Discussion
DISCUSSION

The drugs are administered into the body to produce therapeutic effect and body takes them as foreign substances and eliminates them. The chemical alteration of the drug in the body is called as Biotransformation. Within the body drugs could undergo three possible fates, metabolic degradation by enzymes, spontaneous changes into other substances and excretion without undergoing any change. Majority of the drugs are metabolized by the enzymes of the liver, resulting in their activation, inactivation or modification. The reactions, which bring about these changes, are oxidation, reduction, hydrolysis and synthetic reactions (conjugation with glucuronic acid, sulphate, glycine etc.).

The biotransformation of drugs is essentially a detoxification process. However, during the initial stages of metabolism of certain drugs, active and even toxic compounds may be produced. The enzymes, which metabolize the drugs, are distinct from those, which function in the intermediary metabolism. These enzymes are located in the liver microsomes. They form a part of the endoplasmic reticulum of the hepatic cells and alter drugs mainly to make them polar and water soluble forms so that the kidneys can easily excrete them. Many drugs are metabolized by the cytochrome P450 enzyme system. A drug bound to cytochrome P450 may be either oxidised or reduced. Drugs are also metabolized by enzymes, which are of non-microsomal origin, present in liver, plasma and tissues (Satoskar, 2001).
Various secondary metabolites of the plants act as the active constituents against the ailments. Sometimes a single constituent may possess wide spectra of activities (for example, reserpine is both a depressant and an antihypertensive drug) or a plant may contain different phytoconstituents with different pharmacological actions (for example quinidine is an antiarrhythmic and quinine has an antimalarial action). In the present investigation also *Diospyros cordifolia* is claimed to be useful in treating various ailments, such as jaundice, wounds, insomnia, pain, inflammation etc. The another plant species *Pterocarpus marsupium* is also claimed to be useful in treating wounds, fever, stomachache, toothache, diabetes, jaundice and skin diseases. The therapeutic efficacy of these species might be due to the presence of a single constituent in higher concentration which is responsible for different activities or the plant might contain different phytoconstituents acting as additives on specific disorder. Therefore to ascertain this aspect, different solvent extracts of the stem bark of the plant *D. cordifolia* and *P. marsupium* were subjected for evaluation of the following pharmacological activities such as hepatoprotective, wound healing, analgesic, anti-convulsant and CNS depressant.

Singh, *et. al.*, (1971) have reported the spasmolytic and hypotensive activity of the alcoholic extract of the plant *D. cordifolia*. Similarly Kohli, *et. al.*, (1972) evaluated the different fractions of the alcoholic extract for anti-inflammatory and anti-pyretic activities.
Suresh Chandra, et. al., (1989) have isolated ursolic acid and lupeol from the leaves of *D. cordifolia*. In the present study, the same constituents have been isolated from the stem bark of *D. cordifolia*. In addition another three constituents namely betulin, betulinic acid and diospyrin have been isolated from the stem bark of this species. However Kapil, *et. al.*, (1961) have isolated these constituents (except ursolic acid) from the stem bark of a relative species *D. montana*.

Ursolic acid inhibited 12-o-teradecanoyal-phorbal-13-acetate induced Epstein-Barr virus activation (Ohigashi, *et. al.*, 1986). It also showed potent inhibitory activity against HIV-I protease (Singh, *et. al.*, 1994), suppressed the tumor promoter induced inflammation (Hirota, *et. al.*, 1990) and increased the blood sugar concentration, glycogen and ATP contents in muscles, heart and uterus (Golovina, *et. al.*, 1976).

Misra, *et. al.*, (1989) have evaluated the anti-tumor activity of betulin, betulinic acid and lupeol. They were also found that to be active against the Walker-Carcinoma-256 (intramuscular) tumor system. Betulinic acid inhibited P-388 leukemia growth (Chen, *et. al.*, 1989). It was also found to possess highly selective activity against human melanoma *in vitro* and *in vivo* (Cordel, 1995). The investigation of Hazra, *et. al.*, (1986) showed that diospyrin exhibited the *in vitro* activity against the protozoan *Leishmania donovani*.

The ethyl acetate extract of *P. marsupium* heartwood and its flavonoid constituents, marsupin, pterosupin and liquiritigenin were screened for
antihyperlipidemic activity (Jahromi, *et. al.*, 1993). Hypoglycemic activity of the aqueous extract of *P. marsupium* wood has been evaluated on normal and alloxan induced diabetic rats (Vats, *et. al.*, 2002).

In the present study four triterpenoids and a naphthoquinone have been isolated from the petroleum ether and carbon tetrachloride extracts of the stem bark of the plant *D. cordifolia*. The IR, $^1$HNMR and Mass spectral analysis of the isolated constituents confirmed the structures of ursolic acid, lupeol, betulin, betulinic acid and diospyrin.

So, in view of the high medicinal value of these two species, the pharmacological investigation was undertaken to evaluate the therapeutic efficacy of the crude extracts (methanol and aqueous extracts of *P. marsupium*, petroleum ether, aqueous and carbon tetrachloride extracts of *D. cordifolia*) and the isolated constituents (ursolic acid, lupeol, betulin, betulinic acid and diospyrin) for the hepatoprotective, wound healing, analgesic, anti-convulsant and CNS depressant activities.

Liver is one of the vital organs in the body. It is most concerned with the vital functions of the body such as metabolism and detoxification. Moreover, it is one of the most frequently exposed organ and liable for damage in the body and it is indeed fortunate that it has enormous functional routes (Bhat, *et. al.*, 1996). Many hepatotoxic chemicals like paracetamol (Wang, 1983), alcohol (Tripathi, 1991) and CCl$_4$ (Wagner, 1986) cause damage to hepatocytes. Among these toxins the hepatic damage caused by CCl$_4$ is more and it
simulates the human hepatitis (Recknagel, 1989). Hence CCl₄ was used as a tool to induce toxic hepatitis in the experimental models.

According to Robbins (2001), in toxic condition formation of free radicals takes place. Free radicals are chemical species that have a single unpaired electron in an outer orbital. In such a state, the radical is extremely reactive, unstable and enters into the reactions with organic/inorganic chemicals like proteins, lipids and carbohydrates particularly with key molecules in membranes and nucleic acids. They initiate autocatalytic reactions whereby molecules with which they react are themselves converted into free radicals to propagate that chain of damage.

Free radicals may be initiated within cells, by absorption of radiant energy and endogenous oxidative reactions. The toxic effect of CCl₄ is due to its conversion to highly reactive toxic free radical CCl₃ by cytochrome P₄₅₀. CCl₃ causes cell injury by (a) lipid peroxidation of membranes usually by oxygen derived free radicals, (b) oxidative modification of proteins and (c) lesions in DNA.

\[ \text{CCl}_4^+ + e^- \rightarrow \text{CCl}_3 + \text{Cl}^- \]

The free radicals produced locally, cause auto-oxidation of polyenic fatty acids present within membrane phospholipids. Their oxidative decomposition of lipid is initiated and organic peroxides are formed after reacting with oxygen. Thus rapid breakdown of structure and function of endoplasmic reticulum takes place is due to decomposition of lipid.
CCL₄ in liver cells

\[ \text{SER} \]

\[ \text{CCl}_3^- \]

Microsomal polyenoic fatty acid

Lipid radicals

\[ + \text{O}_2 \]

Lipid peroxidation (autocatalytic spread)

- Membrane damage to RER
- Polysome detachment
- Decreased Lipid acceptor
- Protein synthesis
- Fatty liver
- Release of products of lipid oxidation
- Damage to plasma membrane
- Increased permeability to Na⁺, H₂O, Ca²⁺
- Cell swelling
- Massive influx of Ca²⁺
- Inactivation of mitochondria, cell enzymes, denaturation of proteins.

CCL₄ induced liver cell injury is severe and extremely rapid in onset.

Within 30 minutes there is decline in hepatic protein synthesis and within 2
hours, swelling of smooth endoplasmic reticulum (SER) and dissociation of ribosomes from rough endoplasmic reticulum (RER) takes place (Robbins, 2001). Accumulation of lipid ensues, owing to inability of cells to synthesize lipoprotein from triglycerides and 'lipid acceptor protein'. This leads to the fatty liver due to CCl₄ poisoning. Mitochondrial injury then occurs, and this is followed by progressive swelling of cell owing to increased permeability of plasma membrane. Further, plasma membrane damage is thought to be caused by relatively stable fatty aldehydes, which are produced by lipid peroxidation in smooth endoplasmic reticulum but are able to act at distant sites. This is followed by massive influx of calcium and cell death.

Herbal drugs are playing an important role in health care programmes worldwide, and there is a resurgence of interest in the herbal medicines for the treatment of various ailments, like hepatitis, epilepsy, insomnia etc., for which there is no specific treatment available. Many authors have reported the hepatoprotective activity of number of medicinal plants like, Phyllanthus niruri (Venkateswaran, et. al., 1987), Picrorhiza kurrooa (Dwivedi, 1990), Trianthema portulacastrum (Mehta, et. al., 1999), Mallotus japonicus (Hwa-Kyung Lim, et. al., 2000), Croton oblongifolius (Ahmed, et. al., 2002), Nigella sativa Linn (Mohideen, et. al., 2003), Wrightia tinctoria Roxb. (Chandrashekar, et. al., 2004) etc. However only a few constituents isolated from different medicinal plants have been screened for their hepatoprotective activity. To name some of them are wedelolactone and dimethylwedelolactone isolated from Wedelia calendulacea (Wagner, et. al., 1986), Baicalin from
Scutellaria rivularis (Lin, et. al., 1997), Berberine from Berberis aristata (Janbaz, et. al., 2000), Lignans (aretigenin, traxillagenin and demethyltraxillagenin) isolated from the bark of Torreya nucifera (Kim, et. al., 2003) etc.

There are many serum markers available to assess the functional efficacy of the liver and the hepatoprotective activity of the drugs. The stem bark extracts of the plants D. cordifolia and P. marsupium are traditionally used in the treatment of jaundice, but there are no reports on the clinical evaluation of the hepatoprotective activity of these plants. Hence experiments were designed to screen the hepatoprotective effect of the different extracts of these plant species and the isolated constituents.

In the present investigation, the animals of group II (CCL4 treated) showed significant loss of appetite and reduction in their body weight when compared to control. The same observation was also made in the animals treated with the carbon tetrachloride extract of the stem bark of D. cordifolia. While all the animals treated with the petroleum ether, aqueous extracts of D. cordifolia, isolated constituents ursolic acid, lupeol, betulin showed normal behaviour. The animals treated with methanol and aqueous extracts of the stem bark of P. marsupium also showed normal behaviour and food consumption.

The estimation of serum markers like total bilirubin, total protein, enzymes like, aspartate aminotransaminase, alanine aminotransaminase and alkaline phosphatase activities indicate the functioning status of liver. When
there is a damage to the lipid membranes of the hepatocytes by the pathogenic viruses or by the toxic effect of the chemicals leads to the release of abnormal concentrations of serum markers into the blood stream. The increase may be the clear indication of cellular leakage and loss of functional integrity of the cell membrane (Saraswath, et. al., 1993).

Estimation of serum bilirubin is the most sensitive test because it confirms the intensity of the hepatic damage. In toxic hepatitis the extent of excretion of bilirubin through the intestines is very less. The bilirubin is excreted into the canaliculi and then regurgitated into the blood stream. Hence, hyperbilirubinemia is common in hepatitis condition. In the present investigation, it was observed that concomitant treatment of the animals with all the crude extracts (except carbon tetrachloride extract of D. cordifolia) showed significant reduction in the levels of serum bilirubin. In the animals treated with ursolic acid there was a significant reduction in the serum level of bilirubin. Lupeol and betulin also controlled the serum level of bilirubin. While the other constituents betulinic acid and diospyrin had insignificant effect on reducing the increased level of serum bilirubin. In case of P. marsupium the methanol extract showed therapeutic effect by significantly lowering the levels of bilirubin while the effect of the aqueous extract was comparatively less. The effect of other indigenous medicinal plants Cassia angustifolia (Ilavarasan, et. al., 2001), Pistacia lentiscus (Janakat, et. al., 2002), Foeniculum vulgare (Ozbek, et. al., 2003), Wrightia tinctoria (Chandrashekar, et. al., 2004) etc. on restoring the level of serum bilirubin have been reported.
The hepatoprotective effect of the extracts and the constituents were compared with the standard drug silymarin which is a mixture of silbyn related flavonoids extracted from the seeds of *silybum marianum* (Choudhari, 1999). It is a potent hepatoprotective drug commonly prescribed by the physicians to heal jaundiced condition. Reports are also available for the use of silymarin as a reference standard drug (Chun-Chin Lin, *et. al.*, 1995; Mehta, *et. al.*, 1999).

The changes in the levels of serum protein form the basis in the diagnosis of hepatic diseases (Kagan, 1943 and Henry, 1986). There is correlation between the degree of serum hypoalbuminemia and hyperglobulinemia. In CCl$_4$ treated animals total protein level was decreased due to the alteration of albumin:globulin ratio. The serum albumin level was slightly decreased, while the globulin level was moderately increased. The reversal of the toxic effect of CCl$_4$ was observed in the animals treated with the crude extracts of both the plants (except carbon tetrachloride extract) and the isolated constituents. The petroleum ether extract of *D. cordifolia* and the constituent ursolic acid were found to be most potential in maintaining the normal concentration of total protein. Methanol extract of *P. marsupium* was more effective in retaining the protein concentration. The similar study on the effect of various plants *Luffa echinata* (Bahar Ahmed, *et. al.*, 2001), *Nigella sativa* (Mohideen, *et. al.*, 2003), *Phyllanthus amarus* (Venkatesan, *et. al.*, 2003).
2003), Thespesia populnea (Havarasan, et. al., 2003) etc. on protein levels has been carried out.

Estimation of the serum marker enzymes individually also is helpful in the differential diagnosis of the hepatic damage. In acute hepatitis or toxic hepatitis, the rise in serum level of AST (SGOT) was greater than that of ALT (SGPT) (Kontinnen, 1971). On the contrary elevation of ALT was more than AST in alcoholic cirrhosis of liver (Tripathi, et. al., 1991). Probably the hepatoprotective activity of D. cordifolia may be due to the membrane stabilizing and antioxidant property of the triterpenoids (Alex Heraldo Jeller, 2004).

In the present investigation it was observed that the levels of the serum marker enzymes was significantly increased in the animals treated with CCl4. Concomitant administration of the extracts with CCl4 showed significant reduction in the serum enzyme levels. However, the carbon tetrachloride extract of D. cordifolia failed to protect the liver against the CCl4 damage. Among the isolated constituents of D. cordifolia stem bark, ursolic acid was highly effective in reducing the toxic effect of CCl4 by controlling the levels of the serum marker enzymes ALT, AST, SALP. This effect was comparable to that of the standard drug silymarin. While the therapeutic efficacy was comparatively less in animals treated with lupeol and betulin. The hepatoprotective activity was insignificant in animals treated with the constituents betulinic acid and diospyrin. However, significant reversal of the
enzyme levels was observed in animals treated with the standard drug silymarin. Similar studies have been done on *Trianthema portulacastrum* (Mehta, *et. al.*, 1999), *Piper longum* (Jalalpure, *et. al.*, 2003), *Solanum pseudocapsicum* (Vijyan, *et. al.*, 2003), *Vitex nigundo* (Srinivas, *et. al.*, 2004).

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. The increase in the levels of serum bilirubin, transaminases and alkaline phosphatases was the clear indication of cellular leakage and loss of functional integrity of the cell membrane (Saraswath, *et. al.*, 1993). Histological profile of the control animals showed normal hepatocytes. The sections of 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 showed the hepatic architecture, which was similar to that observed in the vehicle control. Treatment with the petroleum ether and aqueous extracts gave significant protection to the hepatocytes against CCl₄ damage. While carbon tetrachloride extract was devoid any of hepatoprotective activity.

The earlier investigators, Ilavarasan, *et. al.*, (2001) and Chandrashekar, *et. al.*, (2004) etc. have studied the histopathological profile of the liver tissue
and showed the hepatoprotective effect of *Cassia angustifolia* and *Wrightia tinctoria* leaf extracts respectively.

Among the isolated constituents of *D. cordifolia*, ursolic acid exhibited potent hepatoprotective activity as indicated by the presence of normal hepatic cords, absence of necrosis and macrovesicular fatty change. Lupeol and betulin also showed significant hepatoprotective activity. But betulinic acid and diospyrin failed to protect the liver against CCl₄ induced liver injury. Thus it appears that the most potent triterpenoid, ursolic acid might be a safe drug for hepatitis and needs further clinical evaluation on human volunteers.

The sections of the animals treated with methanol extract of the stem bark of *P. marsupium* exhibited significant protection against the toxicant as evident from the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration and this was compared with the standard drug silymarin. Moderate accumulation of fatty lobules was noticed in the sections of the animals treated with the aqueous extract. The preliminary phytochemical investigations of the methanolic extract of *P. marsupium* showed the presence of flavonoids. The reports also indicated that the methanol is a suitable polar solvent for the extraction of flavonoids (Maurya, et. al., 2004). The hepatoprotective effect of methanol extract of *P. marsupium* is probably due to the presence of higher amounts of flavonoids. The literature also revealed that the flavonoids have antioxidant property and was also found to be useful in the treatment of liver damage (Hesham, et. al., 2002).
The investigation of Khalid, et. al., (2002) revealed that Rutin, a well-known flavonoid, isolated from *Artemisia scoparia* Thunb. (Asteraceae) has exhibited hepatoprotective activity by inhibiting cytochrome-P_{450} (CYPs) enzymes, which oxidize CCl_{4} into the toxic radical. Similarly methanol extract of *propolis*, a resinous hive product of the plants possessed stronger 1,1-3 diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and could protect the liver from the toxic effect of D- galactosamine (Arjun, et. al., 2000). Reports also revealed that the triterpenoids have the anti-oxidant property (Alex, et. al., 2004). Hence the hepatoprotective activity of the petroleum ether extract and its constituent ursolic acid, of *D. cordifolia* is probably due to either the anti-oxidant property or inhibition of the activities of cytochrome-P_{450} (CYPs) enzymes.

The word healing means replacement of destroyed tissue by living tissue. The four basic processes, which take place in, wound healing are, inflammation, wound contraction, epithelialization and granulation tissue formation (Somen Das, 2001). Immediately after disruption of tissue integrity, inflammation starts. Platelets become adherent and with clotting factors form haemostatic plug to stop bleeding from the small vessels. The prostaglandins (PGE_{1} and PGE_{2}) are released in the inflamed area and seem to be the final mediators of acute inflammation and may play a chemotactic role for white cells and fibroblasts. Actively motile white cells migrate into the wound and start engulfing and removing cellular debris and injured tissue fragments. Leukotaxin (a peptide formed in the damaged tissue by the enzymatic
destruction of albumin) is thought to be the chemotactic agent, attracting leukocytes into the wounds. Monocytes must be present to create normal fibroblasts production.

Wound contraction begins initially slowly, after 3 or 4 days rapid wound contraction occurs. Collagenation increases rapidly during mid-phase. The myofibroblasts present in the margins of the wounds appear to constitute the machinery for the wound contraction. These are responsible for moving the overlying debris. Epithelialization of the wound mainly occurs by proliferation and migration of the marginal basal cells lying close to the wound margin. The haematoma within the wound is soon replaced by the granulation tissue, which consists of new capillaries and fibroblasts. The newly formed capillary loops leak protein and thus the tissue fluid which is formed is a very suitable medium for fibroblastic growth. Fibroblasts are responsible for the production of the mucopolysaccharide ground substance. The lymphatics develop, new nerve fibres are formed, hyalinization occurs. There is also formation of scar tissue in which collagen turn over increases. The gross appearance of remodeling scars suggests that collagen fibres are altered and rewoven into different architectural patterns with time. Initially the strength of the wound is only that of the clot, which cements the cut surfaces together. Later on various changes take place in the wound healing process as mentioned earlier. At the end the breaking strength of the wound increases correspondingly to the increase in the amount of collagen formation (Robbins, 2001).
The three different models were used in our study to assess the wound healing effect of various extracts and the isolated constituents on various phases of wound healing, which run concurrently, but independent of each other. The standard drug nitrofurazone is used as a standard reference to assess the healing effect of the drug and the constituents against the controls. Jaswanth, et. al, (2001) and Hemalatha, et. al., (2001) also used nitrofurazone as the standard drug to evaluate the wound healing effect of Aegle marmelos and Indigofera enneaphylla Linn. respectively. In our study, significant wound healing activity was observed in the animals treated with aqueous extract of stem bark of D. cordifolia. The healing time required for complete epithelialization of the excision wound was found to be very less in this group of animals. In nitrofurazone treated animals, the epithelialization was complete on 17th post wounding day. While in D. cordifolia aqueous extract treated animals the epithelialization was complete on 18th post wounding day. In case of D. cordifolia carbon tetrachloride extract treated and the control animals, complete wound contraction occurred on 21st and 22nd days respectively.

Among the crude extracts of P. marsupium, the methanol extract exhibited significant wound healing activity and it was on par with that of the D. cordifolia aqueous extract. The rate of wound contraction was faster in these and the complete epithelialization of the excision wound was observed on 18th day. While, in aqueous extract treated animals healing was delayed by three days.
In the animals treated with the isolated constituent lupeol the percentage of wound contraction observed on 19th day was higher than that of the ursolic acid treated animals. The percentage of wound contraction was still lesser in the animals treated with betulin, betulinic acid. It was very least in the animals treated with diospyrin.

The breaking strength is the strength of a healing wound and is measured experimentally by the amount of force required to disrupt it. In the beginning a wound will be having little breaking strength because the clot alone will be holding the edges together. Thereafter breaking strength increases rapidly as collagen deposition increases and cross linkages are formed between collagen fibers. By the 8th to 10th day there is sufficient restoration of breaking strength and stitches can be removed. The breaking strength of a wound is a point of practical importance in surgery.

In the incision wound model, the animals treated with aqueous extract of *D. cordifolia* showed significant increase in the breaking strength on 10th post wounding day. The breaking strength of the animals treated with petroleum ether and CCl₄ extracts of *D. cordifolia* were comparatively less. The animals treated with methanol extract of *P. marsupium* showed significant increase in the breaking strength on 10th post wounding day. The results of this investigation coincides with the works of Tarnahalli, *et.al.*, (1996) on *Trigonella foenum gracum*, Leite, *et.al.*, (2002) on *Veronica scorpioides*, Shirwaikar, *et.al.*, (2003) on *Desmodium triquetrum* leaves and Patil, *et.al.*, on
Eclipta alba linn. In incision wound models also, the constituent lupeol was found to be more effective in increasing the breaking strength (475.13±0.685g) as similar to that of the standard drug nitrofurazone (558.95±1.495g) and others constituents were comparatively less effective.

The presence of the foreign body in the subcutaneous area initiates the formation of granulation tissue around it. Initially new blood vessels are formed accompanied by lymphatics. These arise from the preserved lymphatics at the margins of the wound. In the initial three days of the injury the intercellular spaces are filled with proteinous fluid. Later the fluid becomes gelatinous and shows increasing quantities of mucopolysaccharides which are either produced locally by fibroblasts or mast cells or come from the blood. The intercellular fibres are laid down in the wound fluid from the 4th day onwards and the concentration of mucopolysaccharides starts declining. At first these are fine thread like, later they coarsen and thicken. In the beginning collagen fibres run parallel and in one plane but soon their arrangement is remodelled to suit local mechanical stresses. Finally it forms a tough membrane of laminated collagen, which is the essential material for healing of the wound. By this time fibroblasts decrease in number and appear as shrunken in conspicuous fusiform cells in between rows of collagen fibres. The concentration of mucopolysaccharides becomes normal or even low. The wound has now acquired significant tensile strength (Somen Das, 2001).
The newly formed tissue is known as granulation tissue because it has the appearance of pink granules protruding from the floor of the wound. Microscopically these granules show newly formed capillaries, fibroblasts and leukocytes. Because of more vascularization, granulation tissue bleeds easily on the slightest trauma. It lacks nerves and therefore insensitive. It is also resistant to infection because of macrophages present in its interstices. As more and more collagen fibres are laid down, vascularization in the granulation tissue decreases. The conversion of granulation tissue into fibrous scar tissue is known as cicatrization. The breaking strength of the granulation tissue increases proportionately with the collagen deposition. The hydroxyproline is an imino acid distributed mainly in the collagen tissue. The hydroxyproline content of granulation tissue also increases with the increased collagenation.

The effects of oral administration of suspensions of the stem bark extracts on the dead space wound models were also assessed by the increase in the weight of granulation tissue, by the increase in its breaking strength and hydroxyproline content of the granulation tissue. 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. Among these treated animals the response was shown to be the best in *D. cordifolia* aqueous extract and lupeol treated animals. The result was assessed by the increase in granulation tissue weight, its breaking strength and the hydroxyproline content. Diospyrin is being the least potent among the remaining constituents. Among
the animals treated with the extracts of *P. marsupium*, the response was more marked in the animals treated with the methanol extract.

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. 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* showed complete epithelialization, fibrosis and collagen formation. Whereas, in the carbon tetrachloride extract treated animals the healing activity was comparatively less and it is indicated by the presence of the monocytes and fibroblasts.

Histopathological study of the granulation tissue evidenced the wound healing property of the stem bark of *P. marsupium*. The sections of the granulation tissue of the animals treated with methanol extract showed complete epithelialization, fibrosis and collagen formation. Whereas, in the aqueous extract treated animals the healing activity was comparatively less. From these findings it was concluded that methanol extract of stem bark exhibited significant wound healing activity. These results also supported the ethnomedical use of *P. marsupium* in healing of wounds.

Histopathological examination of the sections of the granulation tissues of the animals treated with lupeol showed lesser monocytes, fibroblasts and
increased collagen deposition. This fact suggests its potent wound healing property. Comparatively lesser collagen formation was observed in the animals treated with ursolic acid. While, the wound healing potency was still lesser in case of betulin, betulinic acid and minimum with diospyrin.

Similar type of investigation has been reported by Leite, et.al., (2002) on Vernonia scorpioides leaves, Shirwaikar, et. al., (2003) on Desmodium triquetrum leaves. There are reports that the plants having antioxidant property would also enhance wound healing activity (Shirwaikar, et. al., 2003). Hence the better wound healing activity was observed in aqueous and petroleum ether extracts of D. cordifolia. The content of the triterpenoids was more in these extracts. The variation in the potency of the constituents may be due to their structural changes. In P. marsupium, the methanol extract contains higher amounts of flavonoids, which are also having antioxidant property. This fact confirmed the better wound healing potency of the methanol extract.

It has been claimed that the alcoholic extract of the plant D. cordifolia has CNS depressant (Singh, et. al., 1971) and analgesic (Kohli, et. al., 1972) activity. In the present study the different extracts of D. cordifolia and P. marsupium and the isolated constituents of D. cordifolia were screened for analgesic activity by tail-flick, tail-immersion and acetic acid induced writhing methods.

Analgesics are of two types; opioid and non-opioid. The term 'opioid' is used to denote all naturally occurring (morphine, codeine), semisynthetic
heroin, dihydroxymorphinone, oxymorphone) and synthetic (pethidine, methadone, pentazocine) drugs which have a morphine-like action viz. relief of pain and depression of the CNS. The opioid drugs produce their effects by combining with opioid receptors, which are widely distributed in the CNS and other tissues. The opioid receptors have been classified into mu, delta, kappa (K1 and K2) and sigma types. The vast majority of opioid drugs used as analgesics are agonists at mu receptors. The major drawbacks of these opioid analgesics are the development of tolerance and physical as well as psychological dependence.

The nonopioid analgesics relieve pain without interacting with opioid receptors, reduce elevated body temperature, possess anti-inflammatory property and are called as ‘Non Steroidal Anti-inflammatory Drugs (NSAIDs) and are non-addicting drugs. These effects are achieved with doses that do not produce significant depression of CNS. The NSAIDs can be classified mainly into two groups, namely, non-selective COX inhibitors (acetyl salicylic acid, paracetamol, phenylbutazone, diclofenac, ibuprofen, piroxicam etc) and selective COX-2 inhibitors (nimesulide, meloxicam, celecoxib, rofecoxib). Though these drugs have different chemical structures, they produce qualitatively similar actions. During inflammation, pain and fever, arachidonic acid is liberated from phospholipid fraction of the cell membrane. This acid is then converted via cyclo-oxygenase (COX-1 and COX-2) pathways to prostaglandins. These prostaglandins sensitize blood vessels to the effects of inflammatory mediators that increase permeability. The prostaglandins
particularly PGE and PGI produce hyperalgesia associated with inflammation. They sensitize the chemical receptors of the afferent pain endings to other mediators such as bradykinin and histamine. Further, release of prostaglandins in the CNS may lower the threshold of the central pain circuits. Prostaglandins are also involved in the pyretic response in man and PGE might be a final mediator of CNS fever mechanism.

Aspirin and other NSAIDs block the synthesis of prostaglandins by inhibiting COX-1 and COX-2 producing beneficial therapeutic effects. These drugs are effective as analgesics only in pathological conditions and in experimental models where prostaglandins are synthesised locally.

The commonest adverse effects of aspirin and other NSAIDs are dyspepsia, nausea, vomiting, heartburn and ulceration. All NSAIDs can cause acute or chronic renal damage following repeated use, sometimes as short as two weeks. They may cause acute renal failure, mild renal impairment and chronic renal impairment due to papillary necrosis or interstitial fibrosis and serious hyperkalemia. Inhibition of synthesis of prostaglandins within the kidney has several effects. The protective intrarenal vasodilator effect is lost, renal blood flow and glomerular filtration rate are reduced, the natriuretic effects of PGE-2 on the renal medulla is lost with consequent sodium retention, hyperkalemia occurs due to reduced aldosterone synthesis secondary to inhibition of renin synthesis by NSAIDs. The sodium retention caused by NSAIDs may cause edema and congestive heart failure.
Recent studies indicate that aspirin is perhaps safer in that its chronic use is less associated with analgesic nephropathy. The possibility of chronic ingestion of analgesics should always be borne in mind while dealing with unexplained chronic renal damage.

The analgesic activity can be determined in laboratory animals, by noting the reaction time to the painful stimulus. Painful reaction in experimental animals can be produced by applying noxious stimuli such as (i) Thermal (radiant heat as a source of pain), (ii) chemical (irritants such as acetic acid and bradykinin) and (iii) physical pressure (tail compression). In the laboratory, commonly used methods are tail-flick (tail-withdrawal from the radiant heat) using analgesiometer, caudal immersion of rats' tail in hot water and acetic acid induced writhing. We have adopted all these three methods for screening the efficacy of the test drugs.

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. This was followed by aqueous extract. The response was insignificant with carbon tetrachloride extract when compared to other extracts. This might be due to the presence of higher contents of the triterpenoids in the petroleum ether extract. In case of *P. marsupium* both the methanol and aqueous extracts were devoid any analgesic activity. Similarly Dongmo, *et al.*, 

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(2003) has reported the analgesic effects of the stem bark extract of *Mitragyna ciliata* and Choudhary, *et al.*, (2004) have studied the analgesic activity of *Capparis zeylanica* root extract.

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 earlier reports also indicated that the purified mixture of ursolic, oleanolic and micromeric acids isolated from ethanol extract of dried leaves of *Bouchea fluminensis* exhibited significant analgesic activity (Costa, *et al.*, 2003). They also observed that naloxone reverted the analgesic activity of the said mixture of triterpenes. This suggested that the central action of the triterpenes is probably occurring either through the central opioid receptors or by promoting the release of endogenous opiopeptides.

Epilepsy is a collective term for a group of chronic seizure disorders characterized by sudden and transient episodes of loss or disturbance of consciousness. This is associated with characteristic body movements (convulsions) and sometimes with autonomic hyperactivity.

The cause of epilepsy may be due to occasional, sudden, excessive, rapid, local discharges of gray matter. Modern electrophysiology has confirmed this. The characteristic pathophysiology event in a seizure is believed to be paroxysmal depolarization shift of neuronal membrane potential and associated burst discharge.
Neurotransmitters are believed to play a vital role in the initiation and spread of the seizure discharges in epilepsy. Excitatory neurotransmitters such as aspartate, glutamate and acetylcholine are thought to be involved in the initiation and spread of seizure discharge. Inhibitory neurotransmitters, such as glycine, norepinephrine and gamma-aminobutyric acid (GABA) are believed to be responsible for termination of seizure activity.

The normal brain contains billions of neurons, which fire asynchronously. Inhibitory feedback loops in the normal brain regulate the frequency of firing of the individual neurons and prevent synchronization. When such inhibitory feedback is defective, a large number of cells in a given area of the brain fire at the same time and produce a self-regenerative electrical impulse. Such an area constitutes an “epileptic focus”.

An ideal antiepileptic drug should have favourable profile not only in terms of clinical efficacy but also from the pharmacokinetic, toxicological, pharmaceutical and economical point of view and choice of drug for different epilepsies therefore differs. The goal of anti epileptic drug therapy is to achieve complete seizure control with a single drug taken once or twice a day, without side effects. Approximately 70-80% of patients who develop epilepsy may expect to have their seizures controlled with a single drug, while others need combination therapy for improved seizure control (Duncan, et. al., 1995).
Till today the drug therapy of epilepsy is not satisfactory, probably due to incomplete understanding of exact biochemical anomaly, for its pathogenesis. Further, a combination of anti-epileptic drugs suffers high toxicity and drug interactions, which may complicate clinical management (Kulkarni, et. al., 1999; Leppik, et.al.,1993). Even normal therapeutic doses of anti-epileptic drugs produce hepatomegaly, jaundice, megaloblastic anemia, aplastic anemia, alopecia, bone marrow depression, hepatitis and skin disorders. Many anti-epileptic drugs have pharmacokinetic interactions and interfere with the metabolism of other drugs e.g. Phenytoin, carbamazepine, phenobarbitone and valproic acid. Withdrawal of any anti-epileptic drug obviously can give rise to seizures (Delantry, et. al., 1998).

Different types of epilepsies i.e. grand mal, petit mal or psychomotor type can be studied in laboratory animals. The maximal electroshock (MES) induced convulsions in animals represent grand mal type of epilepsy. Similarly chemo-convulsions due to strychnine which produce clonic-type of convulsions resemble petit mal type convulsions in man. These are the two procedures used to study convulsions and to test anticonvulsant drugs in laboratory animals.

In MES convulsions electric shock is applied through the electrodes to induce convulsions in the experimental animals. The MES convulsions are divided into five phases such as a) tonic flexion b) tonic extensor c) clonic convulsions d) stupor, and e) recovery and death. A substance is known to possess anti-convulsant property if it reduces or abolishes the extensor phase of MES-convulsions.
Strychnine is a central nervous system stimulant. It produces jerky type of clonic convulsions in rats. The convulsive effect of this drug is considered to be analogous to petit mal type of convulsions in man. Strychnine produces jerky movements of the whole body and powerful tonic convulsions of the body and limbs. As a result when the animal dies, its body becomes opisthotonus (arch-shaped).

The petroleum ether extract of *D. cordifolia* has exhibited anti-convulsant property by reducing the extensor phase and faster recovery of MES-induced convulsions postponed the onset of convulsions induced by strychnine but failed to give protection against mortality in the experimental animals. While the aqueous extract was found to be less potent in this respect. Carbon tetrachloride extract of *D. cordifolia* did not show any effect. Both the extracts of *P. marsupium* were devoid of any anti-convulsant activity. Among the constituents isolated from the *D. cordifolia*, only ursolic acid and lupeol exhibited some anti-convulsant activity. The standard drugs, phenobarbitone abolished the extensor phase of convulsions and the recovery was faster and diazepam abolished the strychnine-induced convulsions. In these animals, the mortality was zero percent.

Physiologically, sleep is regarded as absence of wakefulness. Every one needs sleep. It is believed that restoration of natural balance among the neuronal centres in the brain take place chiefly during sleep, and the association between sleep and growth in the early years of life is generally accepted.

Insomnia may also be caused by drugs such as, ephedrine, chloroquine, metronidazole, diuretics, systemic glucocorticoids etc. The commonest cause of insomnia in the elderly is age-related changes in the sleep cycles, anxiety and loss of family support. In such cases only some extent of improvement can be achieved rather than total relief of insomnia.

The commonly used hypnotics are barbiturates and benzodiazepines. The barbiturates facilitate inhibitory neuro-transmission in the CNS, presumably by interacting with the alpha subunits of the GABA-A receptors to open chloride ion channels and hyperpolarize neuronal membrane. They also inhibit calcium current in the neurons. They depress the polysynaptic responses and delay synaptic recovery. The barbiturates such as phenobarbitone, pentobarbitone, thiobarbitone, hexobarbitone etc. have the risk of drug automatism, suicidal tendency, rapid development of tolerance and drug dependence. Benzodiazepines act on the alpha subunits of the GABA-A receptors surrounding the chloride ion channel in the CNS, a site different from that of barbiturate action. Their action is more selective than that of barbiturates. Hence, the benzodiazepines are the drugs of choice as hypnotics.
However, the benzodiazepines also can cause drug dependence, tolerance anterograde amnesia and rebound insomnia.

Most of the drug activities on CNS influence the loco motor activity in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the loco motor activity while the CNS stimulants such as caffeine and amphetamines increase the activity. In other words, the loco motor activity can be an index of wakefulness (alertness) of mental activity. The locomotor activity can be easily measured using an actophotometer, which operates on photoelectric cells, which are connected in circuit with a counter. When the animal cuts off a beam of light falling on the photocell, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves.

In the present investigation also, significant reduction in locomotor activity was observed in petroleum ether extract of *D. cordifolia* and the isolated constituent betulin, but carbon tetrachloride extract was less potent in this respect. Methanol extract of *P. marsupium* was more effective than the aqueous extract. Chlorpromazine is a selective depressant of CNS and used as a reference standard during the experiments. It also reduced the spontaneous motor activity significantly. The effect of the crude extracts of the medicinal plants on spontaneous motor activity has been studied on *Cistanche deserticola* (Ming-Chin Lu, 1998), *Dalbergia malabarica* (Nagarajan, et. al., 2003), *Ficus platyphylla* (Chindo, et.al., 2003).
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. The loss of righting reflex indicates the onset of action. While, the duration between the loss of righting reflex and the regain of righting reflex indicates the 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 betulin respectively. The sedative effect of the constituent betulin may be applicable in controlling the aggressive and destructive behaviour of the patients.

Similar type of assessment of sleeping time has been reported on the crude extracts of *Cistanche deserticola* (Ming-Chi Lu, 1998), *Diospyros mespiliformis* (Adzu, et. al., 2002) and *Zizyphus spina-christi* (Adzu, et. al., 2002).

The effect of these crude extracts and the active constituents may be due to their ability to increase the concentration of gamma-amino-butyric 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). This may be otherwise due to the 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.