VI. SUMMARY

India is well known as the emporium of medicinal plants. The use of plants to treat diseases dates back to the times of Rigveda (3500 to 1800 B.C.). Over the years, medicinal plants are treated as subject of serious study and intense research work has been carried in different disciplines like, agriculture, biotechnology, biochemistry, botany, pharmacology, pharmacy, phytochemistry etc. It is being globally recognized that, medicinal plants play a significant role in providing health as well as prevention and management of diseases. The inherent disadvantages of synthetic (chemical) drugs have made the medical scientists to search for natural, easily available and safe remedies in the management of challenging diseases like aids, cancer, diabetes, hepatitis, etc.

Presently there has been more demand all over the world regarding the use of the herbal drugs in the place of modern drugs. But the way in which the drug acts and the manner in which important organ of the body are stimulated has to be proved through practical and clinical experimental evidences.

The state of Karnataka is endowed with a very rich flora. A large portion of the mighty Western Ghats traverses through the state. Due to the various physiographic and physiognomical factors, the vegetation exhibits different types of tropical forests, from the evergreen to moist deciduous to dry deciduous and finally the scrub jungle types. Several places of this region like, Bababudangiri, Bagavathi forest range, Biligirirangana Hills, Bisle Ghats, Gangamula, Joldal, Kemmanagundi, Kollur reserve forest, Kudremukh, Shettyhalli Range, Sharavathi range etc were endowed with a rich diversity of medicinal herbs and have been a frequent hunting ground for the traditional practitioners for the collection of plant based drugs. This intern with the deforestation and natural calamities resulted in the elimination of many valuable medicinal plants.
A survey on medicinal plants of Western Ghats of Karnataka enlightened us that the traditional practitioners of Malnad region (Joldhal forest, Karnataka) are using the aerial parts of *Elephantopus scaber* Linn to cure cut wounds and hepatitis. The present investigation is focusing on an indigenous herb *Elephantopus* scaber Linn that belongs to the family Asteraceae. The ethnomedical review indicated that the plant has been claimed for curing liver disorders, tumors and treating wound. Survey of the literature also enlightened us that so far no investigations were carried out on the *in vitro* propagation of this species. Further, pharmacological evaluation of the crude extracts and the isolated constituents for wound healing activity and isolated constituent for hepatoprotective and anti-inflammatory activities have not worked out so far. In view of the high medicinal value of this species, the present investigations were undertaken.

*In vitro* technique is a promising area of research in conservation of medicinal plants and is the only technique in assisting the sustainable maintenance of rapidly dwindling germplasm in the long term. Keeping this in view, derivation of protocol for rapid regeneration of plantlets has been standardized.

In the preliminary experiment influence of different nutrients were tested on the morphogenic potentialities of the leaf explants of *Elephantopus scaber*. The nutrient media selected for the purpose were MS (Murashige and Skoog, 1962), LS medium (Linsemaier and Skoog, 1965) and B5 medium (Gamborg, *et al*., 1968). Among these, MS medium showed better response and it was selected as the basal medium for further experiments.

In culture of leaf explants on MS medium the luxuriant callus proliferation was observed at the mid dorsal vein of the basal leaf segments and
initiation of callus occurred within seven days of inoculation and later it proceeded all over the surface of the lamina.

Among different growth regulators tested, 2, 4-D (2 to 3 mg/l) was found to be the prime hormone for callogenesis. In the combination with lower concentrations of BAP (0.5 mg/l) callogenesis was enhanced. On contrary, in the presence of only BAP, organogenesis of shoot initials taken place from the callus and from the uncallused explants. The maximum proliferation of callus was observed at the concentration of 2.5 mg/l of 2, 4-D + 0.5 mg/l of BAP.

Though, higher concentration of auxin (2 to 3mg/l of 2, 4-D) and lower concentrations of BAP (0.5 to 7.5 mg/l) favored callogenesis from different explants, its intensity varied depending upon the type of explants. Among leaf, stem, meristem, root, peduncle explants cultured, callogenic potency was more in meristem explants, which is followed by leaf and stem explants. This may be due to the presence of varied concentrations of endogenous hormones.

In the culture of calli of all the explants, interaction of BAP at the range of 2 to 3 mg/l and NAA at the range of 0.5 to 7.5 mg/l has provoked shoot bud organogenesis from the calli. But the frequency of the shoot production was varied. The highest number of shoots (34.2±1.14) and highest number of root intact plantlets (30.4±1.35) were recovered from the meristem callus. In stem and leaf calli the number of shoot buds were 26.3±1.16 and 24.0±1.05 respectively.

After three weeks, root intact plantlets were washed with running tap water and agar sticking to the roots removed. The plantlets with fully expanded leaves and well-developed roots were first transferred to the plastic containers containing 1:1 mixture of sterile sand and soil. The regenerated plantlets were recovered with a thin perforated transparent polythene bags to maintain
humidity. The plantlets were watered with 1/10\textsuperscript{th} strength of MS salt solution and later they were transferred to the field condition. The percentage of field acclimatized plantlets derived from the calli of root, leaf, stem and peduncle were analyzed separately. Morphology of the regenerants were compared with that of \textit{in vivo} plants and found to be similar. The regenerants were maintained in the departmental garden for future studies.

In the present investigation micropropagation, protocol was standardized for the culture of vegetative explants leaf, stem, meristem, peduncle, roots. Attempt was also done on the culture of anther, petals and sepals. Due to the lack of time, availability of floral materials and intense contaminations, regeneration of plantlets was not achieved from the floral explants. This can be worked out in future.

The qualitative chemical tests conducted on the different solvent extracts of \textit{Elephantopus scaber} revealed the presence of carbohydrates, glycosides, proteins, steroids and flavonoids from the aqueous extract. The ethanol extract showed the presence of terpenoids. The earlier reports indicated that the genus \textit{Elephantopus scaber} is a good source of sesquiterpenoids. So, ethanol extract was successively fractionated with different solvents like, hexane, and methanol. From the methanol fraction of ethanol extract a sesquiterpene lactone was isolated according to the method described by Paul Pui Hay But \textit{et al.}, (1997). The structure of the compound was confirmed by melting point, UV, IR and MASS spectral analysis. These spectral data were compared with the literature values and the compound was identified as deoxyelephantopin, having molecular formula C\textsubscript{19}H\textsubscript{20}O\textsubscript{6}.

The different crude extracts and the isolated constituent deoxyelephantopin were comparatively subjected to pharmacological studies
and evaluated on wound healing, hepatoprotective and anti-inflammatory models on experimental rats.

Acute toxicity studies carried out on the different extracts, fractions and isolated compound deoxyelephantopin of *Elephantopus scaber* by ‘Staircase’ method (Ghosh, *et al.*, 1984). The maximum tolerated dose for the aqueous and the ethanol extract were found to be 3000 mg/kg b. w. For the methanol and hexane fractions of ethanol extract, 1000 mg/kg b. w. and for the compound deoxyelephantopin the maximum tolerated dose was 40 mg/kg b. w. Therefore, 1/10th of these doses (300 mg/kg b.w. of each of aqueous and ethanol extract, 100 mg/kg b.w. of each of methanol and hexane fractions of ethanol extract and 4 mg/kg b.w. of the isolated compound deoxyelephantopin) were selected as safer dose for further pharmacological evaluation.

Wound has defined as disruption of normal tissue structure and function. It can be produced by physical, chemical, electrical or microbiological insult to tissue. It may also be caused by mechanical violence, with or without a loss on continuity. In the practical management each wound is treated on an individual basis depending upon the type of wound such as, incised wound, lacerated wound, crushed wound and devitalized wound etc.

Wound healing is a complex bio chemical and cellular event that can be affected by many factors. It depends upon the reparative abilities of the tissues, the type of damage, the extent of damage and general state of health of the tissue. The processes of wound healing are haemostasis, inflammation, epithelization and wound repair. The components of wound repair are, granulation tissue formation, wound contraction, collagen/matrix formation, and scar tissue formation.
Wound healing can be assessed by three different wound models, they are, excision wound model, incision wound model and dead space wound model, in order to monitor the all the phases of wound healing process. Rats of either sex were used for assessing the entire wound healing activities. In the assessment of excision wound model Morten and Malone (1972) method was employed and this model is used to monitor the wound contraction and epithelization time. A circular wound area of 2.5 cm diameter was made on the dorsal thoracic region of the rats, to be tested for wound healing activity. The progressive changes of wound area, after applications of different drug ointments were monitored planimetrically by tracing the wound margin on a graph paper, as well as by taking the photographs of the wound. The area of wound is expressed in mm² units.

The results of this investigation revealed that the animals treated with the isolated compound deoxyelephantopin have showed significant percentage closure of wound contraction, which is comparable with that of the reference drug nitrofurazone treated animals. The percentage closure of wound was significant (98.6±0.37) on the day 16, in the animals treated with the isolated compound deoxyelephantopin, while in control animals it was only (85.8±0.69). Out of the animals treated with different extracts viz aqueous and ethanol extract, methanol fraction of ethanol extract and hexane fraction of ethanol extract, methanol fraction have showed a better response which is almost similar to that of the activity shown by deoxyelephantopin.

The time required for complete epithelization of the excision wound is an important parameter to assess the wound healing process. It was found that the mean time taken for complete epithelization in animals treated with deoxyelephantopin and methanol fraction of the ethanol extract was 15.0±0.26 and 15.6±0.21 days respectively, which is comparatively less than the control (21.0±0.37) and other drug treated animals.
The promotion of wound healing activity is well gazed by tensile strength of the incision wound. Wound healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibres. The breaking strength of the wound tissue is monitored by the instrument Tensiometer. In the investigation, significant increase of tensile strength was noticed in the animals treated with deoxyelephantopin (412.0±11.37) and methanol fraction of ethanol extract (390.6±9.33) respectively. The drug deoxyelephantopin induced the wound tissue for the formation of more collagen that has provided strength to the healing tissue.

In the assessment of dead space wound model, dead space wound were created by subcutaneous implantation of sterilized cylindrical gross piths for a period of 10 days. Oral administrations of different extracts, fractions and deoxyelephantopin were administered at different doses. On the day 10 the granulation tissue formed on the grass piths were harvested and their fresh and dry weights were noted. The buffer extract of the wet granulation tissue was dried and used for the determination of hydroxyproline content (Neuman, et al., 1950).

As observed in other parameters, significant increase in the mean dry weight of the granulation tissue was observed in the animals treated with deoxyelephantopin (74.0±1.15 mg) and in methanol fraction (74.0±1.29 mg). This can be further evidenced by the presence of high protein content of the granulation tissue.

The hydroxyproline content of the granulation tissue of deoxyelephantopin and methanol fraction treated animals were 9.80±0.05 mg and 9.25±0.06 mg respectively. This may be due cross-linking of collagen fibres noticed in the granulation tissue of the treated animals.
Histological observations of the granulation tissue have further evidenced the effective wound healing activity of the deoxeelephantopin and methanol fraction. The sections of these animals showed more fibroblasts, increase in collagen fibres and more number of blood vessels.

The pharmacological studies of the different extracts, fractions and isolated constituent revealed the significant wound healing effect of the deoxeelephantopin, which is followed by the methanol fractions treated animals. Moderate effects of wound healing activity were observed in the aqueous and ethanol extract treated groups and least effect observed in by hexane fraction treated animals.

Liver diseases are the serious health problem and is one of the most frequently exposed organ and liable for damage in the body. The major clinical manifestation of liver disorders is jaundice. In hepatic necrosis an elevated level of serum bilirubin is observed. The rise in level of serum bilirubin may lead to hyperbilirubenaemia.

The change in the concentrations of liver proteins is also one of the parameter to assess the intensity of jaundice. Further, the elevated in the levels of serum enzymes like, AST, ALT and ALP are of the clear indications of hepatocellular necrosis. It was also known that, the hepatic damage caused by CCl4 simulated the human viral hepatitis model. Many investigators have used this toxic chemical as a tool to assess the hepatoprotective effect of herbal drugs. In the present study also CCl4 treated animals showed significant elevation in the levels of serum bilirubin, AST, ALT and ALP as compared to controls. However, the total protein content was decreased by increase in the levels of serum globulin with an alteration in the ratio of albumin and globulin.
On the contrary, concomitant treatment of CCl₄ with the extracts and the constituent, deoxyelephantopin reduced the toxic effects of CCl₄. Among the different treated groups the standard drug silymarin treated animals have showed significant restoration in the levels of serum bilirubin, AST, ALT and ALP as compared to control and CCl₄ treated animals. The enzymes levels were almost restored to the normal. This is an indication of stabilization of plasma membrane as well as repair of hepatic tissue. A moderate hepatoprotective effect was observed in ethanol extract treated animals, whereas, hexane fraction treated animals showed least effect.

Histological examination of the liver section of the rats treated with toxicant, CCl₄ showed hepatic lesions, intense centrlobular necrosis, vacuolization and macrovesicular fatty changes. The rats treated with silymarin and deoxyelephantopin along with toxicant showed sign of protection against this toxicant to a considerable extent as evident from the formation of normal hepatic chords and absence of necrosis vacuoles and fatty globules. In the sections of the liver from the animals treated with aqueous extract and ethanol extract have showed moderate restoration of the liver tissue.

The results of this investigation supported the traditional claims of *Elephantopus scaber* for treating jaundice. The hepatoprotective effect of this plant is due to the presence of active constituent deoxyelephantopin, which possess an active radical, alpha methylene gamma lactone. The earlier investigators also reported the amelioration effect of these radicals on other parameters.

One of the characteristics of living tissue is its ability to react to injury. The reaction of living tissue to injury that comprises a series of changes of the terminal vascular bed, blood and connective tissues, which tends to eliminate the injurious agents to repair the damaged tissue, may be called as
inflammation. Repair begins during the active phase of inflammation, but reaches completion usually after the injurious influence has been neutralized. Destroyed cells and tissues are repaired thereby. Inflammation may be potentially harmful.

Inflammatory reactions underline the genesis of crippling, rheumatoid arthritis, life threatening sensitivity reactions and some forms of fatal glomerular diseases.

Carrageenan induced edema is commonly used as an experimental animal model of acute inflammation and frequently used to assess the anti-edematous effect of natural products (Dellaa Loggia, et al., 1986).

In the present study, the effect of the test drugs, aqueous extract, ethanol extract, deoxyelephantopin and the standard reference drug diclofenac were tested against carrageenan-induced inflammation. It has been observed that, the percentage of inhibition of edema in the animals treated with deoxyelephantopin was 66.66% at 180 min., which is highly effective and comparable with that of the activity of the reference drug diclofenac sodium (73.33%). The percentage of inhibition of edema in the animals treated with aqueous extract and ethanol extract treated animals were found to be moderate when compared to the standard drug treated animals.

In conclusion, the present investigation is the prime report of the establishment of a protocol for the micropropagation of the plant Elephantopus scaber. The screening of medicinal values of the plant through clinical evidences is of immense value. So comparative clinical analyses on the efficacy of the aqueous extract, ethanol extract, various fraction of ethanol extract and the isolated compound deoxyelephantopin of Elephantopus scaber was carried out on wound healing, hepatoprotective and anti-inflammatory
models. The significant pharmacological activities of the isolated constituent deoxyelephantopin, a sesquiterpene lactone is may be due to the presence of alpha methylene gamma lactone moiety, which has a broad range of biological activities. According to Giordano, et al., 1990 and Jean Bruneton, 1999 the compounds having alpha methylene gamma lactone moiety in their structure possess anti ulcerogenic and wound healing properties. The earlier investigators also reports the activities of sesquiterpene lactones for hepatoprotective, (Babalola, et al., 2001; Masayuki Yoshikawa, et al., 2000) antibacterial (Fischer, et al., 1998), antifungal (Alejandro, et al., 2000) and antioxidant (Rekha, et al., 1966) properties. The result of this investigation provides necessary information to the pharmacists for the derivation of the new drugs.