CHAPTER 6

Discussion of Results

The aim of the study was Phytochemical Investigation and Pharmacological Screening of some Indian medicinal plants. A *Leucas aspera* and *Cassia tora* roots were subjected to phyotchemical and various pharmacological screening and results were reported in the previous chapter. In this chapter detail discussion on results is carried out.

6.1 Pharmacognostic Study

Evaluation of anatomical section of *Leucas aspera* and *Cassia tora* roots were carried out to determine the macroscopic and microscopic charters.

6.1.1 Organoleptic Evaluation

In organoleptic evaluation, characters like colour, odour, taste, size, shape are evaluated which is useful to confirm purity, quality and for identification. The observation of organoleptic evaluation of *Leucas aspera* and *Cassia tora* roots is presented in previous chapter which is useful for detection of adulterants, confirmation of quality.

6.1.2 Microscopic evaluation

Microscopic evaluation allows complete examination of a drug and it can be used for identification with the help of histological characters. The observation of microscopic evaluation of *Leucas aspera* and *Cassia tora* roots confirm the identity of roots with the help of histological characters of roots.
6.2 Phytochemical studies

Preliminary qualitative phytochemical studies of MELA revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phytosterols/terpenes, proteins, tannins, saponins and lipids. PELA revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, proteins and tannins. MECT show improved response for alkaloids, glycosides, carbohydrates, flavonoids, phytosterols/terpenes, proteins, tannins and saponins. PECT revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phytosterols/terpenes, proteins and tannins.

6.3 Characterisation of Isolated Compounds (LA-1, CT-1)

The isolated compounds were characterized using physical properties, chemical tests, $R_f$ value and spectral data especially IR, Mass and NMR. The results of characterisation of Isolated Compounds (LA-1, CT-1) are presented in previous chapter. The result confirms that the isolated compound from methanolic extract of *Leucas aspera* (LA-1) is identical with reported Rutin flavonoid and the isolated compound from methanolic extract of *Cassia tora* (CT-1) is identical with reported Flemingin D flavonoid.

6.4 Pharmacological screening

6.4.1 Anti-microbial Activity

Infectious diseases are the most significant cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which compact susceptibility to
antibiotics are continuously increasing. Such rise has been recognized to indiscriminate use of wide spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immune deficiency virus (HIV) infections. This situation provided the momentum to the search for new antimicrobial substances from different source like medicinal plants. The use of plant extracts with known antimicrobial properties can be of vast significance for therapeutic treatment.201

All the extracts at a concentration of 50µg and 100µg per each cup exhibited antibacterial and antifungal activities against one or the other organisms in dose dependent manner. The plants extracts have exhibited significant activity on the tested fungi. *Leucas aspera* and *Cassia tora* methanolic extract had produced good antibacterial activity against gram +ve and gram –ve bacteria and fungal strains when compared to petroleum ether extract. All the tested extracts have shown significant activity with that of the standard drugs.

The above results plainly demonstrated that the extracts had significant and considerable anti-microbial activity against variety of pathogens and this may also be responsible for producing significant anti-inflammatory activity as discussed above and the results obtained in producing significant anti-inflammatory activity supports the folkloric claims for the plants.

All the pure compounds (LA-1 and CT-1) were tested for antimicrobial activity at a concentration of 25µg/cup. All the
compounds have shown significant activity with that of chloramphenicol (10µg/cup), where as these compounds had also shown moderate antifungal activity with that of nystatin (10µg/cup). The compounds were tested for minimum inhibitory concentration and all the tested compounds have shown significant activity with that of the standard drugs.

6.4.2 Anti-oxidant Activity

Antioxidant properties, particularly radical scavenging, are very significant due to the damaging role of free radicals in food and in biological systems. Extreme formation of free radicals encourages the oxidation of lipids in foods and declines food quality and consumer acceptance. So in our work we included methods for rapid evaluation of radical scavenging abilities by using DPPH and superoxide anion radical method can be helpful in lead-identification of novel antioxidants in In-vitro screening of compounds.

The MELA, PELA, MECT and PECT shown significant dose dependant DPPH assay, superoxide anion scavenging activities. There was in vitro antioxidant activity also observed which is may be due to antioxidant principles there in MELA, PELA, MECT and PECT. However, MELA, PELA, MECT and PECT were found to possess more potent in in-vitro antioxidant activity. Therefore, MELA, PELA, MECT and PECT were selected for screening In-vivo antioxidant and hepatoprotective properties.
6.4.3 Acute Toxicity Studies (LD$_{50}$)

It is the standard practice to determine the LD$_{50}$ value, it is significant to study an acute toxicity studies by employing several doses including reasonably high doses. Acute toxicity studies were conducted using a dose of 2000mg/kg, p.o. with MELA, PELA, MECT and PECT in female albino rats according to the OECD guidelines. Even at this high dose MELA, PELA, MECT and PECT did not exhibit any sign or symptoms of toxicity and mortality. Hence low dose (200mg/kg, p.o.) medium dose (400 mg/kg, p.o.) and high dose of MELA, PELA, MECT and PECT (600mg/kg, p.o.) were selected for further studies in animals.

6.4.4 Anti-inflammatory Activity

It is well recognized that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it directly resembles human arthritis.$^{203}$ Injection of formalin subcutaneously into hind paw of rats’ produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response. Thus formalin-induced arthritis is a model used for the assessment of an agent with probable anti-proliferative activity. This experiment is related with the proliferative phase of inflammation. Results with MELA, PELA, MECT and PECT (600mg/kg, p.o.) are showed quite compatible with those of the standard drug diclofenac sodium. Therefore, the drug appears to be effective against formalin-induced arthritis.
The mean response of standard 85.02% was inhibition of increase in paw thickness after 6 days respectively. In this model at 200, 400 and 600 mg/kg dose level of MELA, PELA, MECT and PECT extracts showed significantly inhibition of increase in paw thickness after 6 days.

### 6.4.5 Analgesic Activity

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids. The acetic acid induced writhing response is a sensitive method to evaluate peripherally acting analgesics. The response is considered to be mediated by peritoneal mast cells acid sensing ion channels and the prostaglandin pathway.

The mean response of control and standard was 42.83 ± 2.04 and 17.67 ± 1.11 respectively. The respective test compounds MELA and PELA in its 200, 400 and 600 mg/kg dose, showed mean writhing responses as 28.50 ± 1.47, 24.83 ± 1.44, 20.33 ± 1.25 and 31.50 ± 1.89, 27.33 ± 1.45, 23.50 ± 1.33. In terms of percentage inhibition of writhing by diclofenac sodium was 58.74% while with the test compound it was 33.46%, 42.03%, 52.53% and 26.45%, 36.19%, 45.13% respectively. The respective test compounds MECT and PECT in its 200, 400 and 600 mg/kg dose, showed mean writhing responses as 27.50 ± 1.54, 23.67 ± 1.28, 19.17 ± 1.07 and 29.33 ± 1.52, 26.17 ± 1.35, 22.50 ± 1.17. In terms of percentage inhibition of writhing by
diclofenac sodium was 58.74% while with the test compound it was 35.79%, 44.73%, 55.24% and 31.51%, 38.89%, 47.46% respectively.

6.4.6 Hepatoprotective Activity

In case of toxic liver, wet liver weight and wet liver volumes are improved. Toxicants induced hepatotoxicity produce fatty changes and also it is observed that there is a drop in serum lipids in another series of experiments. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume.\textsuperscript{207} It is reported that liver mass and volume are main parameters in ascertaining the hepatoprotective effect of the drugs.

Treatment with MELA, PELA, MECT and PECT significantly reduced the wet liver weight and wet liver volumes of animals and hence it has statistically considerable hepatoprotective activity.

The hepatoprotective activity was assessed by measuring the biochemical markers like SGPT, SGOT, total protein, bilirubin (total and direct triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), VLDL-Cholesterol (VLDL-C) and ALP in all the four hepatotoxic models (paracetamol and thioacetamide induced hepatotoxicity).

In case of paracetamol induced hepatotoxicity model, paracetamol 2g/kg b.w. block injection caused hepatotoxicity as indicated in the elevation of biochemical markers like SGPT, SGOT, total protein, bilirubin (total and direct triglycerides (TG), total
cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), VLDL-Cholesterol (VLDL-C) and ALP. In addition PCM administration has disrupted the liver architecture.

Treatment with MELA, PELA, MECT and PECT reversed the high levels of all the biochemical markers to the close to normal levels in this model also. Paracetamol was found to increase tissue GSH and decrease the lipid peroxidation. The histopathological parameters of PCM induced hepatotoxicity were normalized by the treatment MELA, PELA, MECT and PECT. These observations indicate that the MELA, PELA, MECT and PECT possess hepatoprotective activity against PCM induced hepatotoxicity.

There are reports that paracetamol induced hepatotoxicity is due to activation of PCM to a toxic electrophile N-acetyl p-benzoquinine amine (NAPQI) by a number of iso-enzyme of CYP-450 namely CYP 2E1, CYP1A2, CYP2A6, CYP3A4, CYP2D6. Generally PCM is eliminated from the body as sulphate and glucuronide to the extents of 95% before oxidation. However, 5% of PCM is undergoing bioactivation by above mentioned isoenzymes of CYP to an extremely reactive NAPQI.\textsuperscript{208}

After the over dosage of paracetamol, routs of sulphation and glucuronidation saturates. As a consequence oxidation of PCM, CYP-450 iso enzymes are improved leading to the enlarged concentration of NAPQI. This NAPQI further loses one electron resulting into the harful radical. This radical interrelate covalently with membrane
macromolecules and harm the membrane. However this reaction is countered by inbuilt tissue antioxidants systems like GSH. Too much concentration of NAPQI radical overpowers the inbuilt protecting mechanisms thereby damages the cell membrane. This results into the leakage of biochemical markers into the serum. It is apparent from the results that treatment with MELA, PELA, MECT and PECT prevents the formation of one electron reduced metabolite of NAPQI (which mediates cytotoxic effects of NAPQI) due to its antioxidant property i.e. hydroxyl and superoxide anion scavenging activities. Further, this may be helpful in retaining the membrane GSH contents, reduced lipid peroxidation and prevents the tissue damage.\textsuperscript{209-210}

Subcutaneous thioacetamide (TAA) 100 mg/kg b.w. for one day has induced hepatotoxicity as indicated by the rise in the biochemical markers SGPT, SGOT, total protein, bilirubin (total and direct triglycerides (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), VLDL-Cholesterol (VLDL-C) and ALP. In addition TAA was found to enlarge tissue GSH and decrease the lipid peroxidation. Treatment with MELA, PELA, MECT and PECT (200, 400 and 600mg/kg.p.o.) significantly reduced dose dependently all the biochemical markers enzymes and increased the tissue GSH levels. The MELA, PELA, MECT and PECT have improved the liver architecture as like to other models of hepatotoxicity. Hence, it can be informed that the test extract possess hepatoprotective activity in this model also.
TAA is metabolised by the liver CYP4502E\textsubscript{1} enzymes reducing sulfones and sulfoxide derivatives which are in fact accountable for inactivation of enzymes and proteins.\textsuperscript{211} In addition there is a information that thioacetamide is metabolised by CYP-450 to thioacetamide 5-oxide, which liable for the change in cell permeability results in enlarged intracellular concentration in Ca\textsuperscript{++} nuclear volume, enlargement of nucleoli and inhibits the mitochondrial activity and consequently leads to cellular death. Further there is information that TAA better the lipid peroxidation and depleted the tissue GSH.\textsuperscript{212}

In the present study TAA has reduced the tissue GSH and increased the lipid peroxidation. Treatment with MELA, PELA, MECT and PECT has reversed the TAA induced elevated lipid peroxidation and decreased tissue GSH.

It seems the protective activities of the plant may be by strengthening the inbuilt antioxidant systems by of the antioxidant principles that are present in the plant. However further studies are required to finally set up the mechanism of hepatoprotective effect of the plants in this model.

Although different enzymatic and non-enzymatic systems have been developed by cell to manage with the ROS and other free radicals, when a condition of oxidative stress establishes, the defence capacities against ROS becomes insufficient.\textsuperscript{213} ROS also affects the antioxidant defence mechanisms, reduces the intracellular concentration of GSH. It has also known to decrease the detoxification
system produced by GST. Increasing facts indicates that oxidative stress leads to liver injury, cirrhosis development and carcinogens. In our studies, it reveals that MELA, PELA, MECT and PECT could restore the activity of both these antioxidant enzymes.

The observed *in-vitro, in-vivo* hepatoprotective activity may be due to presence of phenols, alkoloids, tannins, flavanoids, trierpenoids and sterols present in MELA, PELA, MECT and PECT.