SUMMARY

Plants are the vital source for the existence of all living organisms in the universe. They not only synthesize food necessary for the well being of man, but also manufacture different types of chemicals for human health. Out of about 2,50,000 plant species known, only about 3,000 species are cultivated for food, fibers, medicines and other human needs, rest of the plants are growing wild in different provinces of the world. The use of plants for mitigating the human ailments has been a prehistoric activity. In the early days herbal medicine was inexorably entangled with occultism, witchcraft, astrology and so on and which have been the fore runners of traditional systems of herbal medicine.

Liver is one of the largest organ located in the right thoracic region of the body. It functions as a synthetic site for many enzymes of blood serum, as a storage organ for carbohydrates and proteins, as a remover of toxins and helps in digestion by secreting bile juice for the emulsification of food. But, liver cells are effected easily by hepatitis viruses, toxins and drugs. In hepatitis condition, liver cells are damaged and fails to conjugate bilirubin, consequently excess of bilirubin is exerted and accumulated in the blood stream. In such a condition the patients become physically weak, suffering from fever, giddiness, flapping movement of hands and legs and finally may lapse into coma. Presently, there is no effective allopathic treatment for hepatitis. With the advent of biotechnology vaccines are available for viral hepatitis- B. But after infection effective specific drugs are not available in allopathic system of medicine for healing jaundice.

In indigenous system of medicine, several plants and plant products are known to act as a potent hepatoprotective drugs for healing jaundice such as,

*In vitro studies on Andrographis paniculata Nees. and A. alata Nees.*
Eclipta alba, Emblica officinalis, Flaveria trinervia, Hemidesmus indicus, Phyllanthus amarus, Terminalia bellarica, W. calendulacea, etc. Many traditional practitioners residing in the remote areas of Karnataka were able to cure this deadly disease by using herbal products. Their mode of administration is very simple but its curative effect is more.

The present study is focusing on an antihepatitis plant species Andrographis paniculata Nees., ("Jerathaka," Nelabevu) and Andrographis alata Nees. ("Dodda nelabevu") of Acanthaceae.

In A. paniculata pharmacological composition, therapeutic efficacy and mechanism of hepatoprotective activity, against induced hepatitis was screened by Wang, 1983; Shukla et al., 1992; Visen et al., 1993; Sandberg, 1994; and Weibo et al., 1995. It is also tested for common cold and fever (Caceres et al, 1997).

Phytochemical analysis of the whole plant of A. alata showed the presence of Flavone 2'-glycosides (Damu et al., 1998). The therapeutic efficacy of this plant has not been screened the earlier investigators. The traditional medicinal practitioners residing in the vicinity of forest of Malebennur range Karnataka, India, used the leaf and root extracts of this species to cure infective hepatitis and malaria (Krishna, 1996). Due to its rarity and destruction of the habitat by forest fire and anthropogenic activities the medicine men were experienced the non-availability of the leaves and roots for medicinal purposes.

Biotechnology offers no immediate cure for all diseases but it does hold a promise and facilitates the development of a new phase in the drug production. Plants are the basic source for the establishment of many
pharmaceutical industries. But in their natural occurrence plants are found scattered and it is difficult as well as uneconomical to collect and process these plants for the supply from natural resources. The increasing demand and inadequate supply of these plants have resulted in the increase of prices. Hence, investigation of a rapid propagation method is very essential for these indigenous medicinal plants.

With the discovery of totipotency and development of techniques for regeneration of plantlets either by direct organogenesis or through callus culture, a large number of medicinal plants have been successfully regenerated. However, Literature survey enlightened that there has been no reports on the micropropagation of *A. paniculata* and *A. alata*. So, in the view its medicinal importance the present investigation has been undertaken.

In the preliminary experiments effect of different nutrient formulations such as Murashige Skoog’s (MS) medium, Linsmaier and Skoog’s (LS) medium, Gamborg’s (B₅) medium, White’s medium and Nitsch medium on the viability of both mature and immature seeds of both the taxa have been analyzed. It was found that maximum response was found on MS medium. Further effect of various growth regulators such as 2,4-D, NAA, IAA, BAP and Kn on the callogenic potentialities of immature seed, cotyledon, hypocotyl, root, leaves and stem have been analyzed. It was found that combination of BAP and NAA was most consistent in inducing callogenic response from different explants of *A. paniculata* and *A. alata*. The presence of NAA with BAP induced only a meagre callus. Whereas, Kn at all concentrations tested, failed to exhibit callogenic response from the explants.

In *A. paniculata* and *A. alata* shoot bud organogenesis through the callus was achieved from the culture of cotyledon, hypocotyl, root, leaf and stem.
stem calli. In all the explants the combination of BAP and NAA is most conducive in the induction of shoot bud organogenesis. However, the frequency of shoot bud organogenesis was more in hypocotyl explants than the other explants of both the species. The caulogenic competence of the calli was more at the optimal concentrations of 2μM BAP and 0.5μM NAA. In the above combination when the concentration of BAP decreased by increasing the level of NAA shoot bud differentiating potentialities of the calli was decreased. On the contrary organogenesis of root initials were noticed from the differentiating callus.

Among different explants of *A. paniculata* and *A. alata* cultured *in vitro*, the caulogenic potency was found to be more in calli of seedling explants, especially the hypocotyl-derived calli. The shoot buds, which were at the stage of 3 to 5 mm, were isolated and used for encapsulation into synthetic seeds. On MS basal medium the seeds were grew up successfully into shoots with luster green leaves. Subculturing of these shoots onto the rhizogenic media revealed the development of complete plantlets.

In *A. alata* root explants also showed caulogenic competence with other seedling explants, while, in *A. paniculata* it exhibited only rhizogenic response and failed to differentiate into shoot buds. In leaf explant culture shoot differentiation through the callus was achieved in both the species. In stem culture regenerants were obtained only from stem calli of *A. alata*.

The well grown differentiated shoot buds were aseptically transferred on to the MS half strength semi solid medium containing 0.25 μM-1 μM NAA to induce rhizogenesis. After three weeks of incubation root intact plantlets recovered 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 (v/v). The regenerated plantlets were covered with a thin perforated transparent polythene bags to maintain the humidity. These plantlets were watered with 1/10\textsuperscript{th} strength of MS salt solution and were incubated in the culture room condition for a period of one week, later they were transferred to the field condition. The percentage of field-acclimatized plantlets derived from the calli of cotyledon, hypocotyls, root, leaf and stem explants were analysed separately. Morphology of the regenerants like height of the plant, texture, size and shape of leaves, flower fruits were compared with that of \textit{in vivo} plants.

The regenerants derived from the cotyledon, hypocotyl, root and leaf calli of \textit{A. alata} showed a drastic morphological variation when compared to \textit{in vivo} plants. In cotyledon regenerants formation of reddish brown pigmentation noticed on the leaves. In hypocotyl regenerants leaves were larger in size and ovate glabrous and obtuse, while, in \textit{in vivo} plants leaves are lanceolate, hirsute and acute at both the ends. The stem is also glabrous with narrow internode and stout node. The regenerants derived from root calli possess short internodes and the shape and texture of the leaves similar with that of hypocotyl regenerants.

In \textit{A. paniculata} regenerants derived from hypocotyl and leaf calli showed similarities with the \textit{in vivo} plants in the shape and texture of leaves. The regenerants were maintained in the departmental garden for future studies.

In the present investigation phytochemical analysis was carried out only on the leaves of \textit{A. alata}. The qualitative phytochemical tests conducted on the different solvent extract of leaves of \textit{A. alata} revealed the presence of steroids and diterpenes in petroleum ether extract; flavonoids in ethyl acetate extract; alkaloids in chloroform and alcohol extracts and glycosides in water extract.
From the ethyl acetate extract a flavone compound was isolated and it was subjected to UV, IR, $^1$H-NMR, $^{13}$C-NMR and mass spectral analysis. From these analysis data the compound identified as 5-hydroxy, 7,8,2'-methoxyflavone and the molecular formula C$_{16}$H$_{16}$O$_6$. Mahabubul et al., (1979) isolated this compound in the related species *A. paniculata*.

The main aim of indigenous medicine is to cure disease by administering either single or mixed extract of plant materials, rather than to analyse the active components. But, unless corroborated by clinical experimental evidences the therapeutic effect of the plant parts cannot be confirmed. In *A. paniculata*, Handa *et. al.*, (1990) and Visen *et. al.*, (1993) screened the hepatoprotective activity of the leaf and the isolated compound Andrographolide against toxic hepatitis. The therapeutic efficacy of leaves and its constituents have not been worked out sofar. So in the present investigation aqueous extract of leaves and a Flavone compound isolated from the leaves of *Andrographis alata* were comparatively screened against CCl$_4$ induced toxic hepatitis in experimental Albino rats.

It was known that the injury and dysfunction of the liver caused by the toxic effect of CCl$_4$ stimulated the human infective hepatitis model. It was observed that at the end of every week of treatment, the blood samples of CCl$_4$ treated groups showed highly significant elevation in the levels of serum bilirubin, serum total protein, serum alanine amino transaminase (ALT) serum aspartate, aminotransaminase (AST) and serum alkaline phosphatase (SALP) activities, when compared to normal.

In toxic hepatitis, the presence of bilirubinemia is more common. The concentration of serum bilirubin always depends upon the rate of removal of bilirubin from the destruction of haemoglobin. It was noticed that at the end of
fourth week of treatment the serum of the animals were administered with aqueous extract + CCl₄ and Flavone compound + CCl₄ showed significant decrease in the levels of serum total bilirubin, when compared to CCl₄ treated group. This showed that the degree of hepatic cell damage was invariably less in Flavone isolated compound administered groups. The aqueous extract was less effective in altering the increase in the concentrations of serum bilirubin.

Estimation of serum enzymes namely transaminases (ALT and AST) and alkaline phosphatases (SALP) are stands as the important tools for the diagnosis of hepatocellular necrosis. In the present investigation administration of aqueous extract and the flavone isolated compound extracts lowered the increase in the levels of ALT, AST and SALP. This determines the hepatoprotective activity of the flavone compound and the aqueous extract of *A. alata* Nees.

In conclusion, the present investigation is the prime report of the establishment of a protocol for the micropropagation of *A. paniculata* and *A. alata*. The screening of medicinal value of the plants through clinical evidences is of immense value. So a comparative clinical analysis on the efficacy of the aqueous extract and the flavone compound isolated from the leaves of *A. alata* have been screened for the first time.

Generally, in vitro techniques applied on medicinal plants either to conserve the threatened population or to achieve enhanced quantity of secondary metabolites. In *Andrographis paniculata* and *A.alata* biosynthetic potentialities of the calli derived from different explants by the influence of different concentrations and combinations of growth regulators and the regenerants derived from different explants will be worked out in future.