

Chapter 8

Summary and Conclusion

There is a strong need to adopt modern analytical method for quality control of plant material and herbal remedies. It is important to understand that a plant extract consists of established classes of chemical compounds. These include the primary metabolites, secondary metabolites and inorganic salts and metals. Primary metabolites are compounds like carbohydrates, proteins, lipids etc that are essential for the plant physiology. Secondary metabolites are include alkaloids, flavonoids coumarins, terpenoids, anthocyanins, etc, and we can utilize these secondary metabolites for the identification of plant material as our knowledge of chemistry has advanced sufficiently and through sophisticated analytical techniques we can measure these compounds qualitatively and quantitatively.

A biomarker on the other hand is a group of chemical compounds which are in addition to being unique for that plant material also correlate with biological efficacy. Fingerprinting in essence which means establishing a characteristic chemical pattern for the plant material or its cut or fraction or extract.

Application of fingerprinting technique using modern analytical techniques like HPTLC and HPLC can give high level of quality control of the plant. Chromatographic fingerprinting should be done with emphasis on identification and quantification of specific chemical marker compound representative of specific herb. But nevertheless in its, own limited sense the technique of chromatographic fingerprinting and specific marker compounds are very important tools available to modern analyst as an aid for the total quality control of a medicinal herb.

The present study demonstrated the qualitative and quantitative estimations of phyto constituents present in the *Lagerstroemia speciosa* and *Mangifera indica*. Preliminary chemical tests were conducted to identify the secondary metabolites present in the plant materials. The fingerprinting techniques helped to identify the polyphenols and tannins varieties present in the plant parts. Two compounds were isolated from *M. indica* and identified by various spectral methods like IR, Mass, nmr, C¹³ nmr, 2-D nmr etc. The GC-MS of flower oil showed the percentage of phytoconstituents present in it.

Interest in the search for natural antioxidants has increased over the past few years as the reactive oxygen species (ROS) production and oxidative stress have been shown to play vital role in a number of disorders (Finkel and Holbrook, 2000). Successive extracts

of both the plants were subjected to varieties of *in vitro* antioxidant assays. Based on *invitro* antioxidant studies we have selected two extracts and the total extracts of these were taken and subjected to further experiments. The results showed that among all extracts, total ethanol extract required lesser concentration to inhibit the superoxide, hydroxyl radical and lipid peroxidation. The substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom. Our antioxidant results are compared with curcumin; an isolated plant product and ascorbic acid for some models. FRAP assay reflects total antioxidant power involving the single electron transfer reaction whereas DPPH is based on free radical scavenging activity (Ou *et al.*, 2002). The reducing power is widely used in evaluating the antioxidant activity of polyphenols (Soong and Barlow, 2004). The decrease in absorbance of DPPH caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a colour change from purple to yellow. The lower value of EC₅₀ indicates a higher antioxidant capacity.

The second part of our thesis focused on the pharmacological activity of selected plant extracts and its isolated fractions. The study was performed on both *in vitro* and *in vivo* anti-inflammatory activity of the ethyl acetate and ethanol extracts of *L. speciosa* and *M. indica*. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme, which is responsible for the conversion of arachidonic acid to prostaglandin (PGH). The non-steroidal drugs act either by inhibiting the lysosomal enzymes or by stabilizing lysosomal membranes. Since HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity-induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of drugs. In our study, both the extracts showed higher protection at a concentration of 100 µg/ml.

Carrageenan induced acute inflammation and formalin induced chronic paw edema in animals are the most suitable test procedures to screen anti-inflammatory agents. The formalin induced paw edema is one of the most suitable procedures, as it closely resembled human arthritis (Greenwald, 1991). The effect of our preparations becomes significant within 3 hours, during the phagocytic phase of carrageenan-induced inflammation and it was comparable with earlier reports (Vinegar *et al.*, 1983).

The results suggest the usefulness of *L. speciosa* and *M. indica* in the treatment of inflammation-associated diseases like arthritis. The total ethanol extracts of both the plants has a significant ($p < 0.001$) anti-inflammatory effect against carrageenan and formalin induced paw edema in a dose dependent manner. In conclusion, the higher free radical scavenging ethanol extract showed greater anti-inflammatory activity.

Nitric oxide has been involved in the mechanism of nociception (Duarte *et al.*, 1990) as either a pronociceptive (at low concentrations) or as an antinociceptive (at higher concentrations) (Ferreira *et al.*, 1991). Our extracts also showed the inhibitory action on NO production by *in vitro*. It is concluded that the ethyl acetate and alcohol extracts showed significant anti-nociceptive effects, which has central and peripheral anti-nociceptive activity, which may be partially mediated by opioid receptors as these receptors play an important role in pain sensation. Presence of flavonoids was reported in *M. indica* and these flavonoids are known to inhibit prostaglandin synthetase (Ramaswamy, *et al.*, 1985).

Diuretics are drugs capable of increasing levels of urine, so they are useful in the treatment of diseases related with the retention of fluids. Many herbal diuretics exert their action by directly affecting electrolyte balance of minerals. In the normal rats, diuresis began with low volumes of urine excreted until completing 24 hours. The level of excreted Na^+ and K^+ in urine was equally low. The beginning of urine for the EtOH extract of the *L. speciosa* and *M. indica* were also at 60 minutes post administration, but the volume was smaller than furosemide. The ethyl acetate fraction did not increase urinary excretion when compared with ethanol extracts. All extracts did not increase the Na^+ concentration when compared with the positive controls.

The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect (Bose *et al.*, 2006). The chloride ion excretion was not elevated significantly by the lower dose and the results are indicating that the extract is a potent natriuretic. Further studies like isolation and characterization of diuretic principle from the plant is needed to understand and confirm the exact mechanism of action.

Earlier studies reported that free radical mediated oxidative damage may occur in cisplatin nephrotoxicity as a consequence of decreased renal GSH levels and antioxidant enzyme activity with enhanced lipid peroxidation (Somani *et al.*, 1995). The results of the present study showed the renal SOD, CAT, GPx activities and reduced GSH level were significantly increased by EtOH extract of *L. speciosa* than the *M. indica* in the cisplatin treated group when compared to the control. When compared with the two plants, the EtOAc extract of *L. speciosa* significantly prevents the toxicity of cisplatin where as in EtOH extract treatment study, *M. indica* showed maximum activity and it was more significant than the EtOAc extract of *L. speciosa*.

The recent experimental findings have suggested that the free radicals and reactive oxygen species are involved in gentamicin induced oxidative stress because of depletion of GSH concentration and decreased antioxidant enzyme activity in the kidneys. The results of the nephroprotective investigation indicate that ethanol extract of *M. indica* rendered significant protection against gentamicin induced nephrotoxicity in a dose dependent manner. These observations also support the evidence that part of the mechanism of nephrotoxicity in gentamicin treated animals is related to depletion of antioxidant system (Nitha and Janardhanan, 2008). Treatment of *M. indica* could significantly prevent the depletion of these renal antioxidant systems. CCl₄ induced hepatic damage is due to the cytochrome P-450 enzyme system catalysed hepatic conversion into highly reactive trichloromethyl radical, which upon reaction with oxygen radical gives trichloromethyl peroxide radical. This radical forms covalent bond with sulfhydryl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction (Ahmad *et al.*, 2000).

Pretreatment of rats with *L. speciosa* and *M. indica* extract dose dependently inhibited the increased level of all hepatic marker enzymes in serum, indicating the liver protective activity. Stabilization of serum total bilirubin and total protein levels by the pre administration of *M. indica* extract to rats, dose dependently for 7 days prior to the CCl₄ administration was a clear indication of the improvement of functional status of the hepatic cells and the result was supported by earlier studies (Brent and Rumack, 1993).

Alteration in the activity of alkaline phosphatase may be due to the disturbance in the secretory activity or in the transport of metabolites or may be due to altered synthesis of certain enzymes after CCl₄ administration (Brattin et al., 1985). The extract demonstrated potent superoxide and other free radical scavenging property. Therefore it may be inferred that antioxidant property of the extract may prevent the formation of trichloro methyl peroxide radical. Thereby inhibit the lipid peroxidation and offer hepatoprotection against CCl₄ challenge.

The beneficial health effects from the consumption of diet rich in fruits and vegetables are mainly due to the presence of antioxidants such as polyphenols, carotenoids and anthocyanins. Due to their susceptibility to oxidation, erythrocytes have been used as a cellular model to investigate oxidative damage in biomembranes. Erythrocytes are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the O₂ transport associated with redox active hemoglobin molecules, which are potent promoters of reactive O₂ species. Moreover, the free hemoglobin exposed to H₂O₂ causes heme degradation with the release of iron ions which are catalytically active in initiating free radicals and lipid peroxidation (Puppo and Halliwell, 1988).

Ulcers are thought to be due to the imbalances in gastric offensive and defensive mucosal factors. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda *et al.*, 1993). The ethanol model has been used widely to produce gastric mucosal damage (Anadan *et al.*, 1999). It is well established that gastric acid secretion plays a role in gastric ulcer. Moreover, many anti-ulcerogenic drugs act by reducing the acid secretion.

The gastroprotective effect of *M. indica* might be due to the decrease in gastric motility and increased the gastric emptying time. It is reported that the changes in gastric motility may play a role in the development and prevention of experimental gastric lesions (Mersereau and Hinchey, 1982). Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants on rugal crest. Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to 'mucosal compression' at the site of the greatest mechanical stress

i.e. at the crests of mucosal folds leading to necrosis and ulceration (Mersereau and Hinchey, 1982).

In this study, ranitidine was used as reference drug to delineate in part the mechanism(s), accordance with previous report (Sakai *et al.*, 1989). Polyphenols and tannins prevented ethanol induced gastric damage and increased the mucus significantly. This may be explained with a correlation to strengthen the defense factors of gastric mucosa. Tannins are a group of compounds belonging to the phenolics class of secondary metabolites in plants.

Stress has also been found to decrease the quality and amount of mucus adhering to the gastric mucosa, and it has been suggested that in conditions of emotional tensions, there is only a greater destruction of mucus and decreases the synthesis of its components, but also a quality change affecting the translation, acylation and glycosylation of the ribosomal peptides. These observations reinforce the conclusion that stomach wall mucus is likely to play an important role in stress induced glandular lesions. When exposed to prolonged stress, rats develop gastric ulceration, enhanced colon motility with depletion of its mucin content and signs of physiological and behavioral arousal

Doxorubicin is one of the most active cytotoxic agents in current use. It has proven efficacy in a variety of human malignancies (Fadillioglu and Erdogan, 2003). Free radical generation and lipid peroxidation have been suggested to be responsible for doxorubicin-induced cardiac toxicity (Xu *et al.*, 2001). These oxygen-derived radicals cause severe damage of plasma membranes and interfere with cytoskeletal assembly (Powell and McCay, 1995). Tissues with less developed antioxidant defense reserve such as the heart are highly susceptible to injury by anthracycline-induced oxygen radicals (Olson and Mushlin, 1990). Anti-radical effects has warranted the attention to address whether or not this tannins and polyphenols would ameliorate doxorubicin-induced cardiac toxicity following challenging rats with a cumulative dose of doxorubicin in the current study.

Doxorubicin challenge markedly increased the activities of serum LDH and CPK. Actually, these enzymes are considered important markers of early and late cardiac injury especially during clinical follow-up of doxorubicin therapy (Fadillioglu and Erdogan,

2003). It has been shown that a marked decrease in GSH pool occurs in many tissues after acute and chronic DOX toxicities (Mohamed *et al.*, 2000). The treatment with polyphenols and tannin caused a significant restoration of the anti oxidant enzymes such as CAT, SOD, GPx and G6PDH, where activities of these enzymes were increased in the heart tissues of DOX treated group as compared to normal group. CAT hydrolyses H₂O₂, which has been found to be superior in improving cardiac function to superoxide dismutase (SOD) suggesting that H₂O₂ has a significant role in DOX induced toxicity (Xu *et al.*, 2001). Enzymatic defenses were characterized by enhanced cardiac CAT activity in the DOX administered rats.

DOX causes tissues injury in the kidney and this damage was demonstrated by the biochemical evaluation performed in the present study. DOX toxicity is attributed to its pro-oxidant action. Our study demonstrated that DOX induced lipid peroxidation in kidney tissue samples. ROS attack polyunsaturated fatty acids within membrane lipids as well as proteins and genetic materials. Antioxidant capacity have been important biochemical components to detect tissue damage after DOX toxicity in the kidney tissue. The present data indicate that DOX-induced kidney damage by a possible oxidative injury.

In the present study, the biochemical changes support the histopathological changes, where DOX in chronic administration has produced its characteristic morphological changes in the myocardium. These changes observed in DOX-treated rats were similar to those previously reported (Geeta *et al.*, 1990). The increase in the activities of all these enzymes in the heart tissue might be owing to a compensatory mechanism and an effort made by the myocardium to detoxify the oxygen radicals. Poly phenols and tannins restored the elevated levels of the above enzymes towards normal indicating the beneficial antioxidant potential of these secondary metabolites towards cardiotoxicity (Ayaz *et al.*, 2005).

In conclusion, administration of tannins and polyphenols in ethanol and cold stress induced ulcer models and doxorubicin challenge to male Wistar albino rats ameliorated all the biochemical parameters altered by the cytotoxic agent. Apart from the regulatory role of these compounds on cardiac NO production observed in the current

work, the ulcerprotective, cardioprotective and nephroprotective effects of the tannins and polyphenols could possibly reside for the most part on its anti-radical effects.

The study was extended to find out the tannins and polyphenols on hyperglycaemic and antioxidative properties in STZ-induced diabetic rats. Diabetic complications have been linked to the increased production of free radicals in the tissues. Studies have showed an increase in hepatic and renal TBARS concentration in STZ-induced diabetic rats when compared with the normal rats. The reduction was found in hepatic SOD and CAT level in STZ-induced diabetic rats when compared with normal rats. The level of GSH, the primary endogeneous antioxidant responsible for protecting tissues from oxidative stress was measured and the GSH level in the liver and kidney of diabetic group. After treatment with tannins and polyphenols suggests, some of the components present could up regulate the activity of glutathione.

In our study, concentrations of lipid peroxides were increased in liver and kidneys of diabetic rats, indicating an increase in the generation of free radicals or the concentration of TBARS in the liver and kidney of diabetic animals. The inhibition of these enzymes may affect mitochondrial substrate oxidation resulting in reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of energy production (Savitha, *et al.*, 2006).

Kidney is a major organ involved in diabetic complications. There is difference in kidney mass between STZ diabetic and non-diabetic rats. The increased kidney size accompanies elevation in glomerular filtration rate because of increased glomerular volume, capillary surface area and the elevated creatinine concentration (Yamada *et al.*, 1992). It has been found that GSH levels in diabetic rat kidney were significantly reduced. Our treatments reverse the GSH level when compared with diabetic group. Disturbances in fatty acid metabolism, i.e., elevation of free fatty acids (FFAs), are regarded as one of the major determinants in the pathogenesis of insulin resistance, (Boden *et al.*, 2005) which is the characteristic feature of type 2 diabetes mellitus and is frequently associated with obesity (Li and Yang., 2004; Ping Han *et al.*, 2008)

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in STZ induced diabetic rats (Chakrabarthy *et al.*, 2003). Hyperlipidemia may play a role in the pathogenesis of diabetic complications through enhanced generation of oxygen-

derived free radicals. The concentrations of lipids, such as cholesterol, triglycerides, LDL-C and HDL-C, were significantly higher in diabetic rats than in the control group. Our experiment supports the earlier findings and reduced the LDL and increased the HDL cholesterol by tannins and polyphenols treated groups.

Antioxidant and anti-lipidperoxidative properties of tannins and polyphenols and upregulation of endogenous antioxidant enzymes protected LDL-C from oxidative modifications. These factors prevented the fat accumulation in aortic tissues and subsequent atherogenesis. Present study clearly elucidates tannins and polyphenols as efficient lipid lowering agent.

In this study atherosclerosis in aorta of high fat fed rats were effectively prevented by tannins and polyphenols. The protective effect of these compounds could be possible due to their hypolipidemic property. Significant reduction of lipid levels in blood, liver, aortic tissues clearly indicates their strong hypocholesterolemic and hypotriglyceridemic properties. HFD feeding for 120 days has brought heavy accumulation of fats in liver and which induced fatty changes. High level of cholesterol and triglyceride had been found in control HFD treated group. This clearly indicates the poor mobilization of fats in liver. But tannins and polyphenols treated groups have showed the clearance of lipid and restoration of normal architecture in liver tissues. Prolonged hypercholesterolemia has brought microvesicular fatty changes in hepatic cells. Based on the percentage active constituents, reduction of fatty changes in liver cells, tissue cholesterol and triglyceride content in tannins and polyphenols treated group further substantiate its hypolipidemic effect. It is reported that not only functional phenolic compounds, but also other potent components(s) such as fiber, that are responsible for lipid-lowering action (Park *et al.*, 2002). The best predictor of diabetic retinopathy is the duration of disease. The use of antioxidant therapy may have important implications in preventing diabetes-induced complications.

In the present study, we measured glucose uptake by using an *in vitro* incubated muscle preparations and found that glucose uptake rate is higher in GC-muscle in the presence of insulin incubation. Prolonged exposure of rat skeletal muscle to a moderate concentration of insulin causes a progressive increase in transport of glucose over at least 1 h. The activation of glucose transport is reached maximum at 30 minutes in the

presence of 50 microunits/mL of insulin. Administration of tannins and polyphenols increased the glucose uptake and it may be due to the stimulation of GLUT-4 protein content of the muscle. Study has to be extended to find out the mechanism.

Isolation and structural elucidation of *M. indica* plants extract showed the presence of methyl galate and 1,4 benzene dimethyl ester. Our phytochemical investigations provided the presence of xenobiotics and the variations in the content in different extracts may be the reason for the variation in action in anti-inflammation, nephroprotection and hepatoprotection action. In conclusion, administration of *L. speciosa* and *M. indica* and its crude isolated fractions from the plants like tannins and polyphenols reduced the chemical induced toxicity in gastrointestinal tract, heart muscle etc. The ulcerprotective, cardioprotective and nephroprotective effects of the tannins and polyphenols could possibly reside for the most part on its anti-radical effects.

Hypoglycemic, Hypolipidemic and lipid clearing active principles of these plants can be beneficial to mankind. Along with strong hypolipidemic effect tannins and polyphenols inhibited the atherogenesis. Data is strongly suggestive of anti atherosclerotic and hypolipidemic properties of the phytoconstituents of selected plants.