ABSTRACT

Herbal medicine is the core of traditional medicine. Several plants has been identified and extensively utilized for their therapeutic value from the very beginning of civilization. *Nerium indicum* Mill. (syn. *Nerium oleander* L., *Nerium odorum* Aiton; family: Apocynaceae), commonly known as Oleander, is such an ethnopharmacological plant. It is routinely used in Indian and Chinese traditional medicine for the treatment of various diseases. Indian Ayurvedic medicinal system describes the usage of different parts of the plant to cure numerous aliments. Different groups of researchers have also reported some of its bioactivities. However, in spite of extensive used for therapeutic purpose, many of its claimed medicinal and pharmacological properties has never been studied through a systematic approach. Moreover, most of the previous studies remained confined in the bioactivity of *N. indicum* leaf. Thus, pharmacological properties of *N. indicum* stem and root remained unexplored. The complete phytochemical profile was mostly unknown as well, even though some bioactive constituents were identified and isolated previously.

Therefore, the present study was conducted to evaluate some of the medicinal properties and related pharmacological potentialities of the major parts of *N. indium* i.e. leaf, stem and root. Initial screening was done by evaluating the *in vitro* antioxidant and free radical scavenging activities. The effect of *N. indicum* on immune system was evaluated by its effect on modulation of humoral immune response and macrophage activities. The effect of *N. indicum* leaf, stem and root extracts on various pro- and anti-inflammatory parameters were evaluated to study the possible anti-inflammatory activity as mentioned in ethnopharmacology. The acclaimed anti-diabetic activity of *N. indicum* was investigated on alloxan induced insulin dependent type 1 diabetes mellitus in murine model. The potent hepatoprotective potentiality was studied on haloalkane induced acute hepatic damage in mouse. In addition, detailed phytochemical analysis were also performed to elucidate the major bioactive species in the plant.

The results indicated that the hydroxyl radical, hydrogen peroxide and hypochlorous acid scavenging activities were in the order leaf>stem>root, whereas the DPPH scavenging activity was greatest in stem, followed by root and leaf. The potentiality to scavenge superoxide radical and inhibit lipid peroxidation were in the order of root>leaf>stem. In case of other antioxidant assays, assorted results were observed such as leaf>root>stem for peroxynitrite and iron chelation activity; root>stem>leaf for reducing power and stem>leaf>root for ABTS and nitric oxide scavenging activity. The extracts demonstrated

superior bioactivities than the respective standards in case of hydroxyl, nitric oxide and hypochlorous acid scavenging assays. The estimated high level of phenolic an flavonoid content, also correlated with the antioxidant and free radical scavenging activities of *N. indicum*.

In case of immunomodulatory activities, the extent of stimulation to murine humoral immunity evaluated by Plaque Forming Cell assay, Immunoglobulin M estimation and Hemagglutination (HA) titre were in the order of leaf>root>stem, where the effect of stem was fairly negligible. Among the three extracts, only leaf extract demonstrated significant ($P<0.001$) activity to modulate total macrophage count, phagocytic capacity as well as the respiratory burst activity. Percentage inhibition of cell adhesion property of macrophage and their myeloperoxidase reduction were in the sequence of leaf>stem>root and leaf>root>stem, respectively. Evaluation of anti-inflammatory activities demonstrated up-regulating IL-2, IFN-γ, IL-10 expression and down-regulating IL-4, TNF-α expression *in vitro*. Dose dependent inhibition of nitric oxide was evident in peritoneal exudate macrophage and splenic lymphocytes where root demonstrated better activity than leaf and stem. The effect of leaf extract was most prominent on inhibition on COX activities. The decrease in prostaglandin E_2 level highly correlated with the inhibition of COX activities. Inhibition of inflammation was visually demonstrated and estimated using paw edema model which resulted in decrease of hind paw edema in an order of leaf>root>stem.

N. indicum demonstrated anti-hyperglycaemic activity by inhibition of *in vitro* α-amylase activity and blood glucose level in the degree of leaf>root>stem. The extent of inhibition of lipid peroxidation, restoration of blood insulin and decrease in HbA1c levels were almost equivalent in stem and root, however, predominant in leaf. Restoration of hepatic glycogen was almost comparable in all three extracts. Significant improvement of catalase and peroxidase levels in liver, kidney and skeletal muscle were also demonstrated by *N. indicum* extracts. Percentage decrease in liver marker enzymes and biochemical parameters were significant along with decrease in triglyceride and cholesterol levels, displaying potent antihyperlipidemic activity. Furthermore, excellent improvement of oral glucose tolerance was observed in leaf, followed by stem and root.

The hepatoprotective evaluation of *N. indicum* extracts demonstrated substantial normalization of lever enzymes and biochemical parameters such as ACP, ALP, AST, GGT, ALT, albumin, globulin, bilirubin, urea, uric acid, LDH, cholesterol etc. both in *in vitro* and *in vivo* models. Normalization of hepatic catalase activity was highest in leaf however, all the

extracts demonstrated improvement of equivalent peroxidase activity. Inhibition of lipid peroxidation activity and protection form CCl_4 mediated direct cellular death was highest in leaf. *In vitro* inhibition of the inflammatory markers viz. TNF-α and nitric oxide were in the order of leaf>stem>root. Visual signs of amelioration of CCl_4 toxified liver were documented through histopathological studies, which demonstrated lowering of hepatocellular necrosis, bile duct proliferation, sinusoidal dilatation, inflammation (leukocyte infiltration), vascular congestion, loss of structure of hepatic nodules, hepatocellular fibrosis, fatty infiltration, vacuolar degeneration and calcification in the extract treated groups.

For phytochemical profiling, *N. indicum* leaf, stem and root were initially screened biochemical for the presence/absence of the major classes of phytochemicals such as Tannin, terpenoid, glycoside, phenolics, flavonoid, steroid, anthraquinone, saponin, alkaloid etc. Quantitative estimation of various phytochemicals were performed which revealed the presence of varying degree of alkaloid, flavonoid, saponin, phenolics, riboflavin, thiamine, ascorbic acid, tannin etc. Various functional groups like alcohol, alkane, acid, amide, amine, aliphatic ketone, carbonyl, nitro group etc. belonging to different phytochemicals were identified using FTIR analysis. Various bioactive phenolic and flavonoid compounds such as gallic acid, 4-hydroxybenzoic acid, vanillic acid, jasmonic acid, p-coumaric acid, syringic acid, ferulic acid, myricetin etc. were identified and quantified in *N. indicum* extracts using reverse phase HPLC studies. Moreover, GC-MS analysis revealed the presence of several bioactive compounds with known pharmacological activities like vaccenic acid, phytol, oleic acid, tocopherol, vanillin, myristic acid, stigmasterol, sitosterol, isoeugenol, apocynin, tryptamine, squalene, lupeol etc.

The present investigation thus, demonstrates potent medicinal and pharmacological activities of *N. indicum* as claimed in the traditional therapeutic systems. The immunopharmacological potentialities of *N. indicum* are attributed to the vast array of bioactive constituents identified in the plant.