REVIEW OF LITERATURE

Plant tissue culture is a classic field of biotechnological investigations, in which techniques are employed for the growth of plant organs, tissue and cells in \textit{in vitro} condition. The literary meaning of \textit{‘in vitro’} is in glass, and this is because the culturing is carried out within the glass vessels. This technique has proven its potential for the practical application in the improvement of important and endangered medicinal plants. A large number of plant species can be propagated all round the year and the plant breeder is no longer restricted by season in the production of plantlets or its components (Bajaj, 1986).

Plant synthesizes various medicinally important components such as alkaloids, glycosides steroids, flavonoids etc., similarly the \textit{in vitro} derived calli of plants are also synthesizes medicinally important compounds. Hence, this technique could be used as a tool for rapid multiplication and enhanced production of medicinally important active constituents (Amirato, 1983).

The idea of totipotency and culturing of tissue and cells under controlled condition was visualized by the German botanist Gottlieb Haberlandt (1902). By using this technique many scientists derived the protocol for extensive micro propagation of economically important plant species (Reinert, 1956; Skoog & Miller, 1957; White, 1966; Narayanaswamy, 1980; Bonga, 1984; Bajaj, 1986; Pollard Jeffrey, 1990; Macown & Amos, 1992; Purohit \textit{et al.}, 1993 and Callow, 1997.

The micro propagation involves three stages
1. The selection of suitable explants, their sterilization and transfer to nutrient medium.
2. Shoot bud organogenesis either directly from the explants or indirectly through the callus.
3. The transfer of shoots to the rooting medium, hardening and planting out.

A critical survey of literature enlightened us that the regeneration of plantlets through adventitious organogenesis has been reported in many species of Angiosperms such as *Santalum album* (Bapat & Rao, 1979); *Tectona grandis* (Gupta et al., 1980); *Dalbergia sissoo* (Datta et al., 1983); *Pterocarpus santalinus* (Patri et al., 1988); *Compotonia peregrina* (Louis & Torrey, 1991); *Bauhenia variegata* (Mathur & Kumar, 1992); *Gardenia jasminoides* (George et al., 1993); *Mimosa tenuiflora* (Villarreal & Gabriela, 1996); *Cercies canadensis* (Distabanjong & Geneve, 1997); *Gossypium hirsutum* (Agarwal et al., 1997); *Cumnium cuminum* (Hussein & Batru, 1998); *Vismia guianensis* (Monacelli et al., 1999); *Syzigium travancoricum* (Anand et al., 1999); *Pothomorphe umbellata* (Piereira et al., 2000); *Campanula carpatica* (Sriskandarajah et al., 2001); *Echinacea purpurea* (Koroch et al., 2002) etc.,

A considerable number of reports also published in recent years, indicated the use of various explants such as cotyledon, hypocotyledon, stem, leaf and root segments for callus initiation, maintenance and to know their regenerative potentialities. The regeneration of complete plantlets from stem-derived callus was first achieved by Chaturvedi (1975) in *Dioscorea florifunda*. Similarly successful regeneration of plantlets via callus have been reported by many investigators such as Mantel et al., (1978) in *Dioscorea alata*; Rao and Narayanaswamy (1972) in *Tylophora indica*; Nataraja and Patil (1980) in *Sida* and *Abutilon*; Bermudez et al., (1984) in *Digitalis obscura*; Srivastava et al., (1985) in *Betula pendula*; Kothari & Chandra (1986) in *Tagetes erecta*; Rai & Chandra (1988) in *Dalber gia latifolia*; Lal & Ahuja (1989) in *Rheum embodi*; Tulasidharana & Vaidyanadhan (1990) in *Vicoa indica*; Sudhershana &

A balance between endogenous and exogenous factors controls morphogenesis from the callus. Among which the relative concentrations of auxins and cytokinins are most important. The role of these growth regulators in determining the organogenic response has been well explained by Skoog and Miller (1957); Narayanaswamy (1980); and Razdan (1994).

The *in vitro* grown callus is often used as an alternative to whole plants for the production of useful secondary metabolites (Yamamoto, 1986). Many scientists succeeded in achieving enhanced production of medicinal compounds from the *in vitro* derived calli. Hence, tissue culture technique is employed as a biosynthetic process for the production of medicinally important compounds. Nakagawa and Tabata (1989) noticed that the *in vitro* derived calli of *Thalictrum minus* showed enhanced production of secondary metabolites when compared to *in vivo* plants.

Jojoba (*Simmondsia chinensis*) seedling explants were cultured on a modified medium, supplemented with various concentrations of BA alone combination with silver nitrate (Roussos *et al.*, 1999)
Cassells et al., (1999) noticed that, *Arnica montana* an endangered medicinal species does not give stable yield of pharmaceutical compounds in commercial plantations. While the *in vitro* derived regenerants produce enhance of quantity of secondary metabolite when compared to *in vivo* plants.

*Valeriana jatamansi* is an important endangered medicinal plant. Which is popularly used in indigenous system of medicine. This wild herb is being exploited for its roots and rhizomes which are highly effective against leprosy. Kaur et al., 1999 were successful in inducing rapid and large scale multiplication of *Valeriana jatamansi* by shoot bud culture. Further, they noticed that the shoot induction containing the combination of BAP and NAA was also favorable for the rhizogenesis from the shoots on the same media.

The *in vitro* morphogenesis via organogenesis was achieved from callus cultures derived from hypocotyl, explants of *Acacia sinulata* on Murashige and Skoog’s (1962) medium (Vengadesan et al., 2000).

In the culture of *Pittosporum napanlensis* a rare medicinal plant, (Dhar et al., 2000) noticed the best bud proliferation of shoot buds was achieved on Murashige and Skoog’s medium supplemented with BA and NAA.

Tiwari et al., (2000) described a protocol for rapid and large-scale clonal propagation of the valuable medicinal herb *Centella asiatica* by enhanced axillary bud proliferation from nodal segments. Although bud break was dependent on BA supply, the synergistic combination of BA and NAA induced the optimum frequency of shoot formation as well as shoot number.

The micro propagation procedure could be useful for raising a stock of genetically homogenous plant material for field cultivation. Many
investigations were successful in deriving the protocol for micropropagation of medicinal plant species such as, *Withania somnifera* (Manickum *et al.*, 2000); *Campanula carpatica* (Sriskandarajah *et al.*, 2001) and *Taxus mairei* (Chang *et al.*, 2001).

The development of *in vitro* regeneration systems for medicinal plant species has the potential to radically alter our approach to the production of plant based medicines and *in vitro* propagation allows for selection and clonal multiplication of genetically superior individuals, which may facilitate the development of improved varieties and address some of the above mentioned problems (Murch *et al.*, 2000).

Plants are the storehouse of many chemical constituents, which are synthesized and utilized for its metabolic activities as primary metabolites such as carbohydrates, proteins, lipids etc. or accumulated in plant body as secondary metabolites.

The greatest contribution of plant kingdom to mankind is alleviation of suffering from diseases by providing large variety of potent drugs. There are about 4.5 million species of plants on the earth out of them 2.5 million are Angiosperms and of these about 20,000 species have been identified and screened for the isolation of chemically active compounds which are present in them. Rest of the species of plants are yet to be subjected for the evaluation of phytochemical constituents. Inspite of spectacular advances in synthetic drugs some of the drugs are plant origin have still retained their importance. For example, Ephedrine, catharanthine, morphine, atropine, vincristine, reserpine, dioscogenine, digitalis etc., *Andrographis paniculata* is one such drug commonly used in the Indian system of medicine Ayurveda.
Schwyzer et al., (1952) showed that Andrographolide gives a positive legal test and has UV absorption maxima at 223 nm and addition of acetic anhydride to a solution of andrographolide in pyridine to give diacetyl andrographolide which consists of three double bonds had a melting point 162-163°, which gave positive legal test and showed absorption maxima at 223 and 299 nm. The later being due to the presence of three double bonds in conjugation.

Kleipool (1952) reported the isolation of new lactone Neoandrographolide from *Andrographis paniculata* Nees. He deduced the molecular formula C_{22}H_{38}O_{8} having alpha-beta-unsaturated gamma-lactone it showed a positive legal test and prepared as acetate derivative having melting point 157° C.

Srivastava et al., (1959) described a modified gravimetric method for the estimation of Andrographolide from *Andrographis paniculata* by diluting the alcoholic extract with water and extracting the chlorophyll by benzene and then the andrographolide by ethyl acetate.

Govindachari et al., (1961) isolated a new flavone, 5-hydroxy-7,8,2',3'-tetramethoxy flavone from repeated extraction of the roots of *A. paniculata* with Acetone and their chromatography over silica gel and then serial elution with Benzene : Hexane (3:1), Benzene and Chloroform : Methanol (20: 0.8). The Benzene elution was reported to contain the new flavone.

Subba Rao (1962) had reported the formation of red colour with Potassium hydroxide and andrographolide is unsatisfactory and suggested a chemical method involving lactone titration, as andrographolide is a lactone.

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Gaind *et al.*, (1963) suggested a spectrophotometric method to assay the Andrographolide in *Andrographis paniculata* Nees. by measuring the absorption of methanolic solution at 226 nm. The drug was first extracted with benzene to remove coloring matter and then extracted with chloroform. The chloroform extract was evaporated and the residue dissolved in methanol and absorbance was measured.

In the year 1963, Quadrat-I-Khuda *et al.*, isolated alpha-beta-unsaturated lactone, homoandrographolide (C_{22}H_{32}O_{3}), a sterol Andrographosterol (C_{23}H_{38}O), a hydrocarbon, Andrographane (C_{40}H_{82}), a ketone, Andrographone (C_{32}H_{44}O), a wax panicula wax and two different esters containing hydroxyl groups from Petroleum ether extract of leaves at room temperature.

Chan *et al.*, (1968) proposed the structure for neoandrographolide and the evidence presented indicated the skeletal structure similar to that of andrographolide. Two new diterpenoid glucoside, 14-deoxy andrographolide – 19 beta-glucoside and andrographolide-19-beta-glucoside offened from the more polar fraction of the plants were isolated and their structure was determined by Hu Chang –qi *et al.*, (1982).

Talukdar *et al.*, (1968) suggested an improved method for the estimation of andrographolide involving background correction to the titrimetric method (due to the presence of impurities). Later in the year 1969 they reported a rapid and quantitative method for estimation of andrographolide by TLC in aqueous alcoholic extract of leaves using Chloroform : Benzene : Methanol and Chloroform : Benzene : Ethanol as solvent system.

Chen Weining and Xiaotian (1982) isolated a new diterpenoid glucoside from leaves and its structure is elucidated as deoxyandrographolide-19-beta-D-
glucoside having melting point 201°C-203°C and Uv λ max (methanol) 205 nm.

Gupta et al., (1983) reported the method of isolation and characterization of two new flavonoids, 5-hydroxy-7-8-dimethoxy flavone and 5-hydroxy-3,7,8,2'-tetramethoxyflavone by subjecting column chromatography of the petrol extract of roots of *Andrographis paniculata* Nees.

Testsuro Fujita et al., (1984) had determined the crystal structure of andrographoilide by X-ray crystallographic analysis and reported the absolute configuration of C-14 as R, which was previously undecided. He also reported isolation of three new diterpenoids, andrographonin, andrograpanoside and 14-desoy-12-methoxy-andrographoilide, together with three known compounds, 14-deoxyandrographolide, neandoandrographolide and Andrographolide.

Phytochemical analysis of the whole plant of *A. paniculata* contain lactones such as Andrographolide, 14-deoxy, 11-oxo- andrographolide, 11,12-di, dehydro andrographolide and flavone 2'-glycoside (Bright et al., 2001).

Damu et al., 1998 have identified the chemical component of flavone 2' glycoside from the whole plant extract of *Andrographis alata* Nees. But nowhere in the literature further phytochemical analysis from different parts of *A. alata* has been reported. Hence, in the present investigation an attempt has been made to analyze the phytochemical constituents of leaves of *A. alata*.

In recent years a rapid progress has been noticed in the clinical study of hepatoprotective plants. The investigation of Karandikar et al., (1963), Rubin et al., (1963) and Recnagel et al., (1983) proved the administration of CCl₄ in rats
was known to cause centrolobular hepatic necrosis or toxic hepatitis and the injury caused by this toxic substance is similar to that of human infective hepatitis. Roullier et al., (1964) and Schotz et al., (1964) reported that the fats from the peripheral adipose tissue were translocated to the liver and kidney for accumulation during toxic hepatitis. Similarly the other hepatotoxic substances like alcohol, paracetamol and aflatoxin B1 were also known to cause hepatic cirrhosis in albino rats (Pandey et al., 1990); Dwivedi et al., (1990); Gulati et al., (1991) and Chattopadhay et al., (1992).

As early in 1979, Mahbubul et al., reported the isolation of three new flavones 5-hydroxy-7-8-2 trimethoxy 5,2-dihydroxy –7-8- dimethoxy and 5 hydroxy 7,8- dimethoxy flavones from the leaf callus of A. paniculata.

Ocimum sanctum - a hepatoprotective plant also protected rats against CCl4 induced liver injury. It is reported to contain ursolic acid, apigenin leuteolin, apigenin-7-0-glucuronide, luteolin-7-0-glucuronide orientin and molludistin.

Anand and coworkers (1981) have carried out detailed evaluation of alcoholic extract of aerial part of Indigofera tinctoria. The extract was found to afford significant protection to mice, rats and rabbits against CCl4 induced hepatic injury. It was also found to increase liver weight and bile flow in rats indicating microsomal enzyme induction.

Rege and co-workers (1984) in a series of detailed studies measured biochemical, morphological and histopathological parameters have evaluated the hepatoprotective activity of cyanidanol (+), a isolate from Acacia catechu and milk extract of Piper longum against CCl4 induced hepatic injury.
Sudhir et al., (1986) found that, the alcoholic extract of *Withania somnifera* leaves was found to significantly inhibit the CCl₄ induced alterations in transaminase activity and pentobarbitone sleeping time indicating presence of hepatoprotective activity.

Budhiraja et al., (1986) have identified a withanolide, 3-β-hydroxy-2, 3-dihydro withanolide F, isolated from *Withania coagulans* fruit, which also showed significant hepatoprotective activity against CCl₄ induced liver injury. The activity was assessed by measuring petrobarbitone sleeping time, serum transaminase activity and through histopathological studies.

Wagner et al., (1986) found that the ethyl acetate soluble fraction of *Eclipta alba* and the isolated active constituents of *Wedelia calendulacea* (Wedelolactone and dimethyl Wedelolactone) exhibited hepatoprotective activity. Besides, they observed a significant stimulatory effect on liver cell regeneration.

Dwivedi (1990) observed when aflatoxin-B₁ was given to albino rats caused significant increase in the serum bilirubin and the activities of serum enzymes like alkaline and acid phosphatase, lipase and transaminases. But, the total protein and the albumin concentration in the serum was decreased. When the glycoside fractions of *Picrorhiza kurrooa* was given to the affected rats for seven days, it caused significant reversal toxicant in the induced changes in serum bilirubin and enzyme levels. These results suggest that the hepatoprotective efficacy of *Picrorhiza kurrooa* against aflatoxin – B₁ induced liver damage.

Handa & Anupam Sharma (1990) reported that hepatoprotective effect of andrographolide the major active diterpenoid lactone of the plant
Andrographis paniculata on acute hepatitis induced in rats by single dose of galactosamine, paracetamol. Hepatoprotective activity was monitored by estimating the serum transaminases (GOT & GPT), alkaline phosphatase and bilirubin in serum, hepatic triglycerides and by histopathological changes in the livers of experimental rats.

Visen et al., 1990 and 1993 were isolated Andrographolide from the roots of A. paniculata and successful in comparatively evaluation of the hepatoprotective activity of isolated compound and root extract of A. paniculata.

Picrorhiza kurrooa attracted many scientists for its strong anti-hepatotoxic action. The isolated active constituents containing two iridoid glycosides exhibited significant hepatoprotective and anti-cholestatic activity against CCl₄ induced hepatitis (Dwivedi et al., 1990; Tripathi et al., 1991; Shukla et al., 1993 and Saraswat, 1993).

Gulati et al., (1991) reported that an indigenous herb Boerhaavia diffusa have been reported to be used in chronic alcoholism and jaundice. The root extract of this plant has been claimed to decrease SGOT, SGPT, SALP levels and increased the liver ATP ase activity in albino rats.

Histopathological studies conducted by Chandra et al., (1991) provided further support that the sections of the livers of rats treated with CCl₄ or alcohol showed accumulation of fats with fatcytes. Whereas, in Boerhaavia diffusa treated animal, reduction in the fat deposition was observed.

Andrographolide of Andrographis paniculata produces a significant dose dependent choleretic effect as evidenced by increase in bile flow, bile salt,
and bile acids aim conscious rats and anaesthetized guinea pigs. The paracetamol induced decrease in volume and contents of bile was prevented significantly by andrographolide pretreatment. It was found to be more potent than silymarin- A clinically used hepatoprotective agent (Shukla B. 1992).

Chattopadhyay et al., (1992) found the effect of Ocimum sanctum on paracetamol induced hepatic damage in rats. O. sanctum was found to protect the rats from hepatotoxic action of paracetamol as evidenced by significant reduction in the elevated serum enzyme levels. Histopathological studies showed marked reduction in fatty degeneration in animals receiving O. sanctum extract along with paracetamol as compared to the control group. It is stipulated that the extract treated group was partially protected from hepatic cell damage caused by paracetamol.

The work carried out by Reddy et al., 1993 have showed that the antihepatotoxic activity of alcoholic and chloroform extract of Phyllanthus niruri; Tinospora cordifolia and Ricinus communis was studied on albino rats wherein the extracts have been given after the liver was injured. This study was designed to study whether the herbal constituents could treat the already damaged liver with hepatotoxins like CCl₄ and found that among all the three plant species were tried Phyllanthus niruri was a best in reduction of liver toxicity in tested dose.

The discovery drew the attention of research workers throughout the world towards medicinal plants to search for hepatoprotective agents among them for eg., Picrorhiza kurooa Saraswat, et al., (1993), have observed the hepatoprotective activity with a detailed evaluation of its choleretic effect Picrorhiza kurooa and anticholestatic activity against the potent hepatotoxin carbon tetrachloride (CCl₄) using both conscious and anaesthetized animals.

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The activity is compared with a known hepatoprotective drug *Silymarin*. Significant anticholestatic activity was also observed against CCl₄ induced cholestasis in conscious rats, anaesthetized guinea pigs and cat. Picroliv was more active than the known hepatoprotective drug *Silymarin*.

Visweswaram *et al.*, (1994) stated, Carbon tetrachloride a pharmacological tool to produce liver damage and reduced the urinary excretion of ascorbic acid in rats. *Silymarin* and extract of *Phyllanthus amarus* prevented the CCl₄ induced reduction of ascorbic acid excretion in urine. The results indicate that the measurement of ascorbic acid excretion can be used as a non-invasive test for screening protective substances against CCl₄ induced hepatotoxicity in rats.

Sharma, (1995) studied the hepatoprotective effect of ‘Hepatomed’ an auyrvedic drug containing water extract of 6 medicinal plants has been studied on cumene hydroperoxide induced lipid peroxidation and reduced glutathione content in rat liver homogenate. *In vitro* experiments showed significant reduction in the level of malondialdehyde induced by 1.5 m Mcumene hydroperoxide. Glutathione content was almost did not show any rise in serum GOT and GPT. On similar doses, significant choleric effect was observed without any adverse histological changes after 4 days treatment. The results suggested that ‘Hepatomed’ is a strong hepatoprotective auyrvedic medicine with no detectable adverse effects.

Andrographolide from *Andrographis paniculata* has been studied for its analgesic, antipyretic and antiulcerogenic activities. Andrographolide did not show any analgesic activity in hot plate test in mice, while it showed significant analgesic activity in acetic acid-induced writhing in mice and Randall Selitto’s *In vitro studies on Andrographis paniculata Nees. and A. alata Nees.*
test in rats at 300-mg/kg doses. Andrographolide, produced significant antipyretic effect (Madav et al., 1995).

Rao and Mishra (1997) showed that the powder and different extracts of the roots of *Inula racemosa* were tested for their hepatoprotective activity against CCl₄, paracetamol and rifampicin-induced hepatotoxicities in rats. Powdered roots, total aqueous extract and methanolic extract showed significant hepatoprotective activities, comparable with those of silymarin.

Singh et al., (1998) studied the effect of an oral administration of verbenalin for its hepatoprotective activity. Hexobarbitone – induced sleeping time, zoxazolamine-induced paralysis time, bromosulphalein retention, serum levels of transaminases, bilirubin and total protein were used as tools for liver injury. A significant hepatoprotective effect was observed as evident from shortened hexobarbitone sleeping time and zoxazolamine paralysis time in mice, which were increased by CCl₄ treatment. Further, pre and post-treatment with verbenalin reduced plasma bromosulphalein in mice, serum transaminases and bilirubin in rats in a dose-dependent manner as compared to CCl₄ – treated animals. Verbenalin did not show any sign of toxicity and the minimum lethal dose was greater than 3.0g/kg when given orally or intraperitoneally to mice.

Trivedi and Rawal (2000) proved that *Andrographis paniculata* treatment prevents BHC induced increase in the activities of enzymes γ-Glutamyl transpeptidase, glutathione-S-transferase and lipid peroxidation. The activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and the levels of glutathione were decreased following BHC effect. Administration of *Andrographis paniculata* showed protective effects in the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathion reductase as well as the level of glutathione. The activity of lipid

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peroxidase was also decreased. The result indicates antioxidant and hepatoprotective action of *Andrographis paniculata*.

Shenoy (2001) has proved the elevation in the levels of end products of lipid peroxidation in liver of rats treated with CCl₄ were observed. The increase in malondialdehyde levels in liver suggested the enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Pretreatment with *Ginkgo biloba* significantly reversed these changes. Histopathological studies showed that CCl₄ caused steatosis and hydropic degeneration of the liver tissue.

Nan *et al.*, (2002) was carried out to investigate the antifibrotic effects of methanol extracts from the traditional Chinese medicinal herb, the root of *Scutellaria baicalensis* on liver fibrosis induced by bile duct ligation and scission or carbon tetrachloride in rats. Liver fibrosis was assessed by histological observations and by measuring levels of liver hydroxy proline, lipid peroxidation based on malondialdehyde production, and serum enzyme activities. A methanolic extract of *S. baicalensis* root inhibits fibrosis and lipid peroxidation in rat liver induced by BDL or CCl₄.

Many of the investigators screened the hepatoprotective activities of several plant species. But, so for only a few reports on the therapeutic efficiency of *in vitro* derived calli. Krishna, (1996) comparatively screened the hepatoprotective activity of *in vivo* plant extracts and their *in vitro* derived calli extracts of *Boerhaavia erecta* L. *B. rependa* and *Diospyros cordifolia* against CCl₄ induced toxic hepatitis. It was observed that the hepatoprotective activity was evaluated to more in the root- derived calli of *B. erecta* and *B. rependa* by altering the increased in the levels of ALT, AST, SALP, serum bilirubin, serum protein and albumin/globulin ratio.
Literature survey enlightened that, so far no investigations was carried out on the rapid micropropagation of *A. paniculata* and *A. alata* using both seedling and mature plant explants. Many of the investigators conducted phytochemical studies and clinical studies on the evaluation of hepatoprotective efficacy of active principles of *A. paniculata*. The earlier workers have not reported the therapeutic efficacies of *A. alata*. So, in the view of high medicinal value of these two species the present investigation was undertaken.

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