6.
CONCLUSION

Conclusion

The present study provided scientific evidence of the therapeutic efficiencies of *N. indicum* which were claimed in the ethnopharmacological domain. The entire work was performed with 70% hydromethanolic extract of *N. indicum* because this extraction process is generally considered one of the best method for extraction of the phenolic and flavonoid compounds, which are known responsible for bioactivities of any herbal medicine. Moreover, in ethnopharmacology, the majority of the medicines are actually tinctures, prepared through similar hydroalcoholic solvent extraction process.

Initial screening of NILE, NISE and NIRE was performed through the evaluation of their anti-oxidant and free radical scavenging activities. At first, the overall antioxidant capacity was confirmed by DPPH (IC_{50}**:** 217.15±18.39, 63.56±1.63 and 166.18±6.84 μg/ml) and TEAC (TEAC value**:** 0.316±0.002, 0.396±0.001 and 0.325±0.003) assays, which revealed satisfactory bioactivities of the extracts. Thereafter, selective free radical scavenging assays were performed against different free radicals to find out whether the extracts could demonstrate inhibitory activity on individual free radicals. NILE, NISE and NIRE in case of OH• (IC_{50}**:** 29.65±0.21, 118.68±1.11 and 208.16±6.70 μg/ml) and NO (IC_{50}**:** 46.56±3.42, 23.56±1.16 and 62.43±4.55 μg/ml) and NILE and NISE in case of HOCL (IC_{50}**:** 124.74±1.91 and 162.25±10.31 μg/ml) scavenging activity demonstrated better efficacy than the respective standards (IC_{50}**:** OH• (mannitol) 571.45±20.12 μg/ml; NO (curcumin) 90.82±4.75 μg/ml; HOCL (ascorbic acid) 235.96±5.75 μg/ml). The results of the free radical scavenging assays were convincing, which led us to evaluate possible *in vitro* lipid peroxidation inhibitory (IC_{50}**:** 113.77±8.89, 199.17±33.51 and 110.03±12.75 μg/ml) and iron chelation (IC_{50}**:** 216.70±9.82, 659.95±48.64 and 698.38±39.00 μg/ml) activities of the extracts. Total phenolic (72.62±.08, 81.54±0.05 and 87.38±0.16 mg/ml GA equivalent per 100 mg extract) and flavonoid (93.06±0.03, 67.4±.06 and 64.08±0.002 mg/ml quercetin equivalent per 100 mg extract) contents were also estimated in parallel to the antioxidant assays as the antioxidant capacity of any herbal formulations rely mostly on the load of both classes of phytocompounds. On later part of this study, the protective role of *N. indicum* through its antioxidative properties were also demonstrated under *in vivo* anti-diabetic and hepatoprotective evaluations.

The assessment of immunomodulatory activities of *N. indicum* was divided into two broad sections i.e. the overall immunomodulatory activities and anti-inflammatory activities. The assays were performed both through *in vitro* and *in vivo* methods. The effect of NILE, NISE and NIRE on the murine humoral response was evaluated through PFC (1.57, 1.01 and 1.50 fold increase), HA titre (agglutination up to 1/320, 1/40 and 1/160 dilutions i.e. 8, 0 and 4 fold

elevation) assay and subsequent estimation of IgM levels (1.76, 1.09 and 1.42 fold increase) in serum samples. Among the three extracts, only NILE and NIRE resulted in significant stimulation of the humoral immune system. The extracts were in parallel investigated for their efficiency to modulate the effector functions of the macrophages, isolated from the murine peritoneal cavity. Elevation in the phagocytic capacity was observed only in case of NILE and NIRE (1.43 and 1.18 fold) however, mild increase in macrophage population (1.69, 1.05 and 1.20 fold) were observed for all the extracts. The extracts further demonstrated modulation of cell adhesion property (21.52±2.06, 10.32±1.32 and 9.92±2.41% inhibition) and phagocytosis related respiratory burst (1.70, 1.32 and 1.31 fold increase) and MPO release (16.00±1.64, 7.17±1.68 and 10.64±0.83% reduction) under *in vitro* condition. Majority of the anti-inflammatory study was performed on *in vitro* murine splenic lymphocytes which were stimulated with optimum dose (5 μg/ml) of Con A. Quantification of different pro-and anti-inflammatory markers demonstrated the efficiency of *N. indicum* extracts to down-regulate TNF-α (225.66±11.67, 349.33±15.63 and 327.02±13.45 pg/ml) and IL-4 (1.37±0.15, 2.67±0.14 and 2.37±0.22 pg/ml) in addition up-regulate IL-2 (39.47±2.70, 18.3±3.71 and 25.49±2.63 pg/ml), IL-10 (4388.00±295.48, 3378.00±357.78 and 4439.33±56.88 pg/ml) and IFN-γ (343.33±11.59, 275.33±20.59 and 220.66±28.14 pg/ml) levels. The release of NO from lymphocytes and macrophages demonstrated inhibitory activity of the extracts. The anti-inflammatory activities were further established through inhibition of COX-1 (0.48, 0.80, 0.84 fold), COX-2 (0.39, 0.55, 0.68 fold) activities and associated PGE_2 release (55.94±3.68, 28.71±1.28 and 36.49±2.82% of inhibition) from the lymphocytes. Moreover, inhibition of hind paw edema (2.72, 1.62 and 1.94 fold at 200 mg/kg) under DTH test, provided visual evidence of the anti-inflammatory activity of *N. indicum*.

Under hepatoprotective evaluation, hepatic injury to murine liver was introduced through oral administration of a potent haloalkane hepatotoxin CCl_4 and subsequently treated with *N. indicum* extracts. Biochemical analysis of the serum samples from the experimental animals revealed decrease of various liver marker enzymes and biochemical parameters such as ALT (0.51, 0.64 and 0.75 fold), AST (0.57, 0.72 and 0.80 fold), ACP (0.66, 0.79 and 0.78 fold), ALP (0.49, 0.78 and 0.77 fold), GGT (0.53, 0.80 and 0.73 fold), bilirubin (0.53, 0.78 and 0.87 fold) etc. in the extract treated groups. Outcomes of the *in vitro* results were also in accordance with the *in vivo* studies performed through liver explant cultures. Hepatic catalyse (0.52 fold) and peroxidase (0.48 fold) activities, which were significantly lowered due to CCl_4 toxicity, were found to be up-regulated (catalase: 1.52, 1.27 and 1.26 fold; peroxidase: 1.21, 1.18 and 1.22 fold) due to extract treatment. This also correlated with the lowering of lipid

peroxidation (0.56, 0.79 and 0.78 fold). Two major hepato-inflammatory markers i.e. NO (0.19, 0.44 and 0.50 fold) and TNF-α (0.14, 0.28 and 0.38 fold) were also found to be lowered in case of extract treated groups. Furthermore, histopathological studies of the liver samples provided evidence for lowering of hepatic inflammation, fatty infiltration, bile duct proliferation, hepatocellular necrosis, calcification, sinusoidal dilatation etc. in the extract treated groups.

The experiments of the anti-diabetic study was designed keeping in mind, that diabetes mellitus is a multi-organ disease, though has a pancreatic origin. Initial screening was done using *in vitro* α-amylase activity, demonstrating inhibitory effect of *N. indicum* extracts (IC_{50}**:** 703.01±56.47, 1402.07±232.15 and 899.22±84.11 μg/ml) on primary breakdown of complex carbohydrates. Experimental diabetes was introduced using alloxan and subsequently diabetic animals were treated with the plant extracts. The treatment resulted in significant lowering of blood glucose (73.79, 55 and 67%) and HbA1c (0.72, 0.84 and 0.86 fold) levels which correlated with the increase of insulin (2.07, 1.34 and 1.38 fold) and hepatic glycogen (1.48, 1.25 and 1.39 fold) levels at 200 mg/kg. Normalization of catalase and peroxidase activities in liver, kidney and skeletal muscle corroborated the findings under hepatoprotective study, along with lowering of MDA level (0.69, 0.83 and 0.81 fold) in both cases. Improvement of diabetic liver damage by lowering of ACP (0.56, 0.74 and 0.61 fold), ALP (0.50, 0.66 and 0.58 fold), AST (0.75, 0.86 and 0.74 fold), ALT (0.55, 0.71 and 0.60 fold) and amelioration of diabetic nephropathy due to lowering of urea (0.49, 0.64 and 0.52 fold), creatinine (0.60, 0.77 and 0.71 fold), uric acid (0.75, 0.86 and 0.76 fold) along with improvement in diabetic hyperlipidemia by normalization of triglyceride (0.72, 0.88 and 0.79 fold) and cholesterol (0.72, 0.76 and 0.72 fold) levels were observed due to the extract treatment at 200 mg/kg. Moreover, the efficacy of *N. indicum* extracts at 200 mg/kg in OGTT revealed increased rate for glucose absorption (0.23, 0.41 and 0.46 fold lowering of blood glucose) in the extract treated animals.

The photochemical composition of leaf, stem and root of *N. indicum* was investigated initially through biochemical spectrophotometric methods, which revealed the presence of alkaloid, saponin, polyphenols, flavonoids and vitamins. NILE, NISE and NIRE were further subjected to FTIR analysis which revealed the major chemical groups (alcohol, amide, alkane, nitro, acid, ketone etc.) in the extracts. Numerous polyphenolic metabolites were identified and quantified (rutin, gallic acid, p-coumaric acid, vanillic acid, myricetin syringic acid etc.) through HPLC analysis. Further bi-fractionation of the extracts using DCM and n-hexane and

subsequent GC-MS analysis revealed the presence of different bioactive phytocompounds (vanillin, phytol, vaccenic acid, vitamin E, stigmasterol, β-sitosterol, squalene etc.).

The major findings of the present study may thus, be summarized as follows:

- *N. indicum* possess potent antioxidant activities as demonstrated by DPPH, TEAC and reducing power activities.
- Free radical scavenging potentialities of *N. indicum* was established through inhibition of the oxygen and nitrogen free radicals, which would further prove beneficial in attenuation of oxidative stress in various diseases.
- *In vivo* immunomodulatory studies revealed stimulation of humoral immunity by *N. indicum*.
- *N. indicum* demonstrated profound influence on macrophage activities.
- Modulation of cytokine levels along with inhibition of COX activities, PGE_2 and NO levels would provide protection in inflammatory conditions.
- Normalization of liver biochemical and enzymatic markers may provide beneficial under chronic hepatic damage.
- Inhibition of NO and TNF-α associated lowering of liver injury scores would prove beneficial in hepatic inflammation.
- Elevated catalase and peroxidase activities along with the inhibition of lipid peroxidation would prove beneficial under hepatic damage and diabetic conditions.
- Lowering of blood glucose and HbA1c levels associated with elevation of insulin level would be beneficial in diabetes.
- Bioactivity of *N. indicum* leaf was found higher than that of stem and root in most cases.
- The various phytocompounds identified through HPLC and GC-MS analysis, are responsible for the potent bioactivities of *N. indicum*.