CHAPTER IV
SUMMARY AND CONCLUSIONS

Traditional medicaments play an important role in our day to day life inspite of the overwhelming influence of modern medicine in the treatment of various adverse conditions like hepatic disorders, viral infections, AIDS, rheumatic diseases, etc. Although, there are about 700 plant products used in various polyherbal formulations, only a few could retain a place in modern medicine due to lack of accurate methods for standardisation and evaluation of their therapeutic efficacy. Therefore, in the present studies various plant drugs such as rhizomes of C. orchioides, roots of I. racemosa, whole plants of F. indica, S. acuta, S. cordifolia, S. rhombifolia and fruits, leaves, roots and stem bark of M. pterygosperma were selected based on their utility in traditional systems of medicine and literature. These studies were focussed to evaluate selected drugs for their efficacy against artificially induced inflammation and liver damage.

The selected plant drugs were identified by comparing their diagnostic features with those of standard herbarium specimens preserved in CDRI, Lucknow and the Botany Department, M.S.University, Baroda. Their identity was further confirmed by pharmacognostic studies including proximate analysis and inorganic metal ion content using atomic absorption spectrophotometry. All these plant drugs were found to contain high amounts of sodium, potassium and calcium while lead and nickel were found to be absent. Also

zinc was found to be absent in C. orchioides rhizomes and S. acuta roots, cobalt in I. racemosa roots and copper in S. acuta aerial parts.

The plant drugs were also subjected to preliminary phytochemical screening which indicated comparatively high amounts of semipolar and polar extractive values with positive tests for alkaloids, carbohydrates and glycosides, phytosterols, phenolic compounds and tannins. In addition to these chemical moieties, the roots of I. racemosa were also found to contain volatile oil. The phytoprofiles devised could in turn be utilized as markers in ensuring the quality of the crude drugs and can also provide a base for preparation of selective extracts of these drugs for biological studies.

These extracts, as well as powdered drugs and one marketed preparation containing the drug, were then subjected for preliminary biological studies. These studies were aimed for identification of biologically active extracts and also comparing the drug with that of one of the available marketed preparation prescribed for the purpose. The biological studies, to start with, were first undertaken on all the drugs in powder form, their extracts and one of the marketed preparation for acute toxicity determination.All the test samples and majority of extracts when administered in graded dose range were found practically non-toxic except the

petroleum ether extract of I. racemosa roots, which gave LD_{50} value 1.5 g/kg,p.o. in rats, whereas, others were safe till 10 g/kg,p.o. These studies provided dosage regimen for individual test samples such as 500 mg/kg, p.o. in case of powdered drugs, 100 mg/kg, p.o. in case of extracts and the marketed preparations, 1 ml/kg, p.o. in case of liquid formulations and 100 mg/kg,p.o. in case of solid formulations. All these drug samples were administered in above dosage regimen while screening the claimed biological activities like anti-inflammatory and hepatoprotective activities.

All the test samples were studied for their activities first on preliminary basis against artificially induced inflammation. These preliminary studies were aimed so as to select from amongst the above test samples, effective one which were then subjected to further detailed investigations. These studies showed that out of all the drug samples tested, the petroleum ether extract of the whole plant of F. indica possesses maximum significant ($P < 0.01$) activity (90.7%) followed by the aqueous extract of fruits of M. pterygosperma (80.9%), total aqueous extract of roots of I. racemosa (72.1%), methanolic extract of rhizomes of C. orchioides (97.6%) and powdered aerial parts of S. acuta within two hours of carrageenan induced artificial paw oedema.

Since the selected drugs for present study, form items

of importance in alternative systems of medicine against liver disorders, an approach for screening their hepatoprotective activity was designed using reported techniques for the purpose. Before directly studying the activity on intoxicated liver, an assessment of the possible hepatotoxicity, if any, due to these drugs themselves, was also planned. All the powdered drugs, extracts and one representative formulation were, therefore, subjected to study their effects on normal liver functions by assessing changes caused on serum and urinary biochemical parameters at the selected dosage regimens. From these studies, it was revealed that all the samples tested were practically safe as no significant changes indicating toxicity were noticed.

All the above drug samples were then subjected to assessment of hepatoprotective activity against most common chemical induced intoxication with CCl_4 as model toxicant. The powdered drug, various extracts and one representative marketed preparation of individual drug were administered in the selected dosage regimen to normal rats in different groups followed by administration of CCl_4. The protective activity was assessed in terms of changes in the levels of serum biochemical parameters and comparing them with that of normal group. These studies showed that the aqueous extract of roots of *M. pterygosperma* possesses maximum hepatoprotective activity against CCl_4 intoxication followed by the petroleum ether extract of whole plant of *F. indica*,

powdered roots of S. rhombifolia, powdered roots of I. racemosa and powdered rhizomes of C. orchioides when placed in descending order.

These drug samples were also subjected to evaluation of their hepatoprotective activity against commonly used drugs for long term therapy and identified as hepatotoxicants like paracetamol and rifampicin. The protocol followed remained similar as in the case of CCl_4 as toxicant for assessment of activity. These studies revealed that out of all the test samples screened, the aqueous extract of roots of M. pterygosperma possesses maximum, significant activity ($P < 0.01$) followed by the powdered aerial parts of S. acuta, total aqueous extract of roots of I. racemosa, total aqueous extract of whole plant of F. indica and methanolic extract of rhizomes of C. orchioides against paracetamol intoxication. In the case of rifampicin intoxication, aqueous extract of aerial parts of S. rhombifolia showed maximum, significant activity ($P < 0.01$) among all the test samples tried. The activity was followed by powdered roots of M. pterygosperma, methanolic extract of roots of I. racemosa, methanolic extract of whole plant of F. indica and powdered rhizomes of C. orchioides when placed in descending order. The variation in the activity exhibited by different test samples against the three toxicants may be due to the presence of active chemical moieties present, causing interference in the mechanism of induction of toxicity by individual toxicants.

The confirmation of activities of these identified drug samples were obtained from the histopathological studies of the sections of livers of treated groups and comparing them with the normal liver sections.

From the above studies, the active extracts possessing significant biological activities were chosen. All these extracts were then subjected for isolation of probable active component using various recommended methods. The fractional extraction technique using different solvents was applied after proper treatment to the individual active extract like hydrolysis or saponification, etc. The treated extracts were fractionated and different fractions so obtained were subjected for identification of number of components through thin layer chromatographic studies. The fractions showing the presence of a singular spot were then utilized for isolation of the components through partition coefficient technique, as column chromatography did not provide satisfactory results. The singular components were extracted in different solvents and their purity was checked using TLC. A few of these biologically active extracts could, however, afford singular components which were separated and utilized further for their characterisation. Out of the selected bioactive extracts, petroleum ether extract of rhizomes of C. orchioides yielded two compounds. The petroleum ether and methanolic extracts of whole plant of F. indica yielded one

compound each. The petroleum ether extract of I. racemosa roots yielded one compound. The total aqueous extracts of the stem bark of M. pterygosperma and whole plant of S. cordifolia yielded three and one compounds respectively. These individual chemical compounds were then subjected for their characterization using different physico-chemical as well as spectral studies.

The physiochemical data as obtained from the compounds were recorded and used for their identification. The data available in literature were also taken into consideration before assigning probable chemical structure to the compounds. The compounds C-1 and C-2 from the petroleum ether extract of the rhizomes of C. orchioides were assigned the probable chemical structure as 3,11,16-trihydroxycycloartan-24-one (curculigenin A, $C_{30}H_{50}O_4$) and as 24-methylcycloart-7-ene-3,20-diol (Curculigol, $C_{31}H_{52}O_2$). The residue of methanolic extract of whole plant of F. indica afforded a compound F-1 characterised from spectral data as monomethyl fumarate ($C_5H_6O_4$). The compound F-2 obtained from the methanol insoluble fraction of petroleum ether extract of whole plant of F. indica characterised from spectral data as n-octacosanol ($C_{28}H_{58}O$). The compound I-1 obtained from the methanolic fraction of petroleum ether extract of roots of I. racemosa was characterised as alantolactone ($C_{15}H_{20}O_2$). The compounds M-1, M-2 and M-3 were obtained from different fractions of the total aqueous extract of stem bark of M.

pterygosperma. The compound M-1 obtained from the benzene fraction of the residue of petroleum ether extract of the total aqueous extract after saponification was subjected for various determinations for its structural elucidation. The correct structure, however, could not be assigned for the want of detailed informations. The compound M-2, a hydrolysed product, extracted in diethyl ether was characterised from the spectral data as butenedioic acid or fumaric acid ($C_4H_4O_4$). The compound M-3 obtained from the methanolic fraction of the residue of petroleum ether extract, from total aqueous extract after saponification was characterised as caffeic acid or 3,4-dihydroxycinnamic acid ($C_9H_8O_4$). The compound S-1 from the total aqueous extract of S. cordifolia was characterised as fumaric acid or butenedioic acid ($C_4H_4O_4$).

All these compounds were then subjected to in vitro hepatoprotective activity tests against galactosamine and thioacetamide induced hepatic cytotoxicities. Their nontoxic effects were first, observed on normal hepatocytes. The compound M-2 from stem bark of M. pterygosperma and the compound S-1 from S. cordifolia could offer complete protection against galactosamine induced cytotoxicity at the dose of 1000 ug/ml. The other compounds, however did not show any protection. When tested against thioacetamide induced hepatic cytotoxicity, the compound S-1 from S. cordifolia offered maximum protection while the compound M-3 showed

minimum protection. The compounds C-1 from C. orchioides F-2 from F. indica and I-1 from I. racemosa, however, showed activity at the dose of 100 and 1000 ug/ml respectively against galactosamine induced hepatic cytotoxicity and offered protection against thioacetamide induced cytotoxicity also. Out of all the isolated compounds F-1 from F. indica, I-1 from I. racemosa and S-1 from S. cordifolia were further tested for in vivo activity. The compound S-1 from S. cordifolia showed maximum activity followed by the compound I-1 from I racemosa and the compound F-1 from F. indica against CCl_4 intoxication. The maximum protection against paracetamol intoxication was offered by compound S-1 followed by the compounds F-1 and I-1 while in case of rifampicin intoxication, the compound F-1 showed maximum protection followed by the compounds S-1 and I-1. These results so obtained were also confirmed by histopathological examination of treated livers of rats with different compounds.

The effect of different compounds against carrageenan induced paw oedema revealed peak reduction in oedema caused by all the compounds within one hour of carrageenan injection similar to that shown by ibuprofen. Out of all the compounds tested, the compound I-1 showed most significant reduction ($P<0.01$) in paw oedema followed by the compounds F-1 and S-1 when placed in descending order of activity.

Some of the marketed preparations of these plant drugs

were also studied for their hepatoprotective and anti-inflammatory activities even though their labelled claims are different, since these plant drugs exhibited such activities. All the marketed preparations were found to be safe when administered at the selected dosage regimen. All the formulations were studied for their effects against all the three toxicants, CCl_4, paracetamol and rifampicin. Out of all the tested formulations 'Liv 52' against paracetamol showed most significant recovery followed by 'Safi' against CCl_4 and 'Kumari Asava' against rifampicin. These studies were supported by their histopathological studies.

These marketed preparations when studied against carrageenan induced paw oedema, revealed that highest significant activity was shown by 'Kumari asava'. The formulations like 'Balant Kadha' and 'Livfit syrup' showed insignificant reduction in paw oedema. Since these formulations are multi component systems, the activity exhibited, may however, not be due to the selective drugs of present study but an over all indication on their therapeutic efficacy could be achieved. Thus, the present investigations can provide a wide spectrum of informations with regard to evaluation of therapeutic efficacy of the selected drugs which are already in practice under various alternative systems of medicine either alone or as components of polyherbal formulations. The utility profile as obtained from present study will also form an addition to the existing

literature on phytopharmaceuticals and initiate the exploration of untapped values of various such medicaments which offer protection against hepatic disorders caused due to either hazardous environmental conditions or continuous exposure to xenobiotics. These studies, however, also provide further scope of detailed investigations on the mechanism of action of individual components obtained from these drugs. On the whole, the purpose of standardisation and evaluation of the selected drugs for their therapeutic efficacy could, to some extent, be achieved during present investigations as far as the incorporation of these drugs in traditional systems of medicine is concerned.