V. DISCUSSION

From a time immemorial, plants have served as drugs to cater the medical needs of human beings and animals. Despite the enormous development in allopathic medicines, plants stood a prime position in current therapy. According to Chopra (1965) the traditional and modern medicines were vary widely in their philosophical foundations. Apart from their divergent views on the nature of man’s health and ace, they differ primarily in the mode of development and application of medicine. The men of traditional medicine use plants and plants parts, as such and their preparation are rather simple and crude. On the other hand, the modern medicine involves isolation, purification and sophisticated administration of medicine from a single source.

In traditional system of medicine many plants acts as a potentive drug to cure serious ailments like, cancer, hepatitis, diabetes, arthritis etc. For example Catharanthus roseus contain cytotoxic indole alkaloids vinblastine, vincristine, catherine and strictosides which are therapeutically active against blood cancer (Svoboda and Blake, 1975; Krueger, et al., 1982 and Heijden, et al., 1989). The investigations of Kawasaki, et al., 1987, Hayashi, et al., 1988 and 1991 also stated that Scopalia dulcis possess potent inhibitory activity against the replication of herpes simplex virus type-1 (HSV-1).

The green revolution, in terms of herbal medicine has now achieved astonishing popularity in the world. Many pharmaceutical manufacturers have recognized that plants with folkloric reputations that probably provide the best source of constituents that can serve either as new drugs or as prototypes of them. Compounds from natural sources may have significant roles in modern medicines. They provide a number of extremely useful drugs, many of them are not possible to produce commercially by synthetic means. For example, alkaloid from Papaveraceous and Solanaceous plants, Cardiac glycosides from
Digitalis purpurea, Taxol from *Taxus buccata*, Penicillin from *Penicillium crysogem* etc. Natural sources also supply basic compounds that may be modified slightly to render them more effective or less toxic (ex. morphine). Sometimes the natural products can be utilized as prototypes or models for synthetic drugs possessing physiological activities similar to the originals (ex. Glycopyrrolate is a prototype synthetic product derived from atropine. Similarly, chloroquine from quinine, Ibuprofen from salicin and salicylic acid (James, et al., 1996).

The traditional claims indicated that the plant *Elephantopus scaber* has been useful in treating various ailments such as wound healing, diabetes, skin disorders (Bapalal, 1999), cardio tonic, ulcers, eczema, swelling in stomach (Chopra, et al., 1956) liver troubles, inflammation, blood and in heart diseases, analgesic, diuretic, syphilis, antipyretic, etc (Kirtikar and Basu, 1991). The therapeutic efficacy of this species on different ailments might be due to a specific single constituent or may be due to additive effects, which may be responsible for different activities. So in view of medicinal value, the present investigation is focussing to derive protocol for *in vitro* regeneration of plantlets from different explants, isolation of a sesquiterpene compound deoxyelephantopin from the leaves and comparative pharmacological evaluation of crude extracts, different fractions and isolated constituent against wound healing, hepatoprotective and anti-inflammatory models in rats.

Plant cell and tissue culture is a promising tool for rapid propagation and multiplication of large number of medicinal plants such as, *Withania somnifera* (Jayanthi and Sharma, 1991); *Phyllanthus fraternus* (Rajasubramanian and Saradhi, 1994); *Eclipta alba* (Zafer and Sagar, 1999); *Cunila galiodes* (Fracaro and Echeverrigaray, 2001) etc. Further, *in vitro* technique holds tremendous potential for the production of high quality plant based medicines.
The increasing demand for medicinally valuable natural products such as steroids, alkaloids, glycosides, flavonoids etc. has resulted in increased attention in plant tissue culture (Dziezak, 1986). By using this technique many medicinal plant parts are cultured to obtain enhanced production of secondary metabolites. The investigation of Arellano, et al., (1996) proved that the root callus culture of *Perezia cuernavacona* was used as an alternative for enhance production of sesquiterpenes, quinines perezone.

Although leaves and root parts of *Elephantopus scaber* possess immense medicinal value, protocol for rapid micropropagation has not been standardized by the earlier workers. Therefore in the present investigation an attempt has been made to know the comparative morphogenic potentialities of different explants of *Elephantopus scaber* on *in vitro* condition.

According to Larkin and Scowcroft (1981) culture of plant cell and tissue on a chemically defined media, offers the opportunity to regenerate and select plants with desirable characteristics, which are otherwise difficult to obtain by traditional plant breeding. However, it is not an easy task to propose a generalized medium for achieving totipotency in all plants or even propose a generalized plant. Because, the kind and balance of growth regulators need for various morphogenic phenomenon vary from tissue to tissue depending on their metabolic status (Tisseral, *et al.*, 1985; Hussay, *et al.*, 1986).

In the present investigation, it was found that MS medium was an effective nutrient medium than LS and B5 media used for initiation and maintenance of callus from leaf explants of *Elephantopus scaber*. Most of the earlier reports (Bermudz, *et al.*, 1984; Komalavali and Rao, 1997 and Patnaik and Debata, 1998) also stated that MS was the balanced medium for the culture of dicotyledonous species. When compared to MS medium the ionic concentrations of B5 medium was more and the ionic concentration of
LS medium was less. However, the earlier workers (Maruthi, et al., 2004) observed that LS medium was suitable for the culture of explants of woody species. In the present investigation LS medium is not effective for induction and maintenance of callus.

The results obtained by culturing the excised leaf explants of *Elephantopus scaber* indicated the various parameters regulating the dedifferentiation and also the factors responsible for regeneration in vitro. It was found that MS medium was an effective nutrient medium for the maintenance of callus and regeneration of plantlets from the leaf explant culture. Earlier reports by Bermudez, et al., (1984), Lai and Ahuja (1989) and Omstrup, et al., (1993), Piereira, et al., (2000) and Koroch, et al., (2002) also used MS medium for the propagation of many medicinal plants.

As in the culture of leaf segments of *Flavaria trinervia* (Sudarshana and Shanthamma, 1991) *Boerhaavia erecta* and *B. rependa* (Krishna,1996) *Enicostemma axillare* (Sudhersan, 1998), both endogenous and exogenous hormones played prominent role in the initiation and maintenance of callus. Generally, endogenous auxins are localized at the meristematic regions, particularly in very young leaves. This endogenous hormone level has been shown to decrease towards the apex. The results obtained in the culture of leaf segments of *Elephantopus scaber* indicates that the meristematic activities were more at the mid-dorsal vein of the basal leaf segments and callus initiation was noticed within 7 days of inoculation. This may be due to the occurrence of more endogenous auxins. Whereas, in the middle and apical segments of the leaf, delayed callus initiation was observed. In *Elephantopus scaber* luxuriant callus proliferation was first noticed from the basal segments of the leaf. It was first initiated from the mid-vein in the form of knot like structure. Gradually callusing was proceeded all over the surface of the lamina.
This confirms the existence of more endogenous auxin level at the mid-vein of the leaf.

In the culture of different segments of *Elephantopus scaber* application of NAA and BAP rendered lesser callogenic efficacy than 2, 4-D. The prime hormone 2, 4-D induced callogenesis either alone or in combination of lower concentration of BAP. Among different explant cultured callogenic intensity was less in meristem explants. In the presence of BAP organogenesis of shoot initials occurred both from the callus and from the uncallused explants.

Many authors (Razdan, 2002; Chalupa, 2003) pointed out that the action of growth regulators *in vitro* is species specific and differs in their physiological status and concentrations. In *Elephantopus scaber* maximum growth of the callus was observed at the concentration of 2.5 mg/l. 2, 4-D + 0.5 mg/l of BAP.

The mechanism of differentiation through the callus is almost similar in the culture of all the explants of *Elephantopus scaber*. But the intensity of shoot differentiation differed from explants to explants. This may be due to varied concentrations of endogenous growth regulators. The earlier reports have mentioned that those tissues retained inherent natural auxin in sufficient amount were able to induce differentiation (Sudarshana and Shanthamma, 1989). This view ultimately leads to the question as to whether regeneration can be easily restored by external supplementation of cytokinin alone or in combination with auxins. In most of the plants the decisive factor controlling organogenesis in tissue culture was the balance of cytokinin and auxin ratio. *Phyllanthus amarus* (Sudarshana, 1989), *Trichopus zeylanicus* (Krishnan, et al., 1995), *Centella asiatica*, (Tiwari, et al., 2000) etc. In the present study also auxin and cytokinin interactions provokes the direction of morphogenesis. The interaction of BAP at higher levels and NAA at lower levels induced shoot
bud differentiation form the leaf calli. When the concentration of NAA was increased, the shoot inducing ability of BAP was suppressed. This showed that cytokinins are the causative substances for shoot bud initiation and auxins are the promoter for morphogenic induction (Skoog and Miller, 1957; Torrey, et. al., 1961).

The interaction of BAP and NAA at the range of 2 to 3 mg/l and 0.05 to 1 mg/l respectively provoked shoot bud differentiation from the calli of all the explants. The reports of Laxmisita and Raghavaswamy (1992); Maruthi, et al., (2004) etc. also stated that BAP is an effective cytokinin have been most exploited for the micropropagation of both herbaceous and tree species.

Literature survey also indicated that in many plant species root explants have been cultured successfully to induce organogenesis Bermudez, et al., (1984), Sreenath and Chandra (1989), Sinha and Mallick (1991), Louis and Torrey (1991), Rani and Grover (1999) Ishizaki, et al., (2001), in the present study also plantlets were regenerated from excised root calli of *Elephantopus scaber*.

One of the striking feature observed in the culture of *Elephantopus scaber* was the differentiation of both shoot and root initials from the same differentiating calli. In most of the species the differentiating media is suitable only for caulogenesis. To induce rhizogenesis the microshoots has to be excised aseptically and transferred on to semi solid or liquid media containing optimal concentration of rhizogenic hormone. But in *Elephantopus scaber* differentiation of shoot initial were noticed first, later organogenesis of whitish hair like roots were noticed after three weeks of culture. Some times when the organized shoot comes in contact with the medium, it induces root initials. This feature is more convenient for the large-scale propagation of this species.
The rate of survival of regearants was also high. Though, it is an herbaceous plant endemic to the moist deciduous forests of the tropical countries its reproductive stage is also very short. In Western Ghats of India flowering season completes within September. Due to over exploitation and uprooting, many traditional practitioners faced the unavailability of this species.

In the present investigation protocols were standardized for the regeneration of the plants using vegetative explants. However, due to time constraints the biosynthetic potentialities of the in vitro regenerants derived from different cell lines for varied concentrations of sesquiterpene lactones has not been worked out. The same can be carried out in future.

As early as in 1969, Sim and Lee, have isolated epifriedelinol, lupeol and stigmasterol, potassium chloride and a mixture of triacontan-1-ol and dotriacontan-1-ol. De Silva, et al., (1982), have reported the plant Elephantopus scaber found to contain, a germacranolide dilactone-11, 13-dihydrodeoxyelephantopin. Paul Pui-Hay But, et al., (1997) have isolated sesquiterpene lactones- deoxyelephantopin, isodeoxyelephantopin and a new germacranolide sesquiterpene lactone scabertopin from Elephantopus scaber.

The sesquiterpene lactone, deoxyelephantopin, has showed significant inhibitory activity against the walker 256 carcinosarcoma in rats. This showed its potent anti-tumour activity (Mitsumasa Haruna, 1985). In the present study, sesquiterpene lactone was isolated from the ethanol extract of the aerial parts of Elephantopus sacber. The UV, IR and MASS spectral analysis of the isolated constituent confirmed the structure of the compound as deoxyelephantopin.

Deoxyelephantopin possess alpha- methylene gamma lactone in the structure. The compounds having alpha- methylene gamma lactone moiety
possess, anti-ulcerogenic activity (Giordano, et al., 1990) used externally to treat wounds and has a broad range of biological activities (Jean Bruneton, 1999). The earlier investigators reported the anti-bacterial (Fischer, et al 1998), anti-fungal (Alejandro, et al., 2000) and anti-oxidant (Rekha, et al., 1966), properties of sesquiterpene lactones isolated from other Asteraceae members. The traditional claims indicated that the plant *Elephantopus scaber* (Asteraceae) is being used to heal wounds. The healing property of this species is due to the presence of sesquiterpene lactones.

To ascertain pharmacological aspects, the different solvent extracts of the aerial parts of the *Elephantopus scaber* were subjected for evaluation using three wound healing models.

Wound healing is a fundamental response to tissue injury, by which a damaged tissue restored as closely as possible to its normal size. It is a complex and cellular event, which can be affected by local and systemic factors, out of which, infection is the most important factor causing delayed, wound healing. Infection leads to increased collagenolysis and shortage of essential nutrients for the fibroblasts. The major components of wound healing are haemostatis, inflammation, epithelization and wound repair. Haemostatis is the first step in the process of healing, where reflex vasoplasm secures hemostats of small blood vessels and clotting mechanism comes next to stop continued bleeding. Platelets are critical in haemostatis and wound healing. They release cytokines, considered as a wound hormones which have a vide variety of effects and play a fundamental role in the process of wound repair. Cytokines are also produced from other cells like neutrophils, lymphocytes, macrophages, endothelial cells and smooth muscles.

Cytokines like Transforming growth factor beta (TGFD-B) plays an important role in collagen metabolism, fibroblast proliferation and chemotaxis.
The Platelet derived growth factor (PDGF) does similar function, along with activation of macrophages, and neutrophils.

Epidermal growth factor (EGF) does the function of epithelial cell and proliferation, granulation tissue formation. Basic fibroblast growth factor (b-FGH) carries out the function of growth and differentiation of fibroblasts, proliferation of vascular smooth muscle cells and endothelial cells (Sanjay Azad, 2002).

During inflammation local blood flow increases leading to characteristic local heat and redness. Cellular events are dominant in this stage. The platelets are the first cells to arrive at the site of inflammation. A fibrin network forms over the wound. Inflammation and repair proceed simultaneously with successive formation of granulation tissue, synthesis of wound collagen and ground substance. The wound contraction and scar formations are the final stages in wound healing.

Granulation tissue derives its name from the pink and granular appearance on the wound surface. It is primarily composed of fibroblast and small new blood vessels. The fibroblasts proliferate and some of them acquire the properties of the smooth muscle cells myofibroblasts. These have an important role in wound contraction. The granulation tissue also contains a large number of macrophages, mast cells and some times neutrophils, lymphocytes and eosinophils. The macrophages have a phagocyte function and are responsible for clearing up of debris, foreign matters and fibrin.

The stage of granulation tissue formation smoothly merges in to the stage of collagen and matrix formation with the fibroblasts producing both collagen and matrix. Collagen is the major component of extracellular tissue including soft tissue, ligaments and tendons. The main function of collagen is
support, provide strength and shock absorption. Collagen is composed of three polypeptide chains, containing amino acids- hydroxyproline, proline and glycine.

TGF-B has a significant role in collagen deposition. The matrix is produced by fibroblasts and has various components including collagen, elastin, proteoglycans, hyaluronic acid, laminin and fibronectin. These have different functions including expansion and contraction of tissue, regulation of connective tissue structure and cell adherence. The stage of collagen and matrix formation merges in to the stage of scar formation (cicatrization).

Scar is a metabolically active and dynamic tissue and wound contraction is an essential component in scar formation. Scar remodeling implies a process of reorientation of collagen fibres and the collagen content becomes high. During scar maturation, molecular orientation of the collagen and its chemical cross-linking strengthens the wound healing tissue.

Wound healing can be assessed by three different wound models to monitor all the phases of healing. They are excision wound model, incision wound model and dead space wound model. Excision wounds are of different size and shape at different sites has been employed to study the various physical attributes.

The commonly used animals for wound healing models are rats, rabbits and guinea pigs. Morton and Malone (1972) have employed circular wounds of 2.5 cm diameter made on depilated dorsal thoracic region in rats. The rats are left undressed to the open environment. The drug i.e. the reference standard (0.2% nitrofurazone ointment), simple ointment (control) and the test drug ointment or different other forms are administered till the wound is completely healed. This model is used to monitor wound contraction and epithelization.
time. Wound contraction is calculated as percent reduction in wound area. The progressive changes in wound area are monitored planimetrically by tracing the wound margin on a measurement of wound area on graph paper is expressed as unit (mm²) (Saha, et al., 1997), Udupa, et al., 1994, Bhakta, et al., 1998, Mukhergee, et al., 2000a).

In the present study also, significant wound healing activity in the excision wound was observed in the animals treated with the isolated compound deoxyelephantopin, as similar to that of nitrofurazone treated animals.

In the excision wound model, significant closure of wound was observed on the day 16, in the animals treated with the isolated compound deoxyelephantopin (98.6±0.37), methanol fraction (94.9±0.55) and nitrofurazone ointment (100.0). All these were statistically significant (p<0.01), when compared with the control animals (85.8±0.69). Moderate percentage of wound closure was also observed in aqueous (89.8±0.60) and ethanol extract (92.4±0.64) treated groups and in hexane fraction treated animals, the percentage of wound closure was very insignificant.

The time of epithelization is noted as the number of days after wounding required for the scar to fall off leaving no raw wound behind. The healing time required for complete epethelization of the excision wound was found to be very less in deoxyelephantopin and methanol fraction treated group of animals.

In the animals treated with deoxyelephantopin and methanol fraction, mean time for complete epithelization was 15.0±0.26 and 15.6±0.21 days respectively, which is nearer to the value of standard drug nitrofurazone treated animals (14.0±0.26). The longest period for epithelization was taken in control (21.0±0.37) and hexane fraction (20.0±0.26 days) treated animals.

In the incision wound model, tensile strength of the wound represents the promotion of wound healing. Tensile or breaking strength is the force required for opening the healing skin and is used to measure the amount of healing. Usually wound healing agents promotes gaining of tensile strength, because of increased deposition of collagen content that form cross-linkages between the collagen fibres. The instrument used for this purpose is Tensiometer. One day before performing the experiment the sutures are removed from the stitched wounds of rats after recovery and tensile strength is measured.

The animals treated with deoxyelephantopin and methanol fraction (412.0±11.37 and 390.6±9.33 respectively) showed significant increase in the tensile strength, while the tensile strength of the control animals were comparatively less ((298.6±8.48). A moderate gain in tensile strength was noticed in the animals treated with aqueous and ethanol extract and insignificant effect in hexane fraction treated animals were recorded. The results of methanol fraction and deoxyelephantopin are comparable with that of the standard drug nitrofurazone ointment (420.0±6.35).

A similar type of wound healing activity was observed by Tamahalli, *et al.*, (1996) on *Trigonella foenum gracecum* and Leite, *et al.*, (2002) on *Vernonia scorpiodes*.

In dead space wound model, granulation being an essential basic event in wound repair, quantitative estimation of granulation tissue formed on foreign bodies implanted in dead space wounds has been shown to be reliable
parameter in the study of wound healing. Granulation tissue is also used to estimate tensile strength, collagen content and for histological studies.

The granulation tissues formed on the piths were harvested on the 10\textsuperscript{th} post-wounding day. The buffer extract of the wet granulation tissue is used for the determination of tensile strength. (Trackman, \textit{et al.}, 1981). Part of the granulation tissue dried and acid hydrolysate was used for the determination of hydroxyproline content (Neuman, \textit{et al.}, 1950).

The changes in the biochemical parameters affecting wound healing in dead space wound model like granulation tissue weight, lysyloxidase activity, hydroxyproline levels were measured, which were increased considerably.

The increase in tensile strength of granulation tissue is not only due to increased cross-linking via lysyloxidase but also due to possible interactions (Non-covalent, electrostatics) with the ground substance as evidenced by high significant increase in hexosamine and hydroxyproline content. (Peacock, 1996).

In the present study, the effects of oral suspensions of the different drug formulations and control on the dead space wound model were assessed by increase in its tensile strength. Among these treated animals, significant increase in tensile strength was noticed in both deoxyelephantopin (437.0±4.46) and methanol fraction (424.0±6.63) treated animals, indicating enhanced collagen maturation, which was comparable with that of the other test drug treated and untreated control animals.

The gross pith induced granulation tissue was harvested on the 10\textsuperscript{th} post-wounding day. The granulation tissue were kept for drying overnight in incubator at 60\degree C and weighed. Dry weight of the granulation tissue was
expressed as percentage of body weight of corresponding animals on 10th day of wounding.

There was significant (p<0.01) increase in the mean dry weight of the granulation tissue, indicating high protein content, in both deoxyelephantopin (76.0±1.15 mg) and methanol fraction (74.0±1.29 mg) treated groups.

The estimation of hydroxyproline content of the granulation tissue further evidenced the wound healing potency of the extracts. The dried granulation tissue was used for the estimation of the hydroxyproline content. Increased measurement of the breaking strength indicates the effectiveness of the drugs. Hydroxyproline content of granulation tissue was significantly (p<0.01) increased in animals subjected to the oral administration of deoxyelephantopin and methanol fraction treated groups with mean value of 9.80 μg/ml and 9.25 μg/ml respectively. In aqueous and ethanol extract treated groups it was 6.60 μg/ml and 8.20 μg/ml respectively, showing moderate increase in the concentration of hydroxyproline. In hexane fraction treated animals low concentration of 5.41 μg/ml of hydroxyproline was observed. This showed that the isolated compound deoxyelephantopin are highly effective as compared to the control treated groups (5.20 μg/ml) The increased content of hydroxyproline suggest the high protein content which is required for wound healing process.

Histology of granulation tissue has further evidenced the wound healing potency of *Elephantopus scaber*. Here also, significant result was observed in methanol fraction and deoxyelephantopin treated animals.

The granulation tissue of the control treated animals showed tissue with acute inflammatory cells, fibroblastic connective tissue and very little number of blood vessels. Whereas the section of ethanol fraction treated animals,
showed inflammatory cells, loose and scanty connective tissue and more number of capillaries. In deoxyelephantopin treated animals the histology of granulation tissue showed complete healing with more of fibroblasts with marked increase of collagen tissue and increased number of blood vessels.

Similar type of work has been reported on *Vernonia scorpioides* leaves (Leite, *et al.*, 2002), on *Desmodium triquetrum* leaves (Annie Shirwaikar, *et al.*, 2003) and the studies suggested that the plants having anti-oxidant properties may also enhance the wound healing property. The earlier investigators reported the antibacterial (Fischer, *et al.*, 1998), anti-fungal (Alejandro, *et al.*, 2000) and anti-oxidant (Rekha, *et al.*, 1966) properties of related sesquiterpene lactones isolated from Asteraceae members. The traditional claims of *Elephantopus scaber* also revealed that it has been using to cure wounds. Therefore the wound healing property of *Elephantopus scaber* is due to the presence of sesquiterpene lactone deoxyelephantopin.

The liver is the largest organ situated in right side of the body of mammals and is one of the most frequent exposed organs susceptible to the toxic injuries and invention of microbes. Any damage to hepatic cells disturbs body’s metabolism. Certain drugs like tetracycline, paracetamol (Wang, 1983) anti-tubercular drugs, oral contraceptives of hormonal origin, chemicals used as food preservatives and agrochemical may affect the integrity of the liver.

The toxic effect of carbon tetrachloride (CCL4) on experimental liver damages simulated with that of viral hepatitis (James, *et al.*, 1976). It can act directly on hepatic cells and would cause swelling resulting in mechanical obstruction of sinusoidal blood flow. Carbon tetrachloride induces a coagulate necrosis of hepatocytes that does not affect the sinusoid lining cells, permitting the retention of intact vascular pattern.
Toxic injury occurs as a result of its metabolic conversion by a complex of enzymes bound to membrane of smooth surfaced endoplasmic reticulum (SER). Effect of these enzymes is the major mechanism by which toxic compounds are converted to less toxic or non-toxic ones. In some instances nontoxic substances are metabolized to toxic ones as such in the case of carbon tetra chloride.

Carbon tetra chloride is converted by hemolytic cleavage to a highly reactive haloalkane free radical and a free radical as shown in the following reaction,

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

These free radicals in turn react with a variety of intracellular molecules, notably the unsaturated fatty acids that constitute membranes of the cell. For example, Polyenolic fatty acids are converted to organic free radicals, which in turn react with molecular oxygen to form organic peroxides. These compounds are highly unstable and decompose spontaneously to form aldehydes, ketones and other products. Free radicals are also capable of reacting with methylene bridges to form unstable peroxides, which can also form free radicals.

Carbon tetrachloride reacts with sulfhydryl groups, which mediate the function of many cell proteins, including a number of important enzymes and this reaction leads to their alkylation and subsequent loss of function. The radicals formed react with other molecules to form additional free radicals, such reactions are autocatalytic and tend to spread from a small focus to involve large areas of cytoplasm. The earliest change that has been detected in rat liver cells is a functional one that occurs 30 minutes after the intragastric administration of a single dose of 0.25 ml of carbon tetrachloride. It consists of
a rapid decrease in synthesis of the export protein albumin (Zimmerman, 1978; Clauson, 1989).

After the administration of carbon tetrachloride neutral lipid begin to accumulate in the cytoplasm making their first appearance as osmiophilic droplets. The liver becomes grossly enlarged and turns pale because of accumulation of fat. In addition impairment of protein synthesis leads to a rapid diminution of lipoprotein secretion by the liver cells because the synthesis of protein (lipid acceptor protein) is necessary for the coupling of triglycerides to phospholipids to form lipoprotein which has been interrupted. This results in the accumulation of lipids in the form of triglycerides. Since, these can be secreted into the blood from the liver only as lipoproteins. Lipid can also accumulate in the liver by the other mechanism such as by increased mobilization of free fatty acids from depot fat.

Carbon tetrachloride induced liver damage and the preventive action of test drugs has widely been used to assess the hepato protective activity of drugs in general (Clauson, 1989).

Though, liver diseases are among the important diseases affecting mankind, no remedy is available to treat jaundice. However, numbers of medicinal preparations have been advocated in traditional systems of medicine, especially in Ayurveda for treating liver disorders. The search for the hepatoprotective agents started in medicinal plants after the discovery of silymarin, a flavonolignan from *silybum marianum* a proven hepatoprotective drug.

The search was further facilitated by the description of *in vitro* technique involving evaluating test drugs against carbon tetrachloride induced toxicity in cultured hepatocyte by Hikino and Coworkers (1984) and the method described

Further, many investigators were attempted to evaluate the hepatoprotective effect of the constituents isolated from the medicinal plants. For example, wedelolactone and dimethyl wedelolactone from ethyl acetate soluble fractions of *Wedelia calendulacea* (Wagner, *et al*, 1986), andrographoloide from the leaves of *Andrographis paniculata* (Visen, *et al*, 1990), nirocil, phylanthin from *Phyllanthus amarus* (Venkatesan, *et al*, 2003), essential oil from the seeds of *Foeniculum vulgare* (Ozbek, *et al*, 2003), silymarin from the seeds and fruits of *Silybum marianum* (Dagaonkar and Shankar, 2002), a flavonoid compound 5-hydroxy 7,8 - 21 methoxy flavona from the leaves of *Andrographis alata* (Nagaraja and Krishna, 2002), triterpenes - ursolic acid Lupeol and betulin from the petroleum ether extract of *Diospyros cordifolia* stem bark (Mankani and Krishna 2004) etc.

In the present investigation also the aqueous extract, ethanol extract and isolated sesquiterpenoid compound deoxyelephantopin of *Elephantopus scaber* were screened on Wister strain rats against CCl4 induced toxic hepatitis.

It was observed that the animals that are treated by CCl4 showed significant loss and reduction in their weight, when compared to the control.
animals. While the animals of group treated with the isolated compound deoxyelephantopin and reference standard drug silymarin have showed normal behavior. The test drugs treated animals of aqueous and ethanol have also showed normal behavior and food consumption.

Many biochemical parameters such as estimation of serum bilirubin, serum protein and serum enzymes are the most widely employed and generally accepted parameters to measure the hepatic injury and the extent of liver damage. Treatment of rats with test drugs prior to and concomitant with challenge of CCl\textsubscript{4} produced an alleviation of the hepatic injury to a considerable extent which is reflected by the ability of the test drugs to lower the elevated serum enzymes activity resulting from the administration of CCl\textsubscript{4} alone.

Estimation of total Bilirubin is based on the Vanden-Bergh reaction where, serum bilirubin is converted into azobilirubin, when coupled with diazotized sulphanilic acid. The total bilirubin is determined in the presence of an accelerator methanol. In the presence of 50% methanolic solution both the water soluble bilirubin glucoronides and the free bilirubin, complexes with albumin reacts fast with the diazotized reagent.

The estimation of serum bilirubin level during the course of jaundice is of clinical importance because serum bilirubin level confirms the intensity of jaundice. In hepatic necrosis, rise in the level of serum bilirubin can be due to both direct an indirect bilirubin. In hepatitis condition, hyperbilirubinemia is commonly occurred.

In the present study, it was observed that concomitant treatment of CCl\textsubscript{4} with the extracts or the constituent showed significant reduction in the level of serum bilirubin. In the animals treated with deoxyelephantopin there was a
significant reduction (0.79±0.02) in the serum bilirubin level. The least increase in the level of serum total bilirubin was observed in the standard reference drug silymarin (0.70±0.02). In ethanol extract (0.86±0.02) and aqueous extracts (1.01±0.04) treated animals the levels of serum bilirubin was slightly increased as compared to the controls. This showed that the hepatic necrosis was reduced in the animals treated with deoxyelephantopin.

Similar type of investigations on the effect of medicinal plants in restoring the normal level of total bilirubin have been reported on Cassia angustifolia (Ilavarasn, et al., 2001), Foeniculum vulgare (Ozbek, et al., 2003), Wrightia tinctoria (Chandrashekhar, et al., 2004).

The estimation of serum total protein is the useful insight for monitoring gross changes in the liver tissue caused by various disease or toxicity. It is usually performed in conjunction with other tests such as estimation of serum albumin and albumin / globulin ratio. Albumin is a major plasma protein synthesized in the liver from amino acids, which are absorbed from the ileum. Its functions include regulation of distribution of extracellular fluid, transportation of various hormones, vitamins and trace metals. The decreased synthesis of albumin is due to dietary, hepatic disease or malabsorption (Krishna, 1996).

In CCl₄ treated animals due to alteration of albumin: globulin ratio, the total protein level decreased from 9.18±0.07 to 5.86±0.07 and it was observed that the serum albumin level was slightly decreased from 6.05±0.05 to 1.83±0.04. In addition serum globulin level was moderately increased from 3.08±0.05 to 4.08±0.05, when compared with the normal control animals. The toxic effect of CCl₄ has been reversed in the animals treated with aqueous, ethanol extract, deoxyelephantopin and standard drug silymarin.
Among these groups in deoxyelephantopin treated animals, the toxic effect of CCl₄ has been reversed. This can be evidenced in the increased levels of total protein from 5.86±0.07 to 8.15±0.08, and the serum albumin level from 1.83±0.04 to 5.26±0.04. Whereas, the globulin level was restored from 4.08±0.05 to 2.90±0.08. This showed that the isolated compound deoxyelephantopin was found to be more potential in restoring the normal levels of total protein. Similar work has been carried out on *Luffa echnata* (Bahar Ahmed, *et al.*, 2001), *Nigella sativa* (Mohideen, *et al.*, 2003), *Phyllanthus amarus* (Venkatesan, *et al.*, 2003). Measurement of serum enzyme level is useful insight for the detection of hepatotoxicity. It is useful for detection of early damage and for demonstration of hepatic injury.

The estimation of AST, ALT, ALP activities are of the most sensitive tests that are considered as the index for diagnosis of liver diseases. Treatment of rats with the test drugs concomitant with the treatment of CCl₄ produced an ameliorations of the hepatic injury to a considerable extent which is reflected by the ability of the test drug to lower the elevated levels of AST and ALT in serum. (Drotmann and Lowhorn, 1978).

In view of this, the test drugs mediated restoration in levels of AST, ALT towards respective normal value is an indication of stabilization of plasma membranes as well as repair of hepatic tissue due to damage caused by CCl₄. This effect is an agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew, *et al.*, 1987).

Alkaline phasphatase (ALP) is the prototype of these enzymes that reflects the pathological alteration in biliary flow (Ploa and Hewitt, 1989). The uses of ALP in chemical induced liver dysfunction are fairly investigated in this study. CCl₄ induced elevation of this enzymatic activity in serum is in line
with high level of serum bilirubin content. The test drug mediated suppression of the increased ALP activity with the concurrent depletion of raised bilirubin level suggests the potentiality of the test drugs being able to stabilize biliary dysfunction in rat liver during chronic hepatic injury with CCl₄.

In the present study also the levels of serum marker enzymes showed significant elevation in the blood samples of CCl₄ treated animals, when compared to normal. Whereas, significant reduction in the levels of AST, ALT, ALP activities was observed, after concomitant administration of the test drugs with CCl₄.

The blood samples of CCl₄ treated groups showed increase in the levels of AST, from 142.0±7.48 to 2106.0±12.71, ALT from 47.98± to 1265.0±5.53 and ALP, from 168.0±2.86 when compared with the normal group of animals. Concomitant treatment of the animals with all the extracts and the constituent showed significant supression in the serum enzyme levels. In the animals treated with deoxyelephantopin there was a significant reduction of AST (214.0±9.30), ALT (102.0±16.0) and ALP (242.0±7.14) levels. Similarly restoration in the levels of AST, ALT, ALP was also observed in the animals treated with reference standard silymarin. i.e. 198.0±8.67, 84.0±10.04 and 201.67±5.99 respectively.

Administration of the isolated compound deoxyelephantopin has exhibited effective result in reducing the toxic effects of CCl₄ by controlling the levels of serum marker enzymes. The blood samples of the aqueous and ethanol extract has shown moderate recovery pattern against toxic hepatitis in all the estimations carried out. Similar study on the evaluation of plant extracts by the estimation of liver function enzymes have been reported on *Wedelia calendulacea* (Wagner, *et al.*, 1986), *Diospyrus cardifolia* (Krishna, 1996), *Vitex negundo* (Srinivas, *et al.*, 1999).
The antitoxic effect of the extracts of the *Elephantopus scaber* and the isolated constituent was further supported by histology of liver. The liver section of control group was compared with the toxic effect of the CCl₄ treated animals. The hepatotoxic effect of CCl₄ resulted in intense centrilobular necrosis, vacuolization and totally degeneration in centrilobular areas. This may be due to the peroxidative degradation in the adipose tissue. (Jalalpure, *et al.*, 2003). Further, increased in the levels of serum bilirubin, AST, ALT, ALP was clear indication of cellular leakage and loss of functional integrity of cell membrane (Saraswath, *et al.*, 1993).

In the present study the histological profile of the normal tissue showed regular compact arrangement of cells, whereas, the liver sections of the CCl₄ treated animals showed intense centrilobular necrosis and vacuolization and fatty degeneration. The section of the liver taken from the animals treated with aqueous and ethanol extract showed moderate accumulation of fatty lobules. The sections of deoxyelephantopin treated animals showed significant signs of amelioration of the liver damage, as evident from the presence of normal hepatic cords, absence of necrosis and degree of fatty infiltration similar with that of the normal untreated animals.

The results of this investigation supported the traditional claims of *Elephantopus scaber* for treating jaundice. The earlier investigators Lin, *et al.*, 1995, screened the hepatoprotective effect of aqueous extract. The present investigation proved the hepatoprotective effect of crude extracts and isolated sesquiterpene lactone deoxyelephantopin.

Jean bruneton (1999) postulated that the sesquiterpene lactones possess an active alpha methylene gamma lactone that had a broad range of biological activities. Survey of literature also revealed that the most of the plants used to heal wounds were also used as a hepatoprotective plant (Krishna, et al., 2005; Vidya, et al., 2004).

Inflammation can be described as tissue response to an injury. The damage caused by microbial infection, physical agents such as, heat, radiant energy, electricity or sharp objects, or chemical agents like acids, base and gases. The clinical manifestation of inflammation are, rubor, (redness) calor (warmth), tumor (swelling) caused due to exudation, dolor (pain), due to stretching of nerve endings, change in tissue osmotic pressure and pH and functiolaesa (loss of local function) due to discomfort or destruction of the part involved. Inflammation has the functions, to destroy the injurious agent, if possible, and to remove it and its by-products from the body. If destruction is not possible, to limit the effects on the body by confining or walling off the injurious agent and its by-products and to repair or replace tissue damaged by the injurious agent or its by-products.

During inflammation circulation increases, the leucocytes marginate to the periphery of the vessel lumen and then emigrate into the interstitium. Cellular events are dominant in this stage and follow a chronological order. The platelets are the first cells to arrive at the site of inflammation. The neutrophils and red blood cells follow them and a fibrin network forms over the wound. The concentration of some proteins also changes. The proteins, whose concentration changes markedly during inflammation, are called acute phase protein (Sanjay Azad, 2002).
Carrageenan induced edema is commonly used as an experimental animal model of acute inflammation and is believed to be biphasic. The first phase is due to the release of histamine and serotonin, the second phase is due to the release of bradykinin, protease, prostaglandin and lysosome (Castro, et al., 1968). It has been reported that the second phase of edema is sensitive to most clinically effective anti-inflammatory agents (Smucker, et al., 1967). Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs (Della Loggia, et al., 1986). Depending on the above concept, the effect of test drug/extract against inflammation was studied. By using these models the anti-inflammatory activity of the *Nelumbo nucifera* (Mukhergee, et al., 1997a), *Leucas lavendulaefolia* (Saha, et al., 1996), *Cassia fistula* (Bhakta, et al., 1999), *Cassia tora* (Maity, et al., 1998) were studied.

A variety of anti-inflammatory drugs are flooding the world market today but a very few are relatively non-toxic. Thus the drugs having low toxicity and better therapeutic index is required for the inflammation therapy. Michael Heinrich, et al., (1998) have reported that the plants belongs to the family Asteraceae (Compositae) which possess the sesquiterpene lactones which have anti-inflammatory properties and have the ability to relax smooth muscle thereby relieving gastrointestinal distress. These compounds were readily form adducts with glutathione or free thiols and can thereby affect the metabolism activity and toxicology of a wide array of pharmacological agents.

Anti-inflammatory effects of sesquiterpene lactones from Arnica (Lyss, et al., 1997), Feverfew (Bejaman, et al 2001), *Vernonia colorata* (Cioffi, et al., 2004) were also evaluated on experimental animals. In the present study for the assessment of anti-inflammatory activity carrageenan induced paw edema model was studied and the degree of edema was measured by using plethysmometer (Winter, et al., 1962).
Administration of oral suspensions of the isolated compound deoxyelephantopin significantly inhibited (0.30±0.01 ml) the development of edema formation in rats from the first 30 minutes to 180 minutes after carrageenan injection, as compared to the control rats (0.90±0.02 ml). The standard drug diclofenac sodium also showed much higher inhibition of edema formation (0.24±0.01 ml) at 180 minutes. Whereas, oral suspensions of the aqueous and ethanol extracts have shown moderate reduction in the paw volume, compared to the isolated compound deoxyelephantopin and the standard drug diclofenac sodium treated animals. The edema volume was significantly decreased in the deoxyelephantopin treated animals and is comparable to the standard and control treated animals.

From the results it has been observed that in the isolated compound deoxyelephantopin treated animals the percentage inhibition of edema was 66.66%, which is quite compatible with those of the percentage inhibition of edema of the standard drug diclofenac sodium (73.33%). The percentage inhibition of edema in aqueous and ethanol treated animals was 53.33% and 57.77% respectively, which was moderate when compared to deoxyelephantopin.

The traditional claims indicated that the plant *Elephantopus scaber* (Asteraceae) is being used to heal wounds, liver disorders and to cure inflammation. So in the present investigations compound deoxyelephantopin was isolated from the plant *Elephantopus scaber* and used for the screening of wound healing, hepatoprotective and anti-inflammatory activities. The positive effect of this constituent may be due to the presence of alpha methylene gamma lactone moiety, which has a broad range of biological activities.