals used for the experiments were molecular biology grade chemicals procured from Merck, Sigma Aldrich and HiMedia (India). Freund's complete adjuvant was procured from Sigma, USA. Reverse transcriptase enzyme was obtained from Thermo Fischer, USA; total RNA was prepared using Trizol (Invitrogen, USA); random hexamer, oligo dT, and dNTP were used from GCC Biotech, India, and SybrGreen reaction mixture was obtained from Roche, USA.

Experimental animal maintenance

Wistar albino male rats (*Rattus norvegicus*) (150 ± 15 g) were procured from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India) registered animal vendor (M/s Chakraborty Enterprise, Kolkata, India) after the approval of the experimental protocols by the Institutional Animal Ethical Committee (IAEC, NBU) (Registration number 840/ac/04/CPCSEA; IAEC protocol number IAEC/NBU/2018/02). The animals were housed in the departmental animal house facility at 24 ± 2 °C with a 12 h/12 h day/night cycle and were fed with standard pellet and water was provided ad libitum. All animals were acclimatized for a period of at least 10 days before the initiation of the experiments. Four or fewer rats were kept per cage during the entire period.

In vitro anti-inflammatory tests

Collection of blood and preparation of erythrocyte suspension for in vitro membrane-stabilizing experiments

Three milliliters (3 ml) of fresh blood was collected in an EDTA vial from a healthy human volunteer who had not taken any NSAID at least 15 days prior to the collection. Erythrocyte suspension was prepared following the protocol described by Anosike et al. [2] and was stored at 4 °C prior to the experiments [2].

The membrane stabilizing activities of *A. vera* gel homogenate were assessed by a method following Shinde et al. [19] with some minor modifications.

Hypotonic solution-induced hemolysis test

Hypotonic solution-induced hemolysis test was undertaken following the methodology of Shinde et al. [19], with some modifications. *Aloe vera* gel homogenates were prepared to attain final concentrations of 600, 800, and 1000 μg of gel/ml either in hypotonic solution (homogenized in distilled water) or in isotonic solution (homogenized in isotonic buffer solution of pH 7.4). The RBC membrane breakage in hypotonic situation was calculated compared to that in the isotonic solution. In standard group, standard drug indomethacin was added to 5 ml isotonic buffer as well as to hypotonic distilled water in separate tubes to attain a final concentration of 200 μg/ml [19]. The absorbance values (OD) of the supernatants were measured at 540 nm. The percentage of inhibition of hemolysis was calculated considering the hemolysis occurring in the hypotonic solution or in distilled water of the control tubes to be 100%. Thus, the percentage of inhibition (PI) of hemolysis was calculated using the following equation:

$$\mathrm{PI} = \left[1 - \left(\frac{\mathrm{OD2} - \mathrm{OD1}}{\mathrm{OD3} - \mathrm{OD1}}\right)\right] \times 100$$

where:

OD1 = absorbance of test sample in isotonic solution

OD2 = absorbance of test sample in hypotonic solution (distilled water)

OD3 = absorbance of control sample in hypotonic solution (distilled water)

Heat-induced hemolysis test

For this test, one untreated control group, three experimental plant extract-treated groups, and one standard drug group were considered following the methodology described by Shinde et al. [19] with some minor modifications. In the experimental groups, *Aloe vera* gel was homogenized in isotonic phosphate buffer solution (pH 7.4) at a final concentration of 600, 800, and 1000 μg/ml. The standard drug group contained indomethacin in 5 ml of isotonic buffer solution at a final concentration 200 μg/ml [19]. Absorbance values (OD) of supernatant was measured at 540 nm. The percent inhibition (PI) of hemolysis was calculated using the following equation [20]:

$$\mathrm{PI} = \frac{(\mathrm{OD2} - \mathrm{OD1})}{\mathrm{OD2}} \times 100$$

where:

OD1 = absorbance of heated test sample (isotonic buffer)

OD2 = absorbance of heated control sample (distilled water)

Protein denaturation-inhibition test

The protein denaturation-inhibition test was done following established protocol [21] with some minor modifications to investigate the protein denaturation-inhibition activity of *Aloe* gel homogenate. For the experiment, along with 0.2 ml of egg albumin and 2.8 ml of PBS (pH 6.4), 2 ml of various concentrations of *Aloe vera* gel homogenates were added to experimental groups

which finally gave rise to 600, 800, and 1000 μg/ml concentration of gel in the groups. Diclofenac sodium was used as standard drug. In the standard drug group, the drug was added in the mixture to achieve a final concentration of 100 μg/ml in the 5 ml mixture which contained 0.2 ml of egg albumin and 2.8 ml of PBS (pH 6.4) [21]. The absorbance of the solutions (OD) was measured spectrophotometrically at 660 nm. The percentage of inhibition (PI) of protein denaturation was calculated using the following equation [22]:

$$\mathrm{PI} = \frac{(\mathrm{OD2} - \mathrm{OD1})}{\mathrm{OD2}} \times 100$$

where:
OD1 = absorbance of heated test sample
OD2 = absorbance of heated control sample

In vivo anti-arthritic tests

The experimental set up consisted of 24 rats distributed in four animal groups each containing 6 rats (n = 6). Animals were randomly distributed in one positive control (PC), one FCA or negative control group (FCA), and two experimental groups of LD and HD respectively, receiving daily oral supplement of *A. vera* gel homogenate at a dose of 0.40 g/kg b.w. and 0.80 g/kg b.w. respectively, once a day from the first day of the experiment till 28th day. Freund's complete adjuvant (Sigma, USA) was administrated (0.1 ml) in the right hind paw of all the experimental rats except PC on the first day. Arthritic swelling was observed within 3–4 days and a booster dose of same amount was given on 14th day. The inflammatory paw swelling of all the groups was observed and was assessed through the measurement of paw circumference with the help of a vernier caliper up to 28th day at a regular interval of 3 days (data not shown). The animals were sacrificed on 28th day following complete anesthesia using diethyl ether. On the day of sacrifice, blood was collected separately from each rat. Total RNA was prepared using Trizol (Invitrogen, USA) following the instruction of the manufacturer. The total RNA prepared separately from each of the animals of each group was then pooled together group-wise. From each group, sufficient amount of cDNA was prepared using reverse-transcriptase enzyme (Thermo Fischer, USA), random hexamer, oligo dT, and dNTP (GCC biotech, India) following the protocol suggested by the manufacturer (Thermo Fischer, USA). The prepared cDNA was used to assess the relative expression of TNF-α and Cox-2 among different groups in Lightcycler 96 real-time quantitative PCR (Roche, Switzerland). The exon-specific primers of TNF-α and Cox-2 was used along with a housekeeping gene GAPDH as endogenous control (Table 1). In brief, 5 μl of cDNA was used and mixed with 10 μl of SybrGreen, 0.3 μl of each of the forward and reverse primers (10 mM) and 1 μl of dNTP. Reaction volume was adjusted up to 20 μl by adding nuclease-free water. Annealing temperature of 59 °C was standardized for all the three genes and 45 amplification cycles were implemented. The relative mRNA expressions of the selected genes were calculated using $2^{-\Delta\Delta Ct}$ method. In this method, the expression of target genes were measured in fold change values which were analyzed with respect to the endogenous control gene expression.

Statistical analysis

The in vitro anti-inflammatory tests were carried out in triplicates for each of the groups. All statistical analyses for anti-inflammatory tests were done using GraphPad prism ver 6.01. All the data were represented as mean ± S.E.M. (standard error mean) and were analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test. The results were considered statistically significant at $P < 0.05$ compared to the control group. The *** denotes significance value at $P < 0.001$.

Results

In vitro anti-inflammatory tests

Effect of Aloe vera gel homogenate on hypotonicity-induced hemolysis of hRBCs

The standard NSAID drug indomethacin showed the highest protection (80.52 ± 0.65%) at the concentration of 200 μg/ml against hypotonicity-induced lysis of RBCs. *A. vera* gel homogenate protected the human RBCs in a concentration-dependent manner (Fig. 1a). Among the experimental groups, highest protective effect was seen in 1000 μg/ml *A. vera* gel homogenate dose group (74.89 ± 1.26%).

Effect of Aloe vera gel homogenate on heat-induced hemolysis of hRBCs

All the doses of *A. vera* crude gel homogenate (600, 800, 1000 μg/ml) showed a significant inhibition activity against

Table 1 List of the primers used in the real time quantitative PCR method

Gene	Forward primer	Reverse primer
GAPDH	ATGACTCTACCCACGGCAAG	CTGGAAGATGGTGATGGGTT
TNF-α	AGCCCTGGTATGAGCCCATGTA	CCGGACTCCGTGATGTCTAAGT
Cox-2	TGTATGCTACCATCTGGCTTCGG	GTTTGGAACAGTCGCTCGTCATC

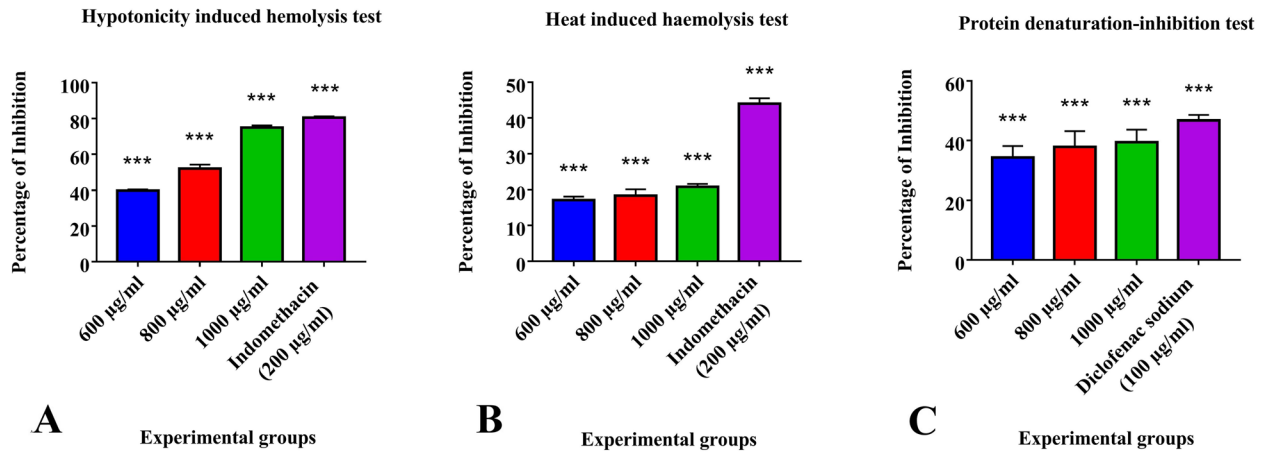

Fig. 1 Results of in vitro anti-inflammatory tests in graph. Hypotonicity-induced hemolysis test of hRBC (**a**), heat-induced hemolysis test of hRBC (**b**), and protein denaturation-inhibition test of hen's albumin (**c**) when treated with *Aloe vera* gel homogenate. All data represents % of inhibition ± SEM. Significance value at $P < 0.001$ is indicated as ***. Indomethacin and diclofenac sodium are used as standard drugs in experiments as indicated. Control group (not shown in the figures) is considered to exert 100% hemolysis

heat-induced hemolysis of hRBCs (Fig. 1b). The PI of hemolysis within experimental groups was dose dependent and maximum inhibition of hemolysis was observed in 1000 µg/ml dose group (20.86 ± 0.77%). Standard NSAID drug indomethacin showed the maximum inhibition of 43.98 ± 1.52% at 200 µg/ml concentration.

Effect of Aloe vera gel homogenate on protein denaturation-inhibition test

All the dose groups of *A. vera* gel homogenate showed significant inhibition of protein denaturation in a dose dependent manner (Fig. 1c). In the experimental groups, *Aloe* dose groups of 600 µg/ml, 800 µg/ml, and 1000 µg/ml showed 34.27 ± 3.86%, 37.82 ± 5.30%, and 39.35 ± 4.25% inhibition of protein denaturation, respectively. The standard NSAID drug diclofenac sodium showed an inhibition of 46.74 ± 1.84% at a concentration of 100 µg/ml.

In vivo anti-arthritic test

The expression of the target and the internal control genes were assessed in the negative control (FCA) and experimental animals (LD and HD) relative to the normal animals (PC) (Figs. 2 and 3). For both the genes, inhibition of expression of target genes in the experimental groups was observed in dose-dependent manner. The TNF-α showed an elevated expression (fold change) in the FCA group (2.271 ± 0.85) compared to PC (1 fold) (Fig. 3a). TNF-α expression was reduced in both the *Aloe* gel-fed groups (1.456 ± 0.11 and 1.396 ± 0.10 folds in LD and HD groups respectively). Cox-2 increased in FCA-treated animals compared to positive control (1.842 ± 0.68) but decreased in the dose groups (1.259 ± 0.11 and 1.198 ± 0.083 folds for LD and HD groups respectively) (Fig. 3b). It is evident that the expression of TNF-α was decreased by 35.88% and 38.52% in LD and HD groups respectively compared to FCA group animals; Cox-2 expression decreased by 31.65% and 34.96% respectively in LD and HD compared to FCA group animals.

Discussion

Erythrocyte membrane stability test is a well-established study to screen the possible anti-inflammatory effect of synthetic drugs as well as of various traditional herbal extracts [19]. During inflammation, lysis of the membranes of the lysosomal vesicles occurs that releases their component enzymes which induce the inflammatory response. Thus, a stabilized membrane prevents the release of its contents as well as the progression of inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) exert their beneficial effects by either stabilizing the lysosomal membranes or by inhibiting the release of lysosomal enzymes [2]. Exposure of RBCs to hypotonic medium or high temperatures results in the lysis of the RBC membranes accompanied by hemolysis and oxidation of hemoglobin. In the hypotonic solution, the hemolytic effect is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Increased body temperatures also cause the rupture of the RBC membrane resulting in hemolysis [23]. In our study, a dose-dependent relationship of *Aloe* gel homogenate against both hypotonicity and heat-induced hemolysis has been found. Therefore, both the doses of the plant extract may inhibit the release of lysosomal content during the inflammatory processes. A possible explanation for the membrane stabilizing activity of plant extracts could be an increase in surface area/

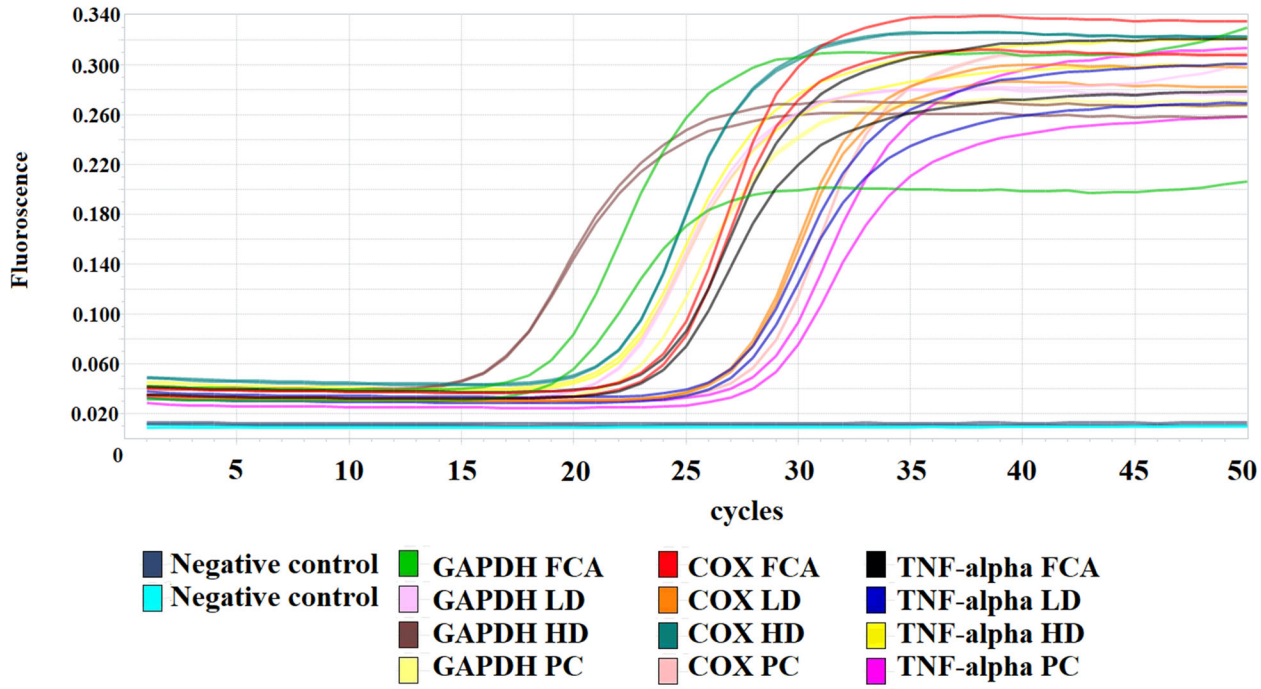

Fig. 2 Amplification curves for all the three selected genes in different experimental groups (in duplicates) in reverse transcription quantitative PCR

volume ratio of the cell [24] or by a stabilization of the skeletal proteins such as tropomyosin [25, 26].

In the protein denaturation-inhibition test, the denaturation of egg albumin was induced by heat treatment. Heat exposure causes the breakage of the functional three-dimensional structure of protein. Protein denaturation is a well-documented feature in chronic inflammatory diseases like RA, especially in the severe stages of the disease [27]. Standard NSAID drugs can inhibit heat-induced protein denaturation [4, 21]. In this study, it has been found that *Aloe* gel homogenate can also inhibit heat-induced protein denaturation. This signifies the anti-inflammatory roles of the plant in vitro and further encourages exploration of its efficacy in inflammatory conditions in vivo.

Cytokines are the key mediators of inflammation. TNF-α is a pro-inflammatory cytokine released from macrophages and monocytes. TNF-α is responsible for the transportation of more immune cells and chemokines to the inflamed region. It also initiates the production of matrix metalloproteinases responsible for cartilage degradation [7]. On the other hand, Cox-2, an immune

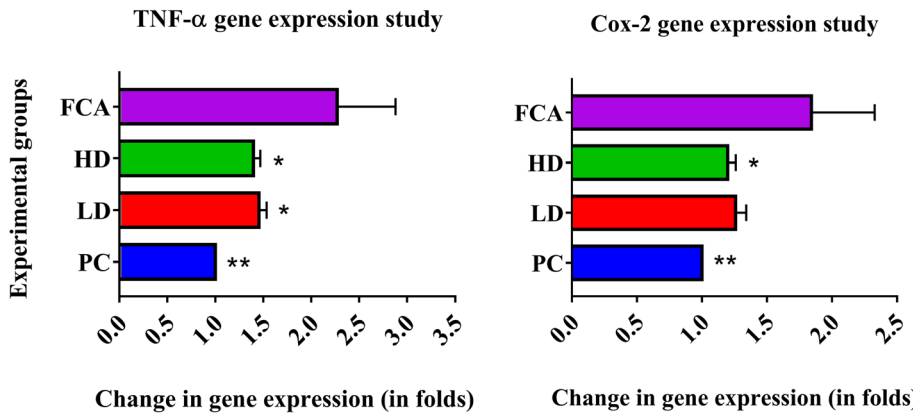

Fig. 3 Results of in vivo anti-inflammatory tests in graph. Relative expression fold changes in the TNF-α (**a**) and Cox-2 (**b**) genes assessed through reverse transcriptase real-time quantitative PCR. The significance value of low dose (LD) and high dose (HD) groups are compared with the experimental group (FCA). Positive control (PC). *P* value at $P < 0.01$ is considered significant

modulator, is responsible for the increased production of prostaglandin E2 which results in increased pain and swelling at the site of inflammation [9]. Cytokines along with Cox-2 are target bio-molecules of the host body which have a regulatory role on inflammation progression. NSAIDs primarily work on cyclooxygenase pathway [28]. Cytokine inhibitors have been introduced in the medication against inflammation as well.

In the present study, our results show that the *A. vera* unprocessed gel homogenate downregulates TNF-α as well as Cox-2 expressions in experimental animals in a dose-dependent manner. The crude unprocessed homogenized form of *A. vera* gel has been orally administered without any post-harvesting processing, a method which is in traditional use in different ethnic communities of Egypt, Rome, Africa, and Asia [29]. In our previous works, we have reported the ameliorative role of unprocessed *A. vera* gel homogenate in the regulation of inflammatory and arthritic symptoms in experimental rats. The paw circumference, serum biochemical parameters, and blood profile were restored to normal levels after oral feeding of unprocessed *Aloe* gel homogenate [5, 30]. The present study now further shows that the unprocessed *A. vera* gel homogenate concomitantly downregulates both TNF-α and Cox-2 in the experimental animal groups. This report can be considered as baseline data describing the efficacy of crude unprocessed plant products against inflammatory arthritis. By not extracting the gel in polar or non-polar solvents, we presume, the natural constituents of *A. vera* gel have been restored in its natural proportion and thus natural synergistic role of the gel has been monitored. Considering the previous works done in this regard, different bioactive compounds of the gel has already been mentioned by other workers which contribute to the efficacy of the plant as an anti-inflammatory resource. Davis et al. [12] has mentioned that the polysaccharides obtained from *Aloe vera* are potent anti-inflammatory agents; Hutter et al. [31] identified 8-[C-beta-D-[2-O-(E)-cinnamoyl]glucopyranosyl]-2-[(R)-2-hydroxypropyl]-7-methoxy-5-methylchromone from *Aloe barbadensis* (Synonym *Aloe vera*) as anti-arthritic agent. Apart from these findings, anthroquinones were identified as potent anti-inflammatory mediators in *Aloe vera* by Kashirsagar and co-workers [15]. The TNF-α downregulating activity of the plant has also been documented by Prabjone et al. [32] in helicobacter pylori-infected rats. It is highly expected that the anti-inflammatory properties of the subject plant in model systems will be equally attributed to the human system as well. Our data showed that the crude *A. vera* gel reduced the extent of lysosomal membrane lysis and protein denaturation in vitro and also regulated the expressions of major pro-inflammatory cytokine TNF-α and a key inflammation modulator Cox-2 in vivo following daily oral consumption in inflammatory RA rat models. To our knowledge, it is the first report on the effect of unprocessed *Aloe vera* gel oral treatment on TNF-α and Cox-2 gene expressions in arthritic animal models.

Conclusions

The study validates the efficacy of unprocessed aqueous *Aloe* gel homogenate in inflammatory disease condition. Our data indicate promising anti-inflammatory activity of *Aloe* gel in the inhibition of lysosomal membrane lysis, protein denaturation in vitro, and downregulation of TNF-α and Cox-2 expression in vivo. The scientific basis of use of raw unprocessed *A. vera* thus has been explored. However, an expression study on a broader spectrum of cytokines would further clarify the scenario.

Abbreviations

A. vera: *Aloe vera*; RA: Rheumatoid arthritis; NSAIDs: Non-steroidal anti-inflammatory drugs; CAMs: Complementary and alternative medicines; WHO: World Health Organization; LD: Low dose; HD: High dose; b.w.: Body weight; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC: Institutional Animal Ethical Committee; ANOVA: One-way analysis of variance; EDTA: Ethylenediaminetetraacetic acid

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Plant authentication

The identification of the plant species was authenticated by the Department of Botany, University of North Bengal, India, and a voucher specimen was deposited in the Departmental herbarium of the Department of Botany, University of North Bengal bearing the Accession No NBU-09884.

Authors' contributions

S.P, D.M, D.N, A.S, and J.R performed the in vitro experiments, analyzed the data, and prepared the tables and figures; S.P, D.M, and S.C performed the in vivo experiments and prepared and analyzed the data. S.P, D.M, and S.C also prepared the manuscript. T.K.C and S.B contributed to the concept and design of the experiments and data analyses, contributed to the critical revision of the manuscript, and gave the final approval of the manuscript for publication.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The work was approved by the Institutional Animal Ethical Committee of the University of North Bengal (IAEC, NBU) (Approval number 840/ac/04/CPCSEA).

Consent for publication

Not applicable.

Competing interests

Authors declare no conflict of interest.

Author details
[1]Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, District Darjeeling, Siliguri, West Bengal 734013, India. [2]Department of Biotechnology, Brainware University, Barasat, Kolkata, West Bengal 700125, India.

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References

1. Chayen J, Bitensky L (1971) Lysosomal enzymes and inflammation with partivular reference to rheumatoid diseases. Ann Rheum Dis 30:522–536
2. Anosike CA, Obidoa O, LUS E (2012) Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). DARU J Pharm Sci 20:76
3. Mizushima Y (1964) Inhibition of pretein denaturation by antirheumatic or antiphlogistic agents. Arch Int Pharmacodyn Ther 149:1–7
4. Saso L, Valentini G, Casini ML, Grippa E, Gatto MT, Leone MG, Silvestrini B (2001) Inhibition of heat-induced denaturation of albumin by nonsteroidal anti-inflammatory drugs (NSAIDs): Pharmacological implications. Arch Pharm Res 24(2):150–158
5. Paul S, Dutta T, Chaudhuri TK, Bhattacharjee S (2017) Curative and protective properties of crude gel of Aloe vera from sub-Himalayan West Bengal in chronic and acute inflammatory rat models. Indian J Tradit Knowl 16(1):121–127
6. Feldmann M, Brennan FM, Maini RN (1996) Role of cytokines in rheumatoid arthritis. Annu Rev Immunol 14:397–440
7. Saleem A, Saleem M, Akhtar MF, Shahzad M, Jahan S (2020) Moringa rivae leaf extract attenuate Complete Freund's adjuvant induced arthritis in Wistar rats via modulation of inflammatory and oxidative stress biomarkers. Inflammopharmacol 28:139–151
8. Crofford LJ (1999) The role of COX-2 in rheumatoid arthritis synovial tissues. Arthritis Res 1(1):S1
9. Sano H (2011) The role of lipid mediators in the pathogenesis of rheumatoid arthritis. Inflamm Regen 31:151–156
10. Parasuraman S, Thung SG, Arumugam S (2014) Polyherbal formulation: concept of Ayurveda. Pharm Rev 8(16):73–80
11. World Health Organization (WHO). General guidelines for methodologies on research and evaluation of traditional medicine. WHO/EDM/TRM 2000.1. http://archives.who.int/tbs/tbs_trm.htmhttp://archives.who.int/tbs/tbs_trm.htm. Accessed 20 July 2019
12. Davis RH, Donato JJ, Hartman GM, Haas RC (1994) Anti-inflammatory and wound healing activity of a growth substance in Aloe vera. J Am Podiatr Med Assoc 84:77–81
13. Davis RH, Leitner MG, Russo JM, Byrne ME (1989) Wound healing: oral and topical activity of Aloe vera. J Am Podiatr Med Assoc 79:559–562
14. Surjushe A, Vasani R, Saple DG (2008) Aloe vera: a short review. Ind J Dermatol 53:163–166
15. Kashirsagar AD, Panchal PV, Harle UN, Nanda RK, Shaikh HM (2014) Anti-inflammatory and antiarthritic activity of anthroquinone derivatives in rodents. Int J Inf Secur 690596. https://doi.org/10.1155/2014/690596
16. Rashed AN, Afifi FU, Disi AM (2003) Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1. J Ethnopharmacol 88:131–136
17. Afolayan AJ (2003) Extracts from the shots of Arctotis arctotoides inhibit the growth of bacteria and fungi. Pharm Biol 41(1):22–25
18. Paul S, Chakraborty AK, Modak D, Sen A, Bhattacharjee S (2018) In vivo and in silico investigations of the toxicological and analgesic properties of unprocessed Aloe vera gel in experimental rat models. Arch Biol Sci 70(4): 727–735
19. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN (1999) Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of Cedrusdeodara wood oil. Fitoterapia 70(3): 251–257
20. Tatiya AU, Saluja AK (2011) Further studies on membrane stabilizing, anti-inflammatory and FCA induced arthritic activity of various fractions of bark of Machilusmacrantha in rats. Rev Bras 21(6):1052–1064
21. Mizushima Y, Kobayashi M (1968) Interaction of antiinflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 20(3):169–173
22. Gupta AK, Parasar D, Sagar A, Choudhary V, Chopra BS, Garg R, Khatri N (2015) Analgesic and anti-inflammatory properties of gelsolin in acetic acid induced writhing, tail immersion and carrageenan induced paw edema in mice. PLoS One 10(8):e0135558
23. Aloni B, Eitan A, Livne A (1977) The erythrocyte membrane site for the effect of temperature on osmotic fragility. Biochim Biophys Acta-Biomem 465(1):46–53
24. Abe H, Katada K, Orita M, Nishikibe M (1991) Effects of calcium antagonists on the erythrocyte membrane. J Pharm Pharmacol 43(1):22–26
25. Chasis JA, Mohandas N (1986) Erythrocyte membrane deformability and stability: two distinct membrane properties that are independently regulated by skeletal protein associations. J Cell Biol 103(2):343–350
26. An X, Salomao M, Guo X, Gratzer W, Mohandas N (2007) Tropomyosin modulates erythrocyte membrane stability. Blood 109(3):1284–1288
27. Rowley M, Tait B, Mackay IR, Cunningham T, Phillips B (1986) Collagen antibodies in rheumatoid arthritis.Significance of antibodies to denatured collagen and their association with HLADR4. Arthritis Rheum 29(2):174–184
28. Efthimiou P, Kukar M (2010) Complementary and alternative medicine use in rheumatoid arthritis: proposed mechanism of action and efficacy of commonly used modalities. Rheumatol Int 30:571–586
29. Shedoeva A, Leavesley D, Upton Z, Fan C (2019) Wound healing and the use of medicinal plants. eCAM 2684108:1–30
30. Guha P, Paul S, Das A, Halder B, Bhattacharjee S, Chaudhuri TK (2014) Analyses of human and rat clinical parameters in Rheumatoid Arthritis raise the possibility of use of crude Aloe vera gel in disease amelioration. Immunome Res 10:081
31. Hutter JA, Salman M, Stavinoha WB, Satsangi N, Williams RF, Streeper RT, Weintraub ST (1996) Antiinflammatory C-glucosyl chromone from *Aloe barbadensis*. J Nat Prod 59(5):541–543
32. Prabjone R, Thong-Ngam D, Wisedopas N, Chatsuwan T, Patumraj S (2006) Anti-inflammatory effects of Aloe vera on leukocyte-endothelium interaction in the gastric microcirculation of helicobacter pylori-infected rats. Clin Hemorheol Microcirc 35(3):359–366

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ORIGINAL ARTICLE

Evaluation of the Effectiveness of *Acmella uliginosa* (Sw.) Cass. Flower Methanolic Extract in Pain Amelioration and Memory Impairment in the Experimental Rat Models: Search for an Alternative Remedy over Opioid Painkillers

Subhashis Paul, Debabrata Modak, Somit Dutta[1], Tapas Kumar Chaudhuri[2], Soumen Bhattacharjee

Department of Zoology, Cell and Molecular Biology Laboratory, University of North Bengal, [1]Department of Botany, Molecular Genetics Laboratory, University of North Bengal, Darjeeling, West Bengal, [2]Department of Zoology, Bodoland University, Kokrajhar, Assam, India

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ABSTRACT

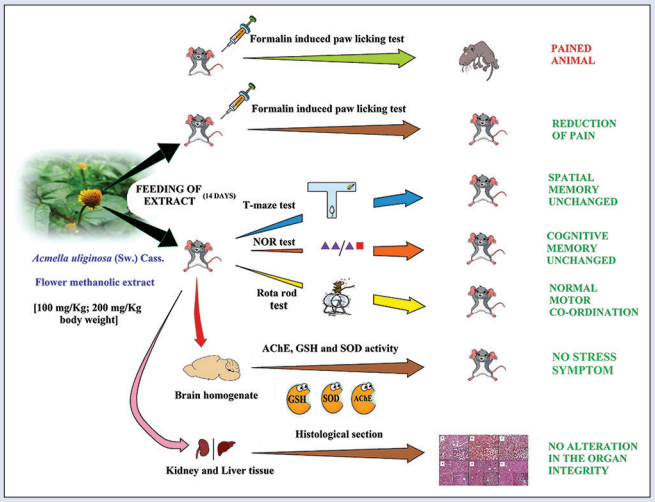
Background: The flower of *Acmella uliginosa* (AU) (Sw.) Cass., a naturally grown herb in India, is consumed as a natural painkiller for its notable analgesic properties. **Objective:** The objective of the study was to establish the role of AU flower methanolic extract in antinociception and its neuromodulatory activities to assess any disadvantage of the drug akin to opioids. **Materials and Methods:** In experimental rats, plant flower extracts were fed at a dose of 100 mg and 200 mg/kg body weight (BW) for 14 days. Analgesic activity was evaluated through formalin-induced paw licking test. T-maze, novel object recognition (NOR), and rotarod tests were done to assess the role of the extract in memory alteration and neuromotor coordination, respectively. Acetylcholinesterase (AChE), reduced glutathione (GSH), and superoxide dismutase (SOD) activity from the brain homogenates were done to assess the induced oxidative stress. **Results:** The plant proved to be a promising analgesic when fed orally up to 200 mg/kg BW dose. No acute toxicity was seen up to 1000 mg/kg. In the T-maze test, extract-fed animals showed a reduction in food searching time. In NOR test, the discrimination index between new and familiar objects was high in extract-fed animals compared to standard group. In rotarod test, the extract did not alter the neuromotor coordination. AChE, GSH, and SOD activities were normal in extract-treated animals. **Conclusion:** Memory alteration and oxidative stress are two major drawbacks associated with opioid drugs. Our results indicate that the AU flower methanolic extract qualifies as a potent painkiller and overcomes the disadvantages of opioid analgesics.

Key words: Acetylcholinesterase, *Acmella uliginosa*, antinociception, cognitive memory, oxidative stress, spatial memory

SUMMARY

- *Acmella uliginosa* is a traditionally used painkilling herb found in the Indian subcontinent
- The plant flower methanolic extract shows potential pain ameliorating activity in formalin-induced pain model of experimental Wistar rats
- The extract overcomes the memory deteriorating activities which are associated with opioid-like painkiller drugs
- The plant extract could be used as an alternative herbal remedy against pain without any possible side effects and might be consumed for a longer time without causing harm in the patient's body.

Abbreviations used: Ach: Acetylcholine; AChE: Acetylcholinesterase; ANOVA: Analysis of variance; ATChI: Acetylthiocholine iodide; AU: *Acmella uliginosa*; AUM: *Acmella uliginosa* methanolic; BW: Body weight; CNS: Central nervous system; Cox: Cyclooxygenase: CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; DI: Discrimination index; DTNB: Dithiobisnitrobenzoic acid; FCA: Freund's complete adjuvant; GSH: Reduced glutathione; IAEC: Institutional Animal Ethical Committee; NOR: Novel object recognition; NSAID: Non-steroidal anti-inflammatory drug; OECD: Organization for Economic Cooperation and Development; PBS: Phosphate-buffered saline; ROS: Reactive oxygen species; SD: Standard deviation; SEM: Standard error mean; SOD: Superoxide dismutase; WHO: World Health Organization.



Correspondence:

Prof. Soumen Bhattacharjee,
Department of Zoology, Cell and Molecular Biology Laboratory, University of North Bengal, Raja Rammohunpur, Darjeeling - 734 013, West Bengal, India.
E-mail: soumenb123@rediffmail.com
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INTRODUCTION

Pain is any unpleasant sensory and emotional experience associated with actual or potential tissue damage. Three major classes of pain have been classified depending on the receptors involved and the response pathways. The types are nociceptive pain, inflammatory pain, and pathological pain.[1] Patients suffering from both inflammatory disorders and autoimmune diseases consume a high dose of painkiller during disease severity.

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Research Article

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ANTI-INFLAMMATORY ACTIVITY OF *ACMELLA ULIGINOSA* (SW.) CASS. FLOWER METHANOLIC EXTRACT ON MEMBRANE STABILIZATION AND PROTEIN DENATURATION: AN *IN- VITRO* EVALUATION

Debabrata Modak, Subhashis Paul and Soumen Bhattacharjee*
Cell and Molecular Biology Laboratory, University of North Bengal, Raja Rammohunpur, Darjeeling 734013, West Bengal, India.

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ABSTRACT
The floral part of *Acmella uliginosa* plant is a common folklore pain-killer used in India and tropical countries. The plant possesses potent anti-arthritic property as well. To establish the anti-inflammatory activity of the methanolic flower extract *in vitro*, the human RBC membrane stabilizing activity and protein denaturation inhibiting activity were assessed. The results showed that the red blood cell suspension treated with flower extract displayed very significant membrane stabilization compared to untreated group. Plant extract, when incubated with RBC suspension at a dose of 400 µg/ml showed 39.37±3.63 % stabilization in hypotonicity induced RBC membrane haemolysis and 25.22±0.98% stabilization in heat induced RBC membrane haemolysis when compared to untreated groups. Similar result was found in protein denaturation inhibition test. The extract treated hen's egg albumin showed significantly less denaturation (26.52±2.39%) compared to untreated group. The present study firmly indicated the role of *Acmella uliginosa* flower extract as a potent anti-inflammatory agent.

Key Words: *Acmella uliginosa*, inflammation, membrane stabilization, protein denaturation, complementary and alternative medicine, haemolysis.

INTRODUCTION

Inflammation is a common complex biological process in response to tissue injury, infection, toxin exposure or chemical irritation. At the site of injury and tissue damage, inflammation is initiated by aggregation of immune cells from blood vessels and release of immune reaction mediators to eliminate foreign pathogens, resolving infection and repairing injured tissues. The main function of inflammation is protective which is rapid and self-limiting, but when prolonged, it may cause various chronic disorders and enhance extensive tissue damage. To treat the consequences inflammation, anti-inflammatory agents like non-steroidal anti-inflammatory drugs (NSAIDs) or steroids are available commercially. However, the consumption of these conventional NSAIDs leads to different side effects such as vomiting, dizziness, bleeding and ulcers in the stomach and intestine [FDA Guideline (www.fda.gov)], liver damage (Bessone, 2010), renal impairment, chronic kidney disease (Nderitu *et al*., 2013) and an increased risk of cardiovascular diseases (Trelle *et al*., 2011).

Herbs play a major role in modern medicines. The herbal resources constitute a major reservoir for potentially active medicinal compounds all over the World. The development and use of herbal medicinal systems known as complementary and alternative medicine (CAM) has come into consideration due to their low side effects, low costing, and ready availability. Countries rich in biodiversity, including India, show herbal medicine practices from ancient era. Ayurveda, Siddha, Unani have been a major remedy against different odd diseases. In CAM system, daily consumption of raw

***Author for correspondence:** Prof. S. Bhattacharjee, E. Mail: soumenb123@rediffmail.com / sbhnbu@gmail.com

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Original Research

ScopeMed

Assessment of anti-inflammatory and anti-arthritic properties of *Acmella uliginosa* (Sw.) Cass. based on experiments in arthritic rat models and qualitative gas chromatography-mass spectrometry analyses

Subhashis Paul[1], Sudeb Sarkar[2], Tanmoy Dutta[1], Soumen Bhattacharjee[1]

[1]Department of Zoology, Cell and Molecular Biology Laboratory, University of North Bengal, Darjeeling, West Bengal, India, [2]Division of Cancer Biology and Inflammatory Disorder, CSIR-Indian Institute of Chemical Biology, Kolkata, West Bengal, India

Address for correspondence:
Soumen Bhattacharjee, Department of Zoology, Cell and Molecular Biology Laboratory, University of North Bengal, Raja Rammohunpur, Darjeeling - 734 013, West Bengal, India.
E-mail: soumenb123@rediffmail.com

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ABSTRACT

Aim: The principle objective of the study was to explore the anti-arthritic properties of *Acmella uliginosa* (AU) (Sw.) Cass. flower in a rat model and to identify potential anti-inflammatory compounds derived from flower extracts. The synergistic role played by a combination of AU flower and *Aloe vera* (AV) gel crude extracts was also investigated. **Materials and Methods:** Male Wistar rats induced with Freund's complete adjuvant (FCA) were used as a disease model of arthritic paw swelling. There were three experimental and two control groups, each consisting of five rats. Paw circumference and serum biochemical parameters were evaluated to investigate the role of the flower extracts in disease amelioration through a feeding schedule spanning 21 days. Gas chromatography/mass spectrometry (GC/MS) analyses were performed to search for the presence of anti-inflammatory compounds in the ethanolic and n-hexane solvent extracts of the flower. **Results:** As a visual cue to the experimental outcomes, FCA-induced paw swelling decreased to the normal level; and hemoglobin, serum protein, and albumin levels were significantly increased in the treated animals. The creatinine level was estimated to be normal in the experimental rats after the treatment. The combination of AU and AV showed the best recovery potential in all the studied parameters, confirming the synergistic efficacy of the herbal formulation. GC/MS analyses revealed the presence of at least 5 anti-inflammatory compounds including 9-octadecenoic acid (Z)-, phenylmethyl ester, astaxanthin, à-N-Normethadol, fenretinide that have reported anti-inflammatory/anti-arthritic properties. **Conclusion:** Our findings indicated that the crude flower homogenate of AU contains potential anti-inflammatory compounds which could be used as an anti-inflammatory/anti-arthritic medication.

KEY WORDS: *Aloe vera*, Freund's complete adjuvant, rheumatoid arthritis

INTRODUCTION

Ethnic people have used herbal resources from ancient times to fulfill their nutritional and medicinal needs. A vast majority of the nutritional supplements and edible medicinal materials are either consumed as such from herbs or synthesized from herbal resources [1]. The interest of producing herbal formulations for medicinal purposes has developed due to their low side effects, low costing, and ready availability in countries such as India and China. In ethnobotanical knowledge, daily consumption of different plant parts and products are said to have disease-modifying and disease-improving activities. However, scientific explanations and working principles of such crude plant parts or herbal formulations are not well-established experimentally. *Acmella uliginosa* (AU) (Sw.) Cass. (Family Asteraceae) is a plant found in the Northern part of West Bengal (known as North Bengal) and has a worldwide distribution [Figure 1]. These herbs grow up to 1 m, generally creep or sometimes stand erect, rooting at nodes, and their stems are sub-glabrous to scabrid-pilose. It has been used as food by many human populations all through the world. The Malay people, as well as the Rajbanshi people from Northern part of West Bengal, consume the plant and its flowers for symptoms such as tooth ache, mouth ulcer, and mouth ache [2]. When consumed, the flower has a characteristic pungent taste which is soon followed by a characteristic tingling and numbness of the tongue. The antinociceptive activity, anti-

Bulletin of the National Research Centre

RESEARCH Open Access

Potent bioactive methanolic extract of wild orange (*Citrus macroptera* Mont.) shows antioxidative, anti-inflammatory, and antimicrobial properties in in vitro, in vivo, and in silico studies

Mousikha Lala[1], Debabrata Modak[2], Subhashis Paul[2], Indrani Sarkar[1], Ankita Dutta[3], Anoop Kumar[3], Soumen Bhattacharjee[2] and Arnab Sen[1*]

Abstract

Background: There is always an increasing demand for natural remedies from natural sources which can substitute the synthetic therapeutic drugs and lessen their side effects. The present study aims to investigate the antioxidant, anti-inflammatory, antimicrobial properties and in silico docking study of *Citrus macroptera* leaf (CML) extract in both in vivo and in vitro aspect.

Material and methods: The antioxidant and anti-inflammatory potential of crude extract was investigated in vitro and in vivo on Wistar albino rat. The antioxidant potentiality also investigated on HepG2 cell line. Antimicrobial activity was evaluated against *Staphylococcus* sp. and *Klebsiella* sp. Chemical compounds of the crude extract were identified by GC-MS analysis. In silico docking was also done against NF-κB protein.

Results: At 200 μg/ml concentration, CML significantly scavenges reactive oxygen species (ROS) which was generated on HepG2 cell line. CML showed 71% anti-inflammatory activity ($p \leq 0.001$) against carrageenan-induced paw edema in albino Wistar rats. CML extract is very effective against *staphylococcus* sp. than *Klebsiella* sp. In the docking analysis, the proximadiol and menthone had − 5.6 kcal/mol and − 5.7 kcal/mol binding affinity with the protein NF-κB.

Conclusion: In the present work, CML provided notable antioxidant, anti-inflammatory, and antimicrobial activity. This activity was confirmed by both in vitro and in vivo followed by in silico docking technique. Overall, the experimental results presented in this study suggest that crude extract of CML could be used as a promising antioxidant and anti-inflammatory candidate with potential benefits.

Keywords: *C. macroptera*, Anti-inflammation, Antibacterial, HepG2 cells, Docking

* Correspondence: senarnab_nbu@hotmail.com
[1]Molecular Cytogenetics Laboratory, Department of Botany, University of North Bengal, Siliguri, West Bengal, India
Full list of author information is available at the end of the article

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RESEARCH ARTICLE Open Access

Validating potent anti-inflammatory and anti-rheumatoid properties of *Drynaria quercifolia* rhizome methanolic extract through in vitro, in vivo, in silico and GC-MS-based profiling

Debabrata Modak[1], Subhashis Paul[1], Sourav Sarkar[1], Subarna Thakur[2] and Soumen Bhattacharjee[1*]

Abstract

Background: The fronds of *Drynaria quercifolia* have traditionally been used in rheumatic pain management. The goal of the present study was to validate the potent anti-inflammatory and anti-rheumatoid properties of the methanolic-extract of its rhizome using in vitro, in vivo and in silico strategies.

Methods: The plant was collected and the methanolic extract was prepared from its rhizome. Protein denaturation test, hypotonicity and heat-induced haemolysis assays were performed in vitro. The in vivo anti-rheumatoid potential was assessed in Freund's complete adjuvant (FCA)-induced Wistar rat model through inflammatory paw-edema, haematological, biochemical, radiological and histopathological measurements. Moreover, metabolites of methanolic extract were screened by gas chromatography-mass spectrometry (GC-MS) and 3D molecular structures of active components were utilized for in silico docking study using AutoDock.

Results: In vitro results evinced a significant ($p < 0.05$) anti-inflammatory activity of the rhizome methanolic extract in a dose-linear response. Further, *Drynaria quercifolia* rhizome methanolic extract (DME) significantly ameliorated rheumatoid arthritis as indicated by the inhibition of arthritic paw-edema (in millimeter) in the rat rheumatoid arthritis models in both the low (57.71 ± 0.99, $p < 0.01$) and high dose groups (54.45 ± 1.30, $p < 0.001$) when compared to arthritic control. Treatment with DME also normalized the haematological (RBC, WBC, platelet counts and hemoglobin contents) and biochemical parameters (total protein, albumin, creatinine and ceruloplasmin) significantly ($p < 0.05$), which were further supported by histopathological and radiological analyses. Furthermore, GC-MS analysis of DME demonstrated the presence of 47 phytochemical compounds. Compounds like Squalene, Gamma Tocopherol, n-Hexadecanoic acid showed potent inhibition of cyclooxygenase-2 (COX-2), tumor necrosis factor (TNF-α), and interleukin (IL-6) in the docking analysis.

(Continued on next page)

* Correspondence: soumenb@nbu.ac.in; soumenb123@rediffmail.com
[1]Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, Darjeeling, West Bengal 734013, India
Full list of author information is available at the end of the article

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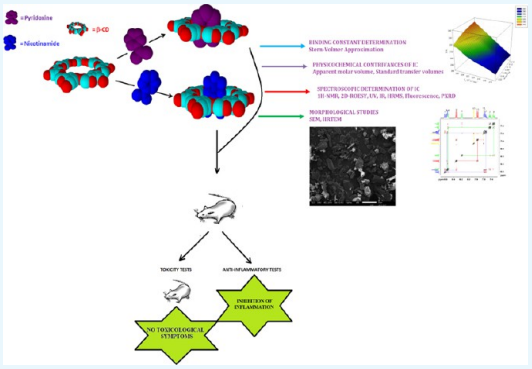
Synthesis, Characterization, and Comparison of Host−Guest Complexes of β-CD with Vitamins Explored through Their Biological Activities

Koyeli Das,[†] Subhashis Paul,[‡] Debabrata Modak,[‡] Beauty Mahato,[†] Soumen Bhattacharjee,[‡] and Mahendra Nath Roy*[,†]

[†]Department of Chemistry and [‡]Cell and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Darjeeling 734013, India

Supporting Information

ABSTRACT: Herein, inclusion complexation of host β-CD with guests, viz. nicotinamide and pyridoxine (two active forms of vitamins), in both aqueous medium and solid state has been explored by means of various spectroscopic and physicochemical procedures. In vivo toxicity and in vitro anti-inflammatory properties in experimental rat models through membrane stabilization and protein denaturation test are studied here. These imperative complexes, when orally consumed, showed no toxic effect in experimental rats equal to the dose (400 mg/kg) of body weight when fed up to 28 days. Binding constants for the inclusion complexes have been designed by the Stern−Volmer approximation method and found to be higher for pyridoxine, elucidated on account of their molecular structural representation. Additionally, molecular docking aided to enlighten the most possible mode of interactions among guests and β-CD. Both of the encapsulated systems could potentially find applications in vitamin B_3 and vitamin B_6 formulation for the purpose of enhancing stability, absorption, and controlled delivery of these imperative vitamins. The use of β-CD as a drug delivery vehicle with vitamin B substituents into eukaryotic cells is well documented and thus increases the bioavailability of diverse therapeutics.



1. INTRODUCTION

Cyclodextrin toroidally fashioned polysaccharides comprised usually six to eight units of glucose. Cyclodextrin cavities comprise internal diameters, permitting them to outline inclusion complexes (ICs) with diverse guests. These have been used extensively to sculpt hydrogen bonds, π−π stacking, hydrophobic interactions, and metal−coordination bond interactions. Vital assets of CDs are to select specific guests that could insert into the cavity to form inclusion complexes. "β-CD−guest" interaction is used as a model for the "enzyme active site", thereby engrossing the attention of several researchers. Studies related to the inclusion of drugs into β-CDs are significant due to progress in solubilization, constancy of the guest, and sustainment of drug release, which represent possible applications in drug constructions.[1−4]

Vitamins are a requisite for average growth and development of multicellular organisms. This investigation comprises two vitamins, which are being used as the guest moiety in the inclusion complex formation. Inflammation is a basic immunological process elicited through the host body in reaction to any foreign material entering the body. Different autoimmune diseases and excess inflammation, however, play a negative role in damaging the host body by numerous means. Diseases such as arthritis, cancer, and diabetes are some examples of inflammatory disarrays. Two vitamins used here are major elements in the inflammatory process. Vitamin B_3 has an active role in the inflammation through regulation of Cox-2 and other proteins and plays a vital part in inflammation regulation. It has been observed that vitamin B6 deficiency is associated by impairment in differentiation, monocyte-derived macrophage production, T-lymphocytes, interleukin-2, and cytokines molecules, which directs to an immunological dysfunction and low level of inflammation in the host body. An adequate supplementary intake of two vitamins is important to regulate the normal immune status.

Nicotinamide is an active form of amide of nicotinic acid. Nicotinamide belongs to the category of water-soluble vitamins. Nicotinamide has anti-inflammatory properties that are possibly beneficial for the patients having inflammatory skin conditions. Animal studies demonstrate that nicotinamide have an anti-anxiety (anxiolytic) property. The formation of nicotinamide is formerly the subject matter of both experiments and theoretical modifications. The $-NH_2$ group in nicotinamide can support the formation of various associations using hydrogen bonding and a variety of tautomers and rotamers in host−guest complexation.

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National (7)

- **Paul, S.** (2012). Evolution of the immune system.

  UGC sponsored National Seminar on "BIODIVERSITY AND SUSTAINABILITY VIS-À-VIS ECONOMIC DEVELOPMENT IN THE NORTHERN PARTS OF WEST BENGAL" August 26-27. 2012 at the Department of Botany, **Raiganj Surendranath Mahavidyalaya, Raigunj, WB**. **[Oral presentation]**

- **Paul, S.** & Chaudhuri, T. K. (2012). Immunopharmacological investigations of the plant *Diascorea alata.*

  UGC Sponsored National seminar on "RECENT ADVANCES IN LIFE SCIENCE APPLICATION" December 8-9, 2012 at the P.G. Department of Zoology, **A.B.N.Seal College, Coochbehar, WB. [Oral presentation]**

- **Paul, S.** & Bhattacharjee, S. (2014). Anti-arthritic properties of crude *Aloe vera* gel in adjuvant induced arthritic rat models.

  UGC Sponsored Seminar on "BIOLOGICAL RESEARCH IN HUMAN WELFARE", February 07, 2014 at Department of Zoology, **University of North Bengal, Siliguri, WB**. [**Poster Presentation**]

- **Paul, S.** & Bhattacharjee, S. (2015). Arthritic diseases: Disease detail, prevention and ways to healthy life.

  22nd WEST BENGAL STATE SCIENCE AND TECHNOLOGY CONGRESS 2015. February 28-March 1, 2015 at the **University of North Bengal, Siliguri, WB**. **[Oral presentation]**

- **Paul, S.** & Bhattacharjee, S. (2015). Anti-Arthritic and protective activities of unprocessed wild *Aloe vera* gel in FCA induced rat Arthritic Model.

  National Seminar on "APPLIED ZOOLOGY IN SUSTAINABLE DEVELOPMENT: AN UPDATE", January 30 to February 02, 2015 at Department of Zoology, **University of North Bengal, Siliguri, WB**. [**Oral Presentation**]