Summary
SUMMARY

The state of complete physical, mental and social well being is called as ‘Health’. Any change from the state of health is known as ‘illness’ and is also referred to as homeostatic imbalance. The causes for this may be the infection, congenital abnormalities, malnutrition, degeneration of certain organs, development of tumors, hypo and hyperfunction of the endocrine glands.

The subject of ‘Drugs’ is as old as illness. It has been man’s heritage from the beginning of his existence, and the search for remedies to combat illness is equally old. The earliest Indian records are Vedas. There are medical descriptions in Rigveda (3000 B.C.). It was Charaka a renowned ancient Indian physician and later Sushruta and Vagbhata described the mode of diagnosis and use of various medicinal preparations in Ayurveda, the science of life.

During last five decades, a phenomenal growth has been achieved in the field of biochemistry, enzymology and molecular biology. These developments have not only changed the face of biomedical research and clinical pharmacology but have made the discovery of drugs more rational and complex. The advent of biotechnology further enriched the pharmacological research in the development of drugs and in the diagnosis of diseases on molecular basis.

In recent years, there has been a constant demand all over the world, for the plant based drugs. In the developed countries people are becoming aware of
the potency and side effects of synthetic drugs and there is an increasing interest in the herbal remedies with a basic approach towards the nature. The mother India is bestowed with natural resources. One third of the diversified flora and fauna are concentrated in the Western Ghats and it has a tremendous wealth of medicinal plants. But only for a few plant species detailed chemical analysis and screening of therapeutic efficacy of the constituents have been worked out. Rest of the medicinal plant species is yet to be subjected for rigorous chemical, pharmacological and clinical investigations.

Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the chemical constituents, which determine the medicinal properties of the plants. It has now been universally accepted fact that the plant medicines and remedies are far more safer than that of synthetic drugs for curing the serious diseases like cancer, AIDS, hepatitis, epilepsy, arthritis, herpes, cancer etc.

The present investigation was aimed on the evaluation of phytochemical constituents and screening of hepatoprotective activity of some indigenous medicinal plants of Karnataka. A survey on medicinal plants of Western Ghats of Karnataka enlightened us that the traditional practitioners of malnad region (Keladi and Joldhal of Karnataka) are using the stem bark of *Diospyros cordifolia* and *Pterocarpus marsupium* for curing infective hepatitis. The traditional claims also revealed the use of these plants for healing wounds, relieving pain (analgesic), and treating epilepsy and sleeplessness (sedative).
So pharmacological experiments were also designed to screen the crude extracts of *D. cordifolia* and *P. marsupium* and the isolated constituents of *D. cordifolia* for wound healing, analgesic, anti-convulsant and CNS depressant activities in addition to hepatoprotective activity.

In the present investigation, four triterpenoids, ursolic acid, lupeol, betulin & betulinic acid and a naphthoquinone, diospyrin have been isolated from the stem bark of *D. cordifolia* for the first time. The isolated constituents were characterized through UV, IR, 'HNMR and Mass spectral studies.

The earlier investigators have screened these triterpenoids (from other plant species) pharmacologically for anti-tumour (Misra, *et al.*, 1989), antileukaemia (Chen, *et al.*, 1989), and anti-inflammatory activity (Recio, *et al.*, 1995). Singh, *et al.*, (1994) have also showed that ursolic acid possesses potent inhibitory activity against HIV-I protease. In *P. marsupium* the aqueous extract of the stem bark extract has been evaluated for antihyperlipidemic (Jahromi, *et al.*, 1993) and hypoglycemic (Vats, *et al.*, 2002) activities. In *P. marsupium*, we have conducted only preliminary phytochemical studies on the methanol and aqueous extracts of the stem bark. It showed the presence of flavonoids, carbohydrates, tannins, saponins, and proteins were found to be present in the aqueous extract of the stem bark of *P. marsupium*. In addition to these constituents, the methanol extract of the stem bark of *P. marsupium* showed the presence of steroids and triterpenoids. These extracts were subjected for evaluation of the above pharmacological activities.
Survey of literature also enlightened us that so far no investigations were carried out on the evaluation of the above said pharmacological activities of the crude extracts of the stem bark of *D. cordifolia* and *P. marsupium*, and the isolated constituents of *D. cordifolia* stem bark. So, in view of the high medicinal value of these two species, the present investigation was undertaken.

Hepatitis is the condition where there is inflammation and/or necrosis of liver cells. It continues to be a major cause of illness and death among all contagious and communicable diseases. Many investigators have screened and reported hepatoprotective activity of a number of plant species on experimental animals, such as *Wedelia chinensis* (Young, et. al., 1986), *Phyllanthus nuriri*, (Venkateswaran, et. al., 1987), *Eclipta alba* (Singh, et. al., 1991), *Ocimum sanctum* (Chattopadhay, et. al., 1992), *Ricinus communis* (Shukla, et. al., 1992), *Diospyros leucomeles* (Recio, et. al., 1995), *Berberis aristata* (Janbaz, et. al., 2000), *Ambrosia maritima* (Bastawy Ahmed, et. al., 2001), *Limonium wrightii* (Aniya, et. al., 2002), *Torreya nucifera* (Kim, et. al., 2003), *Wrightia tinctoria* (Chandrashekar, et. al., 2004) etc.

So far, only limited reports are available on screening of the active constituents isolated from the medicinal herbs against toxic hepatitis e.g. Wedelolactone from *Wedelia calendulacea* (Wagner, et. al., 1986), Andrographolide from *Andrographis paniculata* (Visen, et. al., 1990), Baicalin from *Scutellaria rivularis* (Lin, et. al., 1997), Berberine from *Berberis aristata* (Janbaz, et. al., 2000), Quercetin-3-sephorotrioside from *Pisum sativum*
(Murakami, et. al., 2001), Rutin from *Artemisia scoparia* (Khalid, et. al., 2002), Aretigenin from *Torreya nucifera* (Kim, et. al., 2003).

It was known that the liver injury caused by CCl₄ simulates the human infective hepatitis. In the present study also treatment of animals with CCl₄ for 10 days showed significant elevation in the levels of serum markers namely, bilirubin, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase activities, but the serum total protein level was reduced by altering albumin/globulin ratio (2:1).

In the animals treated with petroleum ether extract of the stem bark of *D. cordifolia*, there was a significant control on increase in the levels of serum bilirubin, transaminases and alkaline phosphatases. The effect of the aqueous extract of *D. cordifolia* was less, where as that of carbon tetrachloride extract of *D. cordifolia* was insignificant. Among the isolated constituents of *D. cordifolia* stem bark, ursolic acid was most effective in maintaining the levels of serum bilirubin and other liver function marker enzymes (AST, ALT & ALP) as similar with that of the reference standard silymarin treated groups. Simultaneously the serum protein level and the albumin/globulin ratio were maintained as similar to the values of the controls. The constituents lupeol and betulin were less effective, while betulinic acid and diospyrin were ineffective in controlling the toxic effect of CCl₄. In *P. marsupium* methanol extract exhibited more hepatoprotective activity than the aqueous extract. This may be due to the presence of higher flavonoid contents in methanol extract.
The histopathological studies of the liver tissue also evidenced the hepatic lesions caused by the effect of CCL₄ and the therapeutic efficacy of the drugs by controlling the toxic effect of CCL₄ which caused peroxidative degradation in the adipose tissue and as a result fatty infiltration occurred in the hepatocytes. Histological profile of the liver section of control animals showed normal hepatocytes. The sections of the liver excised from the animals treated with CCL₄ exhibited severe intense centrilobular necrosis and vacuolization and macrovesicular fatty change. The sections of liver taken from the animals treated with standard drug silymarin, constituent ursolic acid and petroleum ether extract of D. cordifolia showed the hepatic architecture similar to that of the control animals. While the constituent diospyrin and carbon tetrachloride extract of D. cordifolia were devoid any of hepatoprotective activity.

The sections of the liver of animals treated with methanol extract of the stem bark of P. marsupium exhibited significant protection against the damage caused by the toxicant. This is evident from the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration. While moderate accumulation of fatty lobules was noticed in the sections of the liver of aqueous extract treated animals.

The results of this investigation highlighted that the constituent ursolic acid and the petroleum ether extract of D. cordifolia were highly effective in protecting the liver cells against the toxic effect of CCL₄. This may be probably due to anti-oxidant property of these compounds or it may be inhibit the
activities of cytochrome-P_{450} enzymes, which may oxidize CCl\textsubscript{4} into CCl\textsubscript{3} toxic radical.

The three different models used in our study to assess the wound healing effect of *D. cordifolia* and *P. marsupium* were excision, incision and dead space wound. The commercial nitrofurazone ointment was used as a reference standard to assess the healing effect of the various extracts and the constituents.

Among the various crude extracts treated animals significant epithelialization of the excision wound was observed in the animals treated with aqueous extract of *D. cordifolia* and methanol extract of *P. marsupium*. In the animals treated with the isolated constituent lupeol the percentage of wound contraction was higher than that of the ursolic acid treated animals. The effect of betulin and betulinic acid on the percentage of wound contraction was less and diospyrin was least effective.

The breaking strength of the wound is measured experimentally by the amount of force required to disrupt it. In the incision wound model also aqueous extract of *D. cordifolia*, methanol extract of *P. marsupium* and the constituent lupeol were most effective in increasing the skin breaking strength of the incision wound.

The effects of oral administration of suspensions of the stem bark extracts of *D. cordifolia*, methanol extract of *P. marsupium* on the dead space wound models were also assessed by the increase in the weight of granulation
tissue and its hydroxyproline content. The increase in breaking strength of the granulation tissue indicates enhanced collagen maturation by increased cross-linking of collagen fibers. While, an increase in granulation tissue weight indicates the presence of higher protein content.

Histopathological study of the granulation tissue provides further evidences on the wound healing efficacy of the extracts and the constituents. The sections of the granulation tissue of the untreated animals showed monocytes and fibroblasts. On the contrary, incomplete healing was evidenced with lesser epithelialization, fibrosis, and collagen formation. The sections of the granulation tissues of the animals treated with aqueous extract of *D. cordifolia*, constituent lupeol and methanol extract of *P. marsupium* showed complete epithelialization, fibrosis and collagen formation. Whereas, in the carbon tetrachloride extract of *D. cordifolia* treated animals the healing activity was comparatively less and it is indicated by the presence of more monocytes and fibroblasts.

The results of this experiments revealed that the weight of the granulation tissue and its hydroxyproline content was high in the animals treated with aqueous extract of *D. cordifolia* and methanol extract of *P. marsupium* followed by the constituent lupeol.

The investigation of *Desmodium triquetrum* (Shirwaikar, et. al., 2003) revealed that the plants having anti-oxidant property would also possess wound healing activity. The methanol extract of the stem bark of *P. marsupium*
Summary contains flavonoids, which are also screened for anti-oxidant property (Hesham, et. al., 2002). The variations in wound healing efficacy of the different constituents isolated from the stem bark of *D. cordifolia* may be due their structural changes of the triterpenoids.

The analgesic activity can be determined in laboratory animals, by noting the reaction time to the painful stimulus. We have adopted three methods, tail-flick (tail-withdrawal from the radiant heat) using analgesiometer, caudal immersion of rats' tail in hot water and acetic acid induced writhing for screening of the analgesic activity. The results of these experiments have shown that the petroleum ether extract of *D. cordifolia* is the most potent in producing analgesia. This was indicated by the increase in the reaction time in tail-flick, tail-immersion models and by reducing the number of writhings in the acetic acid induced writhing model. The aqueous extract of *D. cordifolia* showed moderate response. While, the response was insignificant in carbon tetrachloride extract treated animals. In case of *P. marsupium* both the methanol and aqueous extracts were devoid any analgesic activity.

Among the three triterpenoids isolated from the petroleum ether extract of *D. cordifolia*, ursolic acid was found to possess significant analgesic activity. Other constituents were devoid of any analgesic activity. The central action of the triterpenes is probably due to their action either through the central opioid receptors or by promoting the release of endogenous opiopeptides.
The anticonvulsant activity was also evaluated against maximal electroshock and strychnine induced convulsions. The petroleum ether extract of *D. cordifolia* has exhibited anti-convulsant property by reducing the extensor phase and faster recovery of MES-induced convulsions. While the aqueous extract was found to be less effective in this respect. Carbon tetrachloride extract of *D. cordifolia* did not show any effect on any of the phases of MES convulsions. Both the extracts of *P. marsupium* were also devoid of any anti-convulsant activity. Among the constituents isolated from the *D. cordifolia*, only ursolic acid exhibited some anti-convulsant activity, which was indicated by the reduction in the extensor and recovery phases of the MES convulsions. Standard drug, phenobarbitone abolished the extensor phase of convulsions and the recovery was faster.

In strychnine-induced convulsions also, only the petroleum ether and aqueous extracts were able to postpone the onset of convulsion but the mortality occurred. Both the extracts of *P. marsupium* were devoid of any effect on strychnine-induced convulsions. However all the constituents failed to protect the animals against the strychnine-induced convulsions, but only ursolic acid was able to postpone the onset of convulsions. On the other hand standard drug, diazepam abolished the strychnine-induced convulsions and the mortality was zero percent.

Most of the drug activities on CNS influence the locomotor activity, which can be an index of wakefulness (alertness) of mental activity.
The locomotor activity can be easily measured by using an actophotometer. The petroleum ether and aqueous extracts of *D. cordifolia* reduced the locomotor activity significantly. Carbon tetrachloride extract was ineffective. Methanol extract of *P. marsupium* also exhibited significant CNS depressant activity, which was indicated by the reduction in the locomotor activity scores. Whereas the aqueous extract was comparatively less effective. Among the constituents isolated from the stem bark of *D. cordifolia*, betulin depressed the CNS to the maximum extent. While others were less potent and diospyrin was devoid of CNS depressant activity. Chlorpromazine is a selective depressant of CNS and used as a reference standard during the experiments. It also significantly reduced the spontaneous motor activity.

Pentobarbitone sodium is a short acting hypnotic drug. Any drug, which depresses CNS, acts synergistically with the pentobarbitone. This is indicated by the potentiation of pentobarbitone sleeping time. Among the different crude extracts and the isolated constituents of the stem bark of *D. cordifolia*, significant sedative effect was noticed in the animals treated with petroleum ether extract and the constituent betulin.

The effect of these crude extracts and the active constituents may be due to their ability to increase the concentration of gamma-aminobutyric acid (GABA, an inhibitory neurotransmitter) in the central synapses of the brain. There are reports that the higher content of flavonoids present in the plant extracts, are responsible for CNS depressant activity (Zetola, *et al.*, 2002).
The CNS depressant activity of these may be otherwise due to their inhibitory effect on GABA metabolism. The extracts and the constituents may inhibit the microsomal enzymes responsible for their metabolism, and consequently prolong the pentobarbitone sleeping time or they may act synergistically with the pentobarbitone.

To conclude, this investigation evaluated the ethnomedical uses of *D. cordifolia* and *P. marsupium*. The most potent triterpene, ursolic acid might be a safe drug for hepatitis, nociception and excitement. Lupeol is more effective in healing of wounds and betulin is effective in reducing spontaneous motor activity.
SCOPE OF THE FUTURE STUDY

This investigation provides a pharmacological evidences on the ethnomedical claims of *D. cordifolia* and *P. marsupium*. The three triterpenoids namely ursolic acid, lupeol and betulin were isolated from the petroleum ether extract of *D. cordifolia*. In addition a triterpenoid, betulinic acid and a naphthoquinone, diospyrin have also been isolated from the carbon tetrachloride extract of *D. cordifolia*. Among these constituents ursolic acid possesses potent hepatoprotective, analgesic and CNS depressant activities. It is also reported for potent inhibitory activity against HIV-I protease, anti-tumor activity and ant-inflammatory activities.

The constituent lupeol showed potent wound healing activity. It is also reported to be effective against Walker-Carcinoma-256 tumor system. So this investigation forms a footstep in medical research. The *in vitro* protocols have to be developed for the enhanced production of these constituents either through the mass multiplication of callus or by the application of bioreactors. So that, the active constituent is obtained in large scale without destroying the natural plant population. The present investigation is a preclinical study carried out on the experimental rats and mice. Further clinical trials on human volunteers are very much essential to confirm the therapeutic efficacy of these constituents.
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<td>Evaluation of wound healing activity of the stem bark of <em>Diospyros cordifolia</em> Roxb.</td>
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<td>8.</td>
<td>Antinociceptive effect of the extracts and the constituents isolated from <em>Diospyros cordifolia</em> Roxb. stem bark.</td>
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