SUMMARY

❖ The highest level of extractive yield, total phenol content and radical scavenging activity was observed in the methanolic leaf extract of *Indigofera caerulea* compared to other solvents extracts.

❖ Methanol was the most suitable solvent for extraction of phenolic compounds from *I. caerulea*.

❖ The data presented in chapter III suggests methanolic extract of *I. caerulea* could be a potential source of natural antioxidant.

❖ RP-HPLC analysis of methanolic extract of *I. caerulea* leaves (MIL) observed peaks close to standard ferulic acid and quercetin.

❖ Intragastric administration of MIL up to 2000 mg/kg bw did not reveal any toxicity and mortality in acute toxicity studies.

❖ Intraperitoneal administration of CCl₄ induced liver damage and protective efficacy of MIL was evaluated by the level of serum marker enzymes, lipids profile, antioxidant enzymes, histology of liver, level of inflammatory mediator’s TNF-α and IL-1β in serum and liver tissue and immunostaining of NF-κB and TNF-α in liver tissue.

❖ Investigation of the underlying mechanism of hepatoprotective activity revealed that treatment of *I. caerulea* was capable of reducing CCl₄ intoxicated inflammation by an antioxidant based defense mechanism that blocks the activation of NF-κB as well as inhibits the release of proinflammatory cytokine TNF-α and IL-1β.
Chromatographic based isolated compounds were subjected to spectroscopic characterization and their assumed structures were: The probable structure of compound MIL-1 (F10) was identified as long hydrocarbon chain with a carbonyl group and a hydroxyl function. Fraction MIL-2 (F-7) was Ethyl linoleate and its molecular formula: C_{20}H_{36}O_{2} and molecular weight: 308.49 g/mol was also obtained. F-12 was identified as fructose molecule.

The bioactive compounds such as ferulic acid and quercetin determined by RP-HPLC and ethyl linoleate characterized by spectroscopic analysis with standard commercial drug silymarin was also selected for molecular docking along with Molecular dynamics (MD) simulation validation studies.

Out of the four ligand molecules quercetin exhibited highly significant G score: -8.80) in suppressing the activity of TNF-α. H-bond formation with GLN:149, GLN:61, TYR:151, and SER:60 was observed and the binding energy of ligand with target protein was found to be -54.18 kcal/mol. Silymarin showed G Score of -5.45, and binding energy of 34.40 kcal/mol with target protein 2AZ5.

Therefore, molecular docking interaction studies revealed that the affinity of quercetin with TNF-α in the active site residues were highly significant than COX-2-quercetin binding interaction.
Observations from the docking interaction of TNF-α with three active ligands revealed that amino acid residue SER: 60 were involved in hydrogen bond formation. In addition, also revealed that quercetin blocked the active site residues of GLN: 149 (2), GLN: 61, TYR: 151(2) and SER: 60 of six H-bonds, Pi-Pi stacking and water mediated interaction.

Molecular dynamics (MD) simulation was performed to evaluate the stability of TNF-α-quercetin complex in duration of 20,000 picoseconds. RMSD, RMSF and energy plot of protein-ligand complex further hydrogen bond interaction with active site residues were analyzed.

The mechanism of action of *I. caerulea* in inflammatory liver ailments was demonstrated in both *in vivo* and *in silico* studies.

The study altogether strongly explores the phytochemical profile of *Indigofera caerulea* and justifies that the use of this plant in traditional medicine as cure for liver diseases which could be of great importance for the treatment of oxidative damage and free radical related diseases.