2.
REVIEW OF LITERATURE

2. Review of literature

The review of literature on *N. indicum* is particularly concentrated on its medicinal and pharmacological aspects, especially highlighting on its ethnomedicinal use and evidence based scientific reports of its pharmacognostic activities. University of North Bengal library web portal (http://10.10.2.100/opac/opac.asp) and manual internet search were performed using various keywords related to the plant such as '*Nerium indicum*', '*Nerium oleander*', 'common Oleander', 'Nerium and traditional medicine, 'Nerium and therapeutic', 'Nerium and anti-cancer' etc. In addition, reference and bibliographies of several published articles were searched for related keywords. Search for published research articles were separately performed in Medline, Scopus, Google Scholar and EBSCO. Different criteria of inclusion were adopted like:

- *Nerium oleander*, common oleander and *Nerium odorum* were considered to be the synonyms of *Nerium indicum.*
- The yellow oleander *Thevetia peruviana* (syn. *Cascabela thevetia*) was considered separate plant.
- All kind of study (*in vitro, in vivo, ex vivo, in silico*) on animals and humans were included.

2.1. Botanical description

Nerium indicum Mill is an evergreen plant belonging to the family Apocynaceae. It is widely cultivated in all over the world, especially in south-west Asia. Classification of the plant is as follows: (Spice and medicinal herbs, 2012)

Kingdom- Planate

Division- Tracheophyta

Class- Magnolipsida

Order- Gentianales

Family- Apocynaceae

Genus- Nerium

Species- Indicum

The plant grows naturally in different parts of the world starting from Mauritania, Morocco, Portugal to the entire Mediterranean region, expanding through Sahara to the Arabian peninsula and southern Asia. But its white flower variety plant, *N. indicum* is exclusively native to India, Bangladesh, Nepal, Myanmar and China.

Figure 10: Picture of white variety of *N. indicum*.

The plant is most commonly known as Oleander in English, Seth Karabi (white flowered variety) in Bengali and Kaneer in Hindi. Different accepted synonyms of the plant includes: *Nerium oleander* L., *Nerium odorum* Aiton, *Nerium japonicum* Gentil, *Nerium flavescens* Spin, *Oleander indica* (Mill.) Medik., *Oleander vulgaris* Medik., N*erium grandiflorum* Desf. etc (The Plant List, 2013). The plant is upright, rounded evergreen sub-tropical to tropical in nature and grows up to 2-6 m in height. The leaves are around 5-21 cm long with narrow shape, willow-like and linear-lanceolate. Leaves are thick, leathery, hairless and dark-green in colour. The erect stems spread outward with maturation and possess greyish bark. The sap is viscous and gummy. Each leaf have a distinct midrib with parallel secondary veins extending towards the leaf margin. Flowers of white, pink or red colour grow in cluster at the end of each branch. They are tubular in shape comprising of five lobes. The fruits are of slender, long, capsular shape consisting of two follicles.

2.2. Ethnopharmacology and traditional use

Medicinal usage of the plant dates back to 1500 BC in ancient Mesopotamia (Isaacs, 2008). Plant based medicines are the pillars of traditional therapeutics. *N. indicum* is one such plant, widely used in ethnomedicinal practices all over the world for the treatment of dermatitis, eczema, psoriasis, herpes, sores, abscesses, warts, corns, skin cancer, ringworm, scabies, epilepsy, asthma, malaria, and heart disease (Nerium Biotechnology, 2015). It is interesting to

note that, in Dan Brown's historical mystery-fiction 'The da Vinci Code', rose is symbolized as the feminine half of God, which highlighted potent healing power as told in Pagan and early Christianity. Perhaps due to the same reason, the plant is mentioned as the 'desert rose' which symbolized its potent medicinal properties (Isaacs, 2007).

N. indicum is a well-known name in traditional medicine in different parts of the world especially in India (Dastur, 1985; Khare, 2007; Ghosh, 2008) and China (Ji, 1999; Ding, *et al*., 2003; Fu, 2005; Gayathri, *et al*., 2013). In ethnomedicinal, the plant is used for the treatment of diverse ailments such as cardiac illnesses, asthma, corns, cancer, and epilepsy (Duke, 1985). In Ayurveda, different polyherbal formulations contained parts of *N. indicum* as active constitutes (Easyayurveda, 2013) such as: Manikya Ras (against fever and allergic dermatitis), Chitrakadi Taila (to treat fistula) and Brihat Marichadi Taila (for the treatment of spondylotic pain, sciatica). The plant has also been mentioned in the writings of Charaka and Sushruta for its medicinal properties. It is prescribed for the treatment of skin disease and inflammatory infections in Sanskrit medicinal literatures (Dutt, 1922). Due to its potent medicinal properties, the plant has also been included in the Indian Ayurvediac pharmacopeia (1978). The detailed description of the therapeutic and medicinal properties along with prescribed doses of *N. indicum* mentioned in Ayurveda are enlisted in different Indian ethnopharmacological books such as Bhavaprakasa Nighantu (Bhavamisra, 1995), Dhanvantari Nighantu (Sharma, 2002), Chakradatta (Sharma, 1994) etc. In Russia and China, *N. indicum* is used for the treatment of cardiac abnormalities (Sreenivasan, *et al*., 2003). A green dye from the flower is used in the treatment of skin diseases and is also claimed to possess wound healing and anti-inflammatory properties. Hot water leaf and seed extracts of the plant are used to cure upper respiratory tract and gastrointestinal infections in Kenya (Nanyingi, 2008). The juice prepared from the stem bark of *N. indicum* is used to cure ear pain in the traditional therapeutic systems in the Kancheepuram district of Tamil Nadu, India (Muthu, *et al*., 2006). In Turkish folklore medicinal system, it is used to treat paralysis, extreme pains, swellings and common cold (Yesilada, *et al*., 1999; 2002). In the folklore medicinal system of Calabria, situated in southern Italy, the plant is used for the treatment of malaria by the natives (Tagarelli, *et al*., 2010). In Trinidad and Tobago, the plant extract is extensively used for reproductive problems (Lans, 2007). It is also used as anti-diabetic medicine in Morocco (Bnouham, *et al*., 2002; Jouad, *et al*., 2001), Pakistan (Hussain, *et al*., 2013), in different parts in Algeria (Rachid, *et al*., 2012) as well as mentioned in Ayurveda for the same properties (Sudha, *et al*., 2011). In Iloilo, Philippines, the plant is used as ethnomedicine to treat fever, headache and dermatological problems (Tantiado, *et al*., 2012).

In the Errachidia province of Morocco, *N. indicum* is used in the treatment of hypertension and diabetes (Tahraoui, 2007). Moreover, it is interesting to note that a recent survey by Saha *et al*., (2014) in Madla district in West Bengal, India, revealed that the leaves of *N. indicum* are used in one of the traditional anti-diabetic formulations by the local indigenous people.

2.3. Pharmacological activities

In past few decades, the focus of medicinal and pharmaceutical science has shifted from antibiotics and synthetic drugs to plant based natural remedies. As a result, extensive research on the medicinal properties of *N. indicum* have revealed its therapeutic potentials which are discussed below:

2.3.1. Antioxidant activity

Singhal and Gupta (2012) demonstrated the free radical scavenging activity of methanolic flower extract by studying reducing power, lipid peroxidation, DPPH, ABTS, superoxide anion, hydroxyl radicals and metal chelation activities *in vitro*. Furthermore, they demonstrated that the same fraction normalized hepatic SOD and inhibited lipid peroxidation in CCl_4 toxified animals. Gayathri *et al*., (2011a) showed the antioxidant activity of glycosidic and nonglycosidic fractions from the flower using ABTS and DPPH assays. However, this is to note that in the complex cascade of free radicals numerous reactive species are generated causing cellular and sub-cellular damage. Therefore, all the major parts of *N. indicum* was required to evaluated for their antioxidant activities concentrating on the free radical scavenging assays.

2.3.2. Antinociceptive activity

Zia, *et al*., (2005) isolated two bioactive fractions namely B1 and B2 from the methanolic extract of *N. indicum* and evaluated their effect on central nervous system and behaviour pattern in mice using p-benzoquinone induced abdominal contraction model. The result indicated that both fractions affected the locomotor activity, rotarod performance and potentiation of hexobarbital sleeping time in the experimental animals. The number of writhing were minimal in the extracts of fresh (15.0±0.6) and dried flowers (15.0±0.7) with an inhibitory ratio of 66.6% ($P<0.001$) in both cases out of the extracts of fresh flower, dried flowers and leaves prepared in methanol and water.

2.3.3. Antibacterial activity

Hussain and Gorsi (2004) evaluated the cold chloroform, ethanol and methanol extracts of and root bark and leaves of *N. indicum* against *Bacillus pumilus*, *Bacilllus subtilis*, *Staphyloccus aureus*, *Escherichia coli*. The zone of inhibition of the chloroform leaf extract resulted in a zone of inhibition of 19 mm throughout the experiment (48-46 h). At 96 h the activity of ethanolic leaf extract was optimum against *B. pumilus* and *S. aureus*, resulting in zone of inhibition of 24 mm each. The methanolic root extract demonstrate comparatively better anti-bacterial activity among the all extracts. Furthermore, Bhuvaneshwari, *et al*., (2007) studied the anti-bacterial activity of benzene, chloroform and alcohol extracts of root, bark and leaves using disc diffusion method. The results revealed that *Staphylococccus aureus, Pseudomonas aeruginosa* and *Salmonella typhimurium* resulted in better growth inhibition having a zone of inhibition of 10 mm, 9 mm and 7 mm respectively. De Britto, *et al*., (2011) also demonstrated the antibacterial activity of the methanolic extract of the leaves. Among various human pathogenic bacteria under evaluation, the growth of only *Pseudomonas vulgaris, Streptococcus aureus, Staphylococcus aureus, Shigella boydii* and *E. coli* were found to be inhibited due to the plant extract.

2.3.4. Anthelmintic activity

The anthelminthic activity of *Nerium* flower was evaluated by two different groups of researchers. Khan and his group (2011) studied the effect of aqueous extract of *Nerium flower* on the time of paralysis and time of death of *Pheretima posthumad* i.e. Indian earthworm, using albendazole as reference compound. At the highest dose of 100 mg/ml, the extract took 03.40±0.29 min to paralyse and 5.58±0.45 min to kill the experimental earthworms. In a similar *in vivo* experiment, Nitave and Patil (2014) demonstrated that the time of paralysis for *Nerium indicum* flower aqueous and ethanolic extracts at 100 mg/ml were 18.50±0.67 min and 8.66±0.49 min, respectively. The time of death were calculated to be 37.83±0.60 min and 20.16±0.65 min, respectively at the same concentration.

2.3.5. Anti-fungal activity

The anti-fungal activity of ethanolic flower extracts of this plant against different fungal pathogens was studied by Hadizadeh, *et al*., (2009). At the optimum concentration of 0.9%, the flower extract resulted in 46.3±0.02%, 70.3±0.05%, 90.3±0.01% and 89.2±0.13% growth reduction for *Alternaria alternate*, *Fusarium oxysporum*, *Fusarium solani* and *Rizoctonia solani* respectively. Moreover, Kalita and Salkla (2012) studied the antifungal activity of 50%

ethanol fraction of *N. indicum* leaves against *Aspergillus niger* and *Candida albican*. The zone of inhibition of 10 mm and 13 mm were found in case of *A. niger* and *C. albican*, respectively.

2.3.6. Antiviral activity

Rajbhandari, *et al*., (2001) demonstrated that the methanolic extract of *N. indicum* possess inhibitory activity against influenza virus and herpes simplex virus. The extract showed IC_{50} value of 10 μg/ml against the influenza virus. Singh and her team studied (2013) the effect of an aqueous extract (Anvirzel™) of the plant on HIV infectivity in human peripheral blood mononuclear cells. They have demonstrated that Anvirzel™ reduced the potentiality of HIV to infect new cells without any alteration in the total number of virus particles. Oleanderin, a cardiac glycoside isolated from the Nerium leaves, down-regulated HIV coat protein g120 expression, which is the primary mediator of HIV infection. As low as 10 μg/ml concentration of Anvirzel™ was potent enough to inhibit the HIV infectivity. However, recently, Avci, *et al*., (2014) reported that the aqueous extract prepared from the leaves had no inhibitory effect on the Blue Tongue Virus under *in vitro* evaluation.

2.3.7. Anti-hyperlipidemic activity

Gayathri, *et al*., (2011b) studied the hypolipidemic activity of ethanolic extract of *N. oleander* flower on mice model which were kept on high fat diet. This resulted in the increase of cardiac lipids, lipoproteins and total body weight. Due to parallel feeding of the plant extract to the animals, the lipid, lipoprotein and body weight gain was significantly lowered in addition, it elevated the plasma and cardiac HDL levels. Besides, oral feeding of the extract also up-regulated the lipolytic enzymes in the experimental animals.

2.3.8. Cardiotonic efficiency

Adome, *et al*., (2003) have demonstrated the cardio-stimulatory effect of a crude ethanolic extract of *N. oleander* leaves on pig cardiac model. The effect of the extract was evaluated on three parameters namely, force of myocardial contraction, heart-beat rate and cardiac blood flow. The results displayed that under the influence of 100 mg/ml extract, the heart beat rate was elevated from 28 beats/min to 41 beats/min, blood flow volume increased from 0.4 ml/min to 1.9 ml/min and amplitude of myocardial contraction increased from 22 mm to 49 mm. The results of the leaf extract were much higher than that of the reference compound acetylcholine and adrenalin.

2.3.9. Effect on myocardial oxidative stress

Gayathri, *et al*., (2011a) studied the effect of a hydro-alcoholic extract of Nerium flower on isoproterenol-induced myocardial oxidative stress on rat model. Isoproterenol is a non-selective β-adrenergic agonist which is routinely used to generate myocardial infraction and hypertrophy in animal models. Pre-treatment of the extract inhibited isoproterenol induced elevation of LDH, GGT, AST, ALT, ALP and creatinine kinase in plasma. The flower extract maintained the levels of anti-oxidant enzymes such as SOD, GPX and GSH which contributed to the inhibition of lipid peroxidation in the experimental animals. The results were further supported by histopathological studies.

2.3.10. Neuroprotective activity

Dunn and his group (2011) investigated the neuroprotective activity of oleanderin and PB-05204 (a CO_2 assisted extract of oleander leaves) through ischemic injury model by studying oxidative damage and glucose deprivation in rats. Results suggested that when treated with 23 µg/mL PBI-05204 (approximately containing 1 µM oleandrin), the yellow fluorescent protein tagged coronal brain slices had more protection from oxygen and glucose deprivation. At 69 µg/mL PBI-05204, the extent of protection was same as 23 µg/mL PBI-05204, whereas 10 fold increase in PBI-05204 concentration (230 µg/mL) decreased the extent of neuroprotection. PBI-05204 also increased levels of α1 and α2 subunits of Na^+/K^+-ATPase in the rat brain slices.

2.3.11. Anti-malarial activity

The larvicidal activity of the ethanol and acetone extracts was investigated by Sharma, *et al*., (2005) who demonstrated the effect of these extracts on the 3rd instar larvae of two malaria causing vectors *Anopheles stephensi* and *Culex quinquefasciatus*. After 24 and 48 h, the ethanolic extract was proved to be less potent than the acetone extract against *C. quinquefasciatus* and vice versa against *A. stephensi*. LC_{50} value of the methanolic extract on *A. stephensi* was 185.99 ppm at 24 h and 184.05 ppm at 48 h, whereas on *C. quinquefasciatus* was 494.07 ppm at 24 h and 194.49 ppm at 48 h. In case of acetone extract, the LD_{50} value was 229.28 ppm at 24 h and 149.43 ppm at 48 h for *A. stephensi* and 209.00 ppm at 24 h and 155.97 ppm at 48h for *C. quinquefasciatus*.

2.3.12. Hepatoprotective activity

The methanolic flower extract of *N. indicum* was tested for its efficiency in CCl_4 mediated hepatotoxic model (Singhal & Gupta, 2012). The results demonstrated that the flower extract ameliorated the injured liver by decreasing the levels of AST, ALT, ALP, bilirubin and MDA levels in the serum of the experimental animals. The level of superoxide dismutase, which gives protection against free radical mediated damage to the liver, was also elevated. Histopathological studies demonstrated that the extract treated livers possessed less signs of inflammation and necrotic tissue as well as the normal liver architecture was restored with increased dose of the flower extract. However, no report exist on a systematic evaluation of hepatoprotective potentiality of any major parts of the plant i.e. leaf, stem and root.

2.3.13. Anti-hyperglycaemic activity

N. indicum is used for its hyperglycaemic activity in traditional medicine in different parts of the world. Sikarwar, *et al*., (2009) in an preliminary study reported anti-diabetic activity of various solvent extracts of *N. indicum* leaf in alloxan induced diabetic model in rats. After one week of treatment the blood glucose levels were 113.33±6.66, 169.33±9.73 mg/ds respectively for chloroform and ethanol extract compared to control (413.50±4.75 mg/dl). *N. indicum* extracts also prevented body weight increase in diabetic animals. Mwafy and Yassin (2011) further studied the anti-diabetic activity of the aqueous extract of the leaves on streptozotocin-induced diabetes model in rats. The results displayed that at the peak of 4^{th} week, the serum glucose level in extract treated group was 238.5±10.3 mg/dl and serum insulin level was 1.10±0.07 mg/dl corresponding to 128% and -18.5% change, respectively. On the contrary, linear correlation analysis between the serum glucose and insulin levels did not resulted in close correlation and they yielded a weak correlation coefficient (r) of -0.3. In the extract treated group, the levels of AST, ALT and ALP in the serum were 96.3±4.7 U/l, 44.4±1.7 U/l and 63.4±2.9 U/l, respectively representing 93.2%, 13.3% and 63.4% change respectively, for the three liver enzymes.

2.3.14. Analgesic activity

The analgesic activity of *N. indicum* flower, root, stem and leave methanolic extract was studied by Ahmed and his team (2006) on acetic acid induced writhing model in rats. At optimum concentration, the extent of writhing were 6.80%, 72.11%, 4.08% and 0.0% respectively for flower, root, stem and leaf extracts, when writhing in the control was considered as 100%. The percentage of inhibition in writhing were 93.20%, 27.89%, 95.92%

and 100%, respectively. Positive control, aminopyrine resulted in 63.27% inhibition in writhing at 50 mg/kg dose.

2.3.15. Antiulcer activity

Patel, *et al*., (2010) demonstrated the anti-ulcer activity of *N. indicum* leaf methanolic extract by studying pylorus ligation and indomethacin induced ulcer *in vivo.* Under indomethacin induced ulcer, the leaf extract at 250 and 500 mg/kg dose resulted in 65.97% and 69.63% protection respectively, with an ulcer index of 5.41±0.20 and 4.83±0.49, respectively based on six parallel experiments. Furthermore, in pylorus ligation induced ulcer in rats, 250 and 500 mg/kg dose resulted in ulcer index of 5.66±0.52 and 4.58±0.86, respectively which was much lower than that of the control (14.08±0.67). Compared to the control (102.43±0.22 meq/1/100 g), the gastric acidity was much lower (63.15±0.29 meq/l/100 g) in the 500 mg/kg dose group with reduced gastric volume.

2.3.16. Immunological effects

In spite of diverse medicinal and pharmacological activities of *N. indicum*, very few work has been performed to study the immunological effect of the plant and its components. Muller, *et al*., (1991) demonstrated that a crude polysaccharide fraction of the leaves possess mild mitogenic activity and stimulated macrophage-mediated cytotoxicity. Manna, *et al*., (2000) demonstrated that a polyphenolic cardiac glycoside oleandrin, derived from the leaves of *N. oleander*, inhibited TNF-α induced NF-κβ activation in time and concentration dependent manner. Oleandrin also had inhibitory effect on activator protein-1 induced TNF-α level as well as TNF-α induced activation of C-Jun NH2-terminal kinase. Furthermore, Sreenivasan, *et al*., (2003) demonstrated that oleandrin supresses NF-κβ activation in human epithelial and lymphoid cells and in insect and murine macrophage cells *in vitro*.

2.3.17. Anti-inflammatory activity

Even though *N. indicum* is used in the treatment of inflammatory diseases in ethnopharmacology, but very limited study has yet been performed. Erdemoglu, *et al*., (2003) studied the anti-inflammatory activity of 500 mg/kg dose of ethanolic and aqueous extracts of the plant on carrageenan induced paw edema model. The measurement of paw thickness were performed at an interval of 90, 180, 270 and 360 min. Among all the fractions, ethanolic extract of the dried leaves resulted in the least amount of inflammatory reaction (37.2±4.9 × 10^{-2} mm thickness at 90 min).

2.3.18. Anti-mitotic activity

Tarkowska (1971) studied the anti-mitotic activity of a complex mixture of glycosides derived from *N. oleander* root tips on *Allium cepa* and endosperm of *Haemanthus katherinae*. The root tips were submerged in the solution of oleander glycosides for 6 to 48 h and the results were observed by microscopic technique. Chromosomes of *A. cepa* was shortened and scattered throughout as visualized by aceto-orcein staining. However, the kinetochore remained integrated. In case of *H. katherinae*, the continuous microtubules were disoriented and were irregularly arranged. Restitution nuclei of varying sizes developed due to partitioning of chromosome and chromatids into uneven groups. Hindrance in the phragmoplast development was also noticed leading to multi-terminal phragmoplasts and abnormal cell plates.

2.3.19. Anti-cancer activity

In ethnopharmacology, the extracts of *N. indicum* is claimed to possess anti-cancer and anti-tumor activities. Even in pharmacology, the oleander extracts and isolated compounds are mostly studied for anti-cancer properties. Smith, *et al*., (2001) demonstrated that oleandrin and oleandrigenin, two bioactive constituent from oleander leaf, inhibited fibroblast growth factor in human prostate cancer cell line DU145 and PC3. Anti-tumor activity of Oleandrin, isolated from the leaves were demonstrated by Afaq, *et al*., (2004), who revealed that external application of oleandrin on mouse skin inhibited 12-O-Tetradecanoylphorbol-13-acetate induced NF-κβ and Ikβα expression. Newman, *et al*., (2005) and his group demonstrated that Oleandrin exposure to BRO human myeloma cells resulted in generation of superoxide radical mediated mitochondrial injury and loss of antioxidative enzymes which ultimately cause loss of cell viability of BRO cells. Ghoneum, *et.al*., (2006) studied the effect of an extract of the leaves, NOE-4, on the susceptibility of Raji cells (human Burkitt's lymphoma) to the natural killer cell mediated cytotoxicity. After 24 h of incubation, the control Raji cells were found to be unaffected by human mononuclear cell (MNCs) mediated cytotoxicity, whereas NOE-4 treated Raji cells were heavily affected in a dose dependent manner. In addition to the increased conjugate formation between Raji cells and MNC or NK-cells, the researchers also found decrease in the expression of Bcl-2 molecules, which gave a clue that NOE-4 perhaps, may be used to treat immune resistant cancers. Anti-leukemic effects of various extracts of the plant was studied on HL60 and K562 cell lines by Turan, *et al*., (2006) which showed that the cytotoxic index on K562 cells were 66.22%, 57.82%, 58.10% and on HL60 cells were 69.33%, 66.50%, 62.81% for leaf, stem and root extracts, respectively. The levels of P-glycoprotein (ATP-binding cassette transporter) was also found to be affected by

the extracts, resulting in toxicity to the K562 cells. Newman, *et al*., (2007) again tested the efficacy of a major glycoside oleandrin on human pancreatic tumor cells PANC-1. Oleandrin not only paused the cell proliferation of PANC-1 cells but also caused cell arrest at G(2)/M stage of cell cycle. The results indicate that oleandrin stimulated death of PANC-1 cells were not mediated by autophagy processes, rather it was governed by apoptotic pathway. Fiebig and his group (2013) studied anti-cancer effect of Breastin, a phytococtail comprising of glycosides, flavonoides and polysaccharides from oleander, against 63 human tumor cell lines. The mean IC_{50} was calculated to be 1.1 μg/ml with much higher anti-cancer potential compared than Cisplatin, 5-Fluoruracil and Cyclophosphamide. Breastin also demonstrated elevated tubuline binding capacity when co-examined with Paclitaxel, and Docetaxel.

2.3.20. Chemotherapy supplementation

Apostolou and Toloudi (2013) have recently shown that Cisplatin supplemented with Anvirzel™ holds enhanced potentiality to breast (MDA-MB 231, T47D, and MCF-7), colon (HCT-116, HT55 and HCT-15), lung (CALU-1, COLO699N and COR-L 105), prostate (PC3, LNCaP and 22Rv1), melanoma (A375) and pancreatic (PANC-1) cancer models *in vitro*. Cisplatin is a chemotherapeutic drug which is derived from platinum. Cancer cells were incubated with 0.1-100 μg/ml Cisplatin supplemented with 0.01-10 ng/ml for Anvirzel™ for 3 consecutive days. Highest toxicity against most of the cell lines were seen in case of co-chemotherapy using 0.1 μg/ml Cisplatin with 0.01 ng/ml Anvirzel™, whereas 1 μg/ml Cisplatin with 0.01 ng/ml Anvirzel™ displayed high efficiency against PC3 and MDA-MB231 cells.

2.3.21. Auditory supplementation

Extracts of *N. indicum* is traditionally used to treat ear pain in Kancheepuram district of Tamil Nadu, India (Muthu, *et al*., 2006). Emanuele, *et al*., (2007) described that oleandrin could be useful for sensorinerual hearing loss on gentamicin induced toxicity through inhibition of the activator protein-1 and C-Jun-terminal Kinase.

2.3.22. Radiotherapy supplementation

Nasu, *et al*., (2002) studied the effect of oleandrin as a supplement to radiotherapy on PC3 (human prostate carcinoma) cell line. PC-3 cells were cultured with 0.05 μg/ml of oleandrin and after 24 h, the cells were irradiated with gradual increasing dose of γ-rays and then studied for colony forming capacity. The test group (oleandrin treated) reduced the colony

forming efficiency from 95% to 21% of the PC-3 cells. Furthermore, the extent of radiosensitivity depended on time for which cells were incubated with oleandrin. Survival rate of cells with 0.05 µg/ml oleandrin exposure prior to radiation was 30% which was lower than the control group (50%). PC-3 cells were found to be more prone to apoptosis when treated with oleandrin prior to radiation compared to individual treatment with oleandrin and radiation.

2.3.23. Mutagenicity

El-Shazly, *et al*., (2000) investigated ethanolic extracts of the leaves containing cardiac glycoside neriifolin for possible mutagenic activity through HPRT (hypoxanthine phosphoribos yl transferase) method. Antichrysen-1, 2401-3, 4-oxide (ACDO) was used as positive mutagenic control. The mutation frequencies were $2.42/10^6$ cells and $3.07/10^6$ cells for 50 ppm and 25 ppm respectively, which were much lower than ACOD, which itself resulted in very high frequency of mutation (1111.83 per 10^6 cells).

2.3.24. Anti-angiogenesis activity

Hu, *et al*., (2009) isolated , three galactooligosaccharides (OJ1–OJ3) from the *N. indicum* leaves by acid hydrolysis method and tested their effect on the HMEC-1 (human microvascular endothelial cell) cells using the tube formation assay. After 16 h incubation, control HMEC-1 cells formed distinct tube-like structures. However, tube formation was disrupted for OJ2 and OJ3 at 100 µM concentration, whereas OJ1 displayed no anti-angiogenesis activity. The authors hypothesised that though all the three polysaccharides possessed backbone of a (1→4)-linked linear galactan chain, but their activity differed due to the difference in their sizes.

2.3.25. Anvirzel™

A Turkish surgeon named Dr. Huseyin Ziya Ozel prepared a phytochemical extract called Anvirzel™ from the leaves of *N. oleander*, which was demonstrated to possess anticancer effects against different cancer cell lines. Dr. Ozel in 1973, successfully treated some critically ill cancer patients with the extract which did not resulted in any side effects associated with chemotherapy such as hair loss or decrease in blood leucocyte count (Ozel, 1973). In the following years further working with the extract, Ozel discovered anti-cancer efficiency of the plant (Ozel, 1974). In 1987, the extract was tested by Sandoz pharmaceutical company (now Novartis) and confirmed that the extracts possess immunomodulatory activity

(Sandoz Labs, 1987). In 1988, a research team from Munich University Pharmacology Institute collaborating with Dr. Ozel isolated some bioactive polysaccharides which were responsible for the tremendous immunomodulatory activity (Carbik, *et al*., 1990; Ozel, *et al*., 1990a). In 1992, the European patent office granted U.S. patent to Dr. Ozel and in 1995, a U.S. pharmaceutical company (formerly known as Pharmaceutical Ventures Trust) registered the extract under the trademark Anvirzel^{TM} and conducted phase I trials at the Cleveland Clinic, Ohio (Mekhail, *et al*., 2011). Today all over the world, various medicinal and pharmaceutical tests are being performed with this extract, which are revealing more of its therapeutic potentialities.

2.3.26. Phytochemical analysis

As a routine phytochemical procedure, different bioactive components were isolated from different parts of the plant. Studies on fatty acid composition was by Kalita and Saikia (2004) performed which revealed the presence of lauric acid (69 g/kg), myristic acid (71 g/kg), palmetic acid (151 g/kg), stearic acid (35 g/kg), oleic acid (127 g/kg), linoleic acid (426 g/kg) and arachidic acid (121 g/kg). Among different bioactive constituents of the plant, two glycosides, nerrin and oleandrin were isolated, which possess cardiostimulatory activities (Jayabalan, *et al*., 1995). The plant is rich in α-tocopherol which possess potent antioxidant and free radical scavenging activities (Hussain & Gorsi, 2004). Different glucosides such as oleandrine, odorosides, adigoside were identified in the seeds and the bark contains glucosidesrosaginoside, nerioside and corteneroside (Zibbu & Batra, 2010). Various pectic polysaccharides primarily composed of galacturonic acid besides rhamnose, arabinose and galactose were identified in a aqueous extract of Nerium leaves (Muller, *et al*., 1991). Many such polysaccharides were demonstrated to possess bioactive properties (Hu, *et al*., 2009; Carbik, *et al*., 1990). The structural characterization of pectic polysaccharides (L-rhamnose, L-arabinose, D-galactose, and D-galacturonic acid) from *N. indicum* flower hot-water extract was done by Dong, *et al*., (2010) and various terpinoids from the plant has also been characterized by different groups of researchers (Begum, *et al*., 1997; Siddiqui, *et al*., 2009). Bai, *et al*., (2007) identified different pregnanes from the leaves among which neridienone A was proved to possess anti-inflammatory activities as well as inhibitory effect on hepatocarcinoma cells. Siddiqui, *et al*., (1986, 1987) isolated two coumaryloxy triterpenoids (neriucoumaric and isoneriucoumaric acid) and two cardiac glycosides (kaneroside and neriumoside) from the leaves. Sharma, *et al*., (2010) identified 4-oxooctyl-2-hydroxyundecanoate and heptacosane-3-enyl-5-hydroxy-hexanoate from the stem. Fu *et al*.,

(2005) isolated seven compounds and methyl esters of urosolic acid and oleanoic acid, which demonstrated anti-inflammatory activities by inhibiting intracellular adhesion molecule-1.

Among different compounds identified and isolated from different parts of the plant, extensive study on the bioactivity was performed only using oleandrin. Besides, in spite of isolation and characterization of different phytocompounds, the overall phytochemical profile of *N. indicum* remained obscure.

2.3.27. Nerium toxicity

In spite of possessing tremendous medicinal properties and being used in traditional medicine for different ailments, the oleander has been considered to be poisonous due to the presence of glycosides like oleandrin, adynerin, digitoxigenin and folineriin (Bandara, *et al*., 2010). However, the amount of cardiac glycosides present in red flowered plants are higher than that of the white flowered plants (Karawya, *et al*., 1973) and thereby making the white flowered oleander less toxic. Some reports also highlighted the lethal nature of the plant (Blum, *et al*., 1987; Haynes, *et al*., 1985; Khan, *et al*., 2010; Adam, *et al*., 2002). However, Shaw and Pearn (1979) reported that, 'the rate of clinical poisoning due to oleander is inconsequential, and mortality is negligible'. Besides, according to the Toxic Exposure Surveillance System (TESS) report of 2002, out of 874 cases of high level exposure to the plant, only 3 cases turned to be lethal (Watson *et al*., 2003).

However, according to the words of Paracelsus, the father of natural toxicology that, "dose makes the poison", higher dose of any compound could prove to be lethal. Interestingly, at present, even snake (Pal, *et al*., 2002; Vyas, *et al*., 2013) and bee (Lee, *et al*., 2005; Wesselius, *et al*., 2005) venoms are being investigated for their medicinal properties. Therefore, utmost importance must be given to explore the medicinal and pharmacological properties of *N. indicum*, which is till date used in ethnomedicine and holds immense potential as therapeutic agent.

2.4. Current research and future prospects

N. indicum is well known in ethnopharmacological domain and used as traditional medicine around the globe. Till date numerous patents has been filed based on its medicinal properties. Ozel (1990b) patented an extract of Nerium which was claimed to ameliorating cell-proliferative disease. Selvaraj, *et al*., (1999) patented an extract of the plant which was efficient in the treatment of cell-proliferative and immune deficient diseases such as cancers

and AIDS. Singh (2003) filed a patent on anti-proliferative activities of nanoparticle compositions containing different glycosides, including oleandrin, claiming the novel formulation to reduce cancer progression and metastasis . Another patent was filled by Singh and Streeper (2004) demonstrating novel water soluble formulations containing some bioactive constituents of Nerium (ex. neriifolin, oleandrin) to possess anti-cancer properties. Addington (2006) patented a method of oleander leaves processing, which contains high amount of oleandrin, an anti-cancer agent from Nerium. Panosyan and Al-Mukarish (2002) patented a novel extract from the leaves which has the potentiality to induce IFN-γ activity. Streeper and Singh (2005) patented pharmaceutical compositions containing different glycosides such as oleandrin, neriifolin etc from *N. oleander* from the treatment of diverse skin diseases in humans and animals. A patent on CO_2 assisted supercritical extraction method to isolate bioactive constituents from *N. oleander* was filled by Addington (2005). Rashan, *et al*., (2006) filed a patent on a method of cold extraction of *N. oleander* phytococtail and its utility against vast array of cancers. Ghoneum and Ozel (2011) patented a therapeutic composition of Nerium coupled with glutathione, which is effective against cell-proliferative diseases, infections, and dementias.

Different clinical trials are been conducted on the therapeutic efficiencies of oleander. M.D. Anderson Cancer Center in collaboration with National Cancer Institute (USA) and Nerium Biotechnology is recruiting patients (since June 2014) for phase I clinical trial (No. NCT01562301) to determine the optimum tolerable dose of Anvirzel which could be given to advance non-small cell lung cancre patients as supplement to carboplatin and docetaxel medications (ClinicalTrials.gov, 2012). The trail also aims to understand the possible anti-inflammatory and immunomodulatory activity of Anvirzel supplementation to the lung cancer patients. In another clinical trial (No. NCT01920841), Pennington Biomedical Research Center in association with Nerium Biotechnology is evaluating (since August 2013) two creams containing phytococtail from different plants including *N. oleander* to stimulate the lipolytic process by stimulating β- adrenergic receptor, which would eventually lead to size reduction in thigh and smooth appearance of the skin (ClinicalTrials.gov, 2014a). Previously (2007-13) M.D. Anderson Cancer Center successfully conducted phase I clinical trial (No. NCT02329717) on an extract of the leaves of *N. oleander* (known as PBI-05204 and or Xenavex™) to determine its maximum tolerated dose on patients with advanced solid tumors. Very recently (December 2014) Phoenix Biotechnology, Inc has started Phase 2 clinical trial (No.: NCT02329717) to evaluate the effect of PBI-05204 on Stage IV metastatic pancreatic cancer patients (ClinicalTrials.gov, 2014b). Xenavex, an ethanolic extract from the oleander

leaves prepared by Shimoda Atlantic Oncology Biosciences was announced in 2005 to undergo phase I and II clinical trials as lung cancer treatment. However, the trials were not yet performed (American cancer society, 2008).

Plant derived medicines could provide a unique opportunity to bioprospect diverse chemical species which would function synergistically on multiple target resulting in a holistic therapeutic approach which would improve the therapeutic efficiency of the drugs. In the global scenario, the shift of the pharmaceutical research towards herbal remedies from modern medicine is very much apparent. In case of plant like *N. indicum*, mechanism based screening of bioactivities focusing on their ethnopharmacological use is of utmost importance. Various issues, which are only applicable to herbal medicine must be considered for proper evaluation of these products (Kunle, *et al*., 2012). Different issues such as:

- Herbal drugs are phytococtails which may contain multiple bioactive species.
- The active constituents are mostly unknown.
- Herbal constituents may be chemically and naturally variable as chemo-variants and chemo-cultivars may be present.
- Appropriate analytical method may not be present.
- Authentication of source and quality of raw material.
- Assurance of quality, efficiency, safety and reproducibility.

Unlike conventional drugs, the botanical drugs are not target specific and therefore, could be used to treat disease associated symptoms of infection and inflammation. Present immunomodulatory strategies have revealed that monovalent approach of isolated drug therapy is unlikely to provide a holistic treatment (Patwardhan & Gautam, 2005). It is very much expected to opt for a strategy which would consider the complex interplay between different biological pathways. Thus, the requirement of designer drugs based on synergistic approach of traditional medicine would be helpful.