CHAPTER - 7

STUDIES ON ANTIULCER ACTIVITY

7.1. Introduction

An ulcer is characterized by disruption of mucosal integrity leading to local defect or excavation due to active inflammation[1]. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *H. pylori* and NSAID's) and local mucosal defensive factors (mucus, bicarbonate, blood flow and prostaglandins). Integrity of gastro duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors[2] Clinically, regulation of gastric acid secretion is considered as major therapeutic target in the management of disease[3]. Among clinically established drugs, H_2 blockers (ranitidine) and proton pump inhibitors (omeprazole) are most widely used as anti-ulcer drugs in addition to the cytoprotective agents like sucralfate and misoprostol. Ulcer healing consists of reconstruction of mucosal architecture and is a dynamic, active process of filling the mucosal defects with epithelial and connective tissue cells. It encompasses cell proliferation, division and migration[4-6]. Prostaglandins (PGs) and growth factors play an important role in healing of ulcers. Synthesis of PGs is governed by the expression of inducible cyclooxygenase-2 (COX-2) isozyme in gastric mucosa during healing process, further, COX-2 expression is enhanced in gastric epithelial cells after treatment with growth factors *in vitro* and *in vivo* during acetic acid induced gastric damage[7-9].

Peptic ulceration is one of the common disease affecting millions of people. Peptic ulcers are localized breaches of the gastric or duodenal mucosa with tissue destruction at least to the depth of the muscularis mucosa. Among different disorders of gastrointestinal system, peptic ulcer is the one which is more prevalent and have greatest clinical impact. It is now considered to be one of the symptom is pain. The most serious complications are bleeding and perforation. Modern age epidemics are affecting nearly 10% of world population. Research advances during last decade have offered new insights in the therapy and prevention of peptic ulceration[10].

Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine known to possess antiulcer properties. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved antiulcer drugs[11]. Herbal medicines are now used by up to 50% of the western population, in a number of instances

for the treatment or prevention of ulceration[12]. Although drug treatment for peptic ulceration has improved in the recent past, its complications still carry a significant mortality. Recently, widespread effort has been launched to identify novel anti-ulcer drugs from natural resources.

Herbal formulations are used by a majority of Indians from ancient times. They are used to treat a wide verity of conditions including hyperacidity and peptic ulcer. Nearly each and every herbal pharmaceutical company has its antacid and antiulcer products in market. These products are most of the times multidrug formulations[13].

There are various plant-originated "gastro protectors" with different composition that have been used in clinical and folk medicine for many centuries due to their beneficial effects on the mucosa of GIT. In China and Japan, polyphenol extracts such as Sopharadin extracts, containing flavonoids and its synthetic flavonoid derivative known as Solon are widely employed in peptic ulcer therapy and also as food additives and nutritional supplements, mainly because of their strong inhibition of prostaglandin (PG) metabolism and vasoconstrictive leukotriene inhibition[14].

The present study was carried out to assess the possible anti-gastric ulcer activity of methanolic extract of *K. rotunda*, *E. cannabinum* and their isolated products which are widely growing in Sikkim, to confirm and extend its uses in traditional medicine.

7.2. Materials and methods

7.2.1. Plant materials

Methanol extracts of rhizomes of *Kaempferia rotunda* Linn. and leaves of *Eupatorium cannabinum* Linn. as well as their isolated compounds were used as test drug in these experiments.

7.2.2. Drugs and chemicals

All the chemicals used in this study were procured from an authorized dealer and were of analytical grade. Methanol was obtained from LOBA chemicals Kolkata, carboxymethyl cellulose was obtained from Zydus-Cadila, India. Aspirin was collected from SD-Fine Chem, Ltd., Mumbai. Ranitidine was obtained from Cipla Ltd., Goa as a gift sample. Methanolic extract of rhizomes of *Kaempferia rotunda* Linn. and leaves of *Eupatorium*

cannbinum Linn. and their isolated compounds used in this study were extracted in our laboratory.

7.2.3. Test compound formulations

Oral suspensions of the rhizome and leaf extract of *K. rotunda* and *E. cannabinum* respectively and the isolated compounds of both the plants were prepared by suspending them separately in 1% solution of sodium carboxy methylcellulose to obtain suitable dosage forms.

7.2.4. Experimental animals

The study was conducted on Wistar albino rats (n=72) weighing 150-200g of either sex and maintained under standard environmental conditions as per a specific design (10% air exhaust in air conditioning unit was maintained along with a relative humidity of 60 ±5% and a temp of 25±3° with 12 h light and dark cycle. Amrut certified rodent diet and tap water (boiled water cooled to room temp) was provided *ad libitum* to the experimental animals. All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) prior to the initiation of the experiment.

7.3. Anti ulcer activity of methanol extract of rhizome and isolated compound of *Kaempferia rotunda Linn.*

7.3.1. Ulcer induction procedure

Gastric ulcers were induced in the experimental animals by administration of Aspirin + pylorus ligation (300mg/kg by gavaging-Model A), Aspirin (300mg/kg by gavaging-Model B).

7.3.2. Model A: Aspirin + Pylorus ligation treatment induced ulcers

Both aspirin treatment as well as pylorus ligation procedure was used to induce peptic ulcers. Effects on gastric lesion and secretions were carried out by ligation of the pyloric end of the stomach by silk suture according to the method described by Shay *et.al.*[15]. Male Wistar rats between 150-200g were selected for pyloric ligation ulcer model. They were fasted in individual cages for 36 h. Care was being taken to avoid coprophagy. For antiulcer investigation animals were divided in to 6 groups (group-I to group-VI). In each group study, 36hrs fasted animals were used. The control animals (group I) were

administered with calculated dose of 1% sodium CMC (Sodium carboxy methyl cellulose) in distilled water. The positive control (group IV) was dosed with ranitidine (20mg/kg suspended in 1% sodium CMC in distilled water. The test group-II and III were dosed with test extract 200 mg/kg, and 400 mg/kg in 1% sodium CMC in distilled water and V, VI were dosed with isolated compound I at 10, 20 mg/kg respectively. All the animals received drug/ extract treatment along with 300 mg/kg of aspirin suspended in 1% sodium CMC once daily for five days. The sixth dose of drug was given 30 minutes prior to pylorus ligation. On the sixth day the 36 hrs fasted rats were subjected to pylorus ligation. They were sacrificed after 4 hrs post surgery and their intact stomachs were excised, observed and the contents were emptied in to a graduated centrifuge. The collected gastric juice was centrifuged at 3000rpm for 30min and the volume of gastric juice was measured. Total acidity in the supernatants was determined with 0.01 M NaOH and expressed as m.Eq/L gastric juice. The stomach was cut open along the greater curvature and pinned on a board for ulcer scoring[16-18].

7.3.3. Model B: Aspirin induced ulcers

In aspirin induced ulcer model[19] the rats were divided into six groups each consisting of three. The Group I served as control, the Group II served as positive control and the Group III, IV, V and VI served as test. Control animals received 1% sodium CMC in distilled water for 8 days and the group II treated with Ranitidine (20mg/kg). The Group III and IV were treated with methanolic extract of *K. rotunda* at 200mg/kg and 400mg/kg body weight respectively.

Group V and VI were dosed with isolated compound 1 at 10 and 20 mg/kg body weight respectively for 8 days orally. After 8 days of treatment animals were fasted for 36 hours. The animals were gavaged with aspirin suspended in 1% sodium CMC. The animals were then left as such for 4 hrs. After which they were sacrificed. The intact stomach was removed from each animal, washed in normal saline and cut along the greater curvature. The inner lining was observed for ulcer formation and ulcers were scored to obtain the ulcer index[20]. All the animal experimental protocols were approved by the Institutional Animal Experimental Committee.

7.4. Antiulcer activity of methanol extract of leaves and isolated compound of *Eupatorium cannabinum* Linn.

The antiulcer activity of methanol extract of *E. cannabinum* leaves and its isolated compound II were determined as per the methods described above in 7.3 for *K. rotunda* Linn.

7.5. Calculation of ulcer index

After sacrificing the rat, the stomach was removed and opened along the greater curvature. The mucosal layer of stomach was observed under a magnifying lens and was checked for ulcers, hemorrhagic areas or perforations. The ulcer index was determined as Ulcer Index =10/X (Where, X= Total area of stomach mucosa / total ulcerated area) [21].

7.6. Statistical analysis

The results of all the assays were reported as (X±SEM). Statistical significant differences between the groups were calculated by means of one way ANOVA. All the results obtained in the study were compared with the control group and positive control group treated with ranitidine. P values < 0.05 were considered statistically significant.

7.7. Results

Peptic ulceration was immensely induced with different intensities in each of the ulcer models. The ulcer index for *Kaempferia rotunda* extracts (200 and 400mg/kg) and isolated compound 1 (10, 20 mg/kg) in Model-A were calculated as 0.67±0.12, 0.45±0.018, 0.63±0.61 and 0.52±0.12 respectively which is summarized in table-7.1 and were found to be statistically significant when compared with control (1.16±0.082) as well as for positive control group (0.41±0.038) at 5% level of significance (P<0.05). The ulcer index for *Eupatorium cannabinum* extracts (200 and 400mg/kg) and isolated compound II (10mg and 20mg/kg) in Model-A were determined as 0.59±0.12, 0.38±0.041, 0.54±0.30 and 0.39±0.19 respectively which is summarized in table-7.3 and were found to be statistically significant when compared with control (1.02±0.082) and for positive control group (0.27±0.016) at 5% level of significance (P<0.05). Similarly the volume of gastric content, total acidity and total acidity output of the groups of animals treated with the extracts of *K. rotunda* and *E. cannabinum* at 200 and 400 mg/kg and their isolated compounds had significantly reduced the values when compared with

control. The group treated with 400 mg/kg for both the plant extract showed significant difference when compared with ranitidine. All the extract treated animal parametric values were comparable with that of standard group. In order to assess the statistical significance in between the groups one way ANOVA was carried out and it authenticates that almost all four parameter were significant. The extracts at the dose level of 200, 400 mg/kg body weight showed dose response antiulcer effect whereas the isolated compound I and II showed the dose response antiulcer effect at the dose level of 10, 20 mg/kg body weight. For the aspirin induced model (Model-B) the ulcer index values are epitomized in table7.2 and 7.4. Pretreatment with the methanolic extract of *Kaempferia rotunda, Eupatorium cannabinum* and their isolated compounds the results were comparable to control and ranitidine treated groups which are verified with one way ANOVA. The datas were found to be highly significant when compared with control and ranitidine group at 5% level of significance.

Table-7.1. Antiulcer activity of *K. rotunda* and its isolated compound-I in rats by Aspirin+pylorus ligation method (Model-A)

Acid secretary parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control (X±SEM)	Plant extract 200mg/kg (X±SEM)	Plant extract 400mg/kg (X±SEM)	Ranitidine 20mg/kg (X±SEM)	Isolated product 1 (10mg/kg) (X±SEM)	Isolated product 1 (20mg/kg) (X±SEM)
Vol. of gastric content (ml/100g)	9.67±0.372	8.78±0.410	7.02±0.27	6.57±0.316	6.89±0.65	6.71±0.09
Total acidity m.Eq/L	72.89±2.17	59.63±3.10	48.53±2.02	47.88±4.71	48.40±0.11	48.01±0.10
Total acidity output m.Eq/100g	183.28±12.44	168.72±31.24	151.84±20.19	148.11±11.81	150.87±0.34	150.78±0.08
Ulcer index	1.16±0.082	0.67±0.12	0.45±0.018	0.41±0.038	0.63±0.61	0.52±0.12
One way ANOVA F 0.036	All values are statistically significant respect to control at 5% level of significance					

Table -7.2. Antiulcer activity of *K. rotunda and* its isolated compound-I in rats by Aspirin induced ulcer method (Model-B)

Group No.	Treatment	Ulcer index (X±SEM)
I	Control (1% sodium CMC)	0.93±0.218
II	Ranitidine(20mg/kg)	0.26±0.17
III	Plant extract (200mg/kg)	0.63±0.192
IV	Plant extract (400mg/kg)	0.32±0.23
V	Isolated compound-1(10mg/kg)	0.53±0.61
VI	Isolated compound- I (20mg/kg)	0.42±0.12

All the values were (X±SEM) significantly different from control and ranitidine at $p < 0.05$ level

Table-7.3. Effect of methanolic extract of *E. cannabinum* and its isolated compound II in rats by Aspirin + pylorus ligation method (Model-A)

Acid secretory parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control (X±SEM)	Plant extract (200mg/kg) (X±SEM)	Plant extract (400mg/kg) (X±SEM)	Ranitidine (20mg/kg) (X±SEM)	Isolated Compound II (10 mg/kg) (X±SEM)	Isolated Compound II (20 mg/kg) (X±SEM)
Vol. of gastric content (ml/100g)	7.03± 0.096	5.64± 0.14	5.49 ± 0.32	5.38 ± 0.29	5.44±0.08	5.41±0.41
Total acidity (m.Eq/l)	66.82± 3.28	56.02± 4.88	41.25± 4.71	37.03 ± 5.62	42.87±0.10	42.03±0.25
Total acidity output (m.Eq/100g)	79.72±18.1	141.22±38.3	132.1±14.9	122.61±22.11	129.87±0.20	128.78±0.26
Ulcer Index	1.02± 0.082	0.59 ± 0.12	0.38± 0.041	0.27 ± 0.016	0.54±0.30	0.39±0.19
One way ANOVA F 0.020			All values are statistically significant respect to control at 5% level of significance			

Table-7.4. Effect of methanolic extract of *E. cannabinum* and its isolated compound II in rats by Aspirin induced ulcer method (Model-B)

Group No.	Treatment	Ulcer Index (X±SEM)
I	Control (1% sodium CMC)	0.81 ± 0.031
II	Ranitidine(20mg/kg)	0.26± 0.170
III	Plant extract (200mg/kg)	0.24 ± 0.033
IV	Plant extract (400mg/kg)	0.21± 0.015
V	Isolated compound II (10mg/kg)	0.20 ± 0.014
VI	Isolated compound II (20mg/kg)	0.18 ± 0.019

All the values were (X±SEM) significantly different from control and ranitidine at $p < 0.05$ level

7.8. References

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