Discussion
5. DISCUSSION

Medicinal plants are the oldest sources of pharmaceutically active compounds and an estimated $\frac{2}{3}$ of the world population rely on plant-derived drugs (Schumaker, 1999). Collections from wild exhibit a wide variation in quality and quantity of the bioactive chemical and are unlikely to meet the growing demand for such products for health care. There are several advantages of the cell culture techniques that can be profitable viz., cheap and desirable production of bioactive molecules, enhanced production of bioactive molecules, enhanced production of medicinally important organic compounds in shorter production cycles, increasing yields by optimizing cell cultures and by genetic transformation / selection.

With growing interest in medicinal herbs and increasing therapeutic usage, availability of renewable resources is major, vital and global problem that cell cultures can solve. In view of this lacunae in tissue culture, a preliminary attempt was made to establish cell cultures of *Blumea mollis* which proved to be successful.

Some of the workers reported high callus induction when media contained NAA along with low concentration of cytokinin. (Neelam A, *et al.*, 1986 & Vani A.K.S., Reddy V.D. 1996). Earlier studies on leaf explants of genotype C-235 showed callusing along with rhizogenesis when cultured on medium containing auxin along with cytokinin. While the present study
showed that all the explants induced calluses when a specific combination of growth regulators. (2,4 – D + Kinetin + NAA) was used.

Mathur. S. and Kumar. S. (1998) stated that leaf and stem explants of *Bacopa monnieri* seems to have self sufficiency of regeneration without addition of phytohormones to the growth medium. On the other hand, plant morphogenetic studies carried out by earlier workers have revealed that an exogenous supply of growth regulators in growth medium is essential in plant growth and organogenesis. In the present study the supplementation of NAA with the combination, of 2, 4 - D and kinetin in calluses induced by leaf explants (Table 1) good percentage of regeneration, was observed.

The main objective of this experiment is to optimize the protocol for molecular fingerprinting analysis using ISSR primers but an interesting feature noted was the difference in the amplified products. The DNA fingerprints of sample DNA 1 & 2 showed some missing bands (Fig. 2). These missing bands were found in DNA of the plant samples brought from different habitats and may be significant in view of cultivar identification and phylogenetic studies.

PCR based DNA finger printing using RAPD markers have revaled high genetic variability among 250 isolates of *M.grisea* populationons at Himachal Pradesh (Sharma *et al.*, 2002b).

Two isolates of *A. Raphani* one each from Canada and France, produced discrete finger prints indicating genetic variation in these isolates (Sharma & Tewari, 1996b, 1988).
Molecular markers are used for the characterization of genetic variability in plant pathogens (Sharma et al., 1999). Using PCR, very closely related strains of a pathogen can be distinguished without prior knowledge of the nature of polymorphic regions by the use of RAPD (Williams et al., 1990). PCR based DNA fingerprinting, particularly with short oligonucleotide primers (Williams et al., 1990) has been used for the analysis of genetic variation in some plant pathogens.

In India, Twenty isolates of A. brassicae collected from geographically distinct regions of the world and different host species with RAPD markers have also been analyzed (Sharma & Tewari, 1995, 1998). Out of the five primers tested, primers OPA 07 and OPA 09 could not distinguish variation among these isolates. However, three primers e.g. OPA 03, OPA 04 18 were efficient in the detection of inter-and intra-regional variation among the isolates of A. brassicae.

Closely linked markers (Woo. S. S. et al., 1994) has been used successfully for the isolation of a number of important plant genes, including genes for disease resistance. (Giraudut et al., 1992 & Song W.Y. et al., 1995). Since RAPD are highly reproducible and dominant markers, the simple sequence repeats (SSR) (Litt M & Luty J.A., 1989 R; Weber J. & May 1989). have been initially employed to detect to detect polymorphism between the parental cultivars. Simple sequence repeats, also known as microsatellites, are based on tandem repeats of short (2-6 bp) DNA sequences. Different alleles
can be detected at a locus by the polymerase chain reaction (PCR), using conserved DNA sequences flanking the SSR as primers.

An assessment of genetic diversity is important for the breeders in order to select the diverse type in crossing programmes and also for the gene bank managers to devise strategies for their conservation. Recently, the identification and characterization of genotypes has assumed greater importance due to reaffirmation of sovereign rights of the countries on their genetic resources (CBD, 1994). Analysis of the cultivars and improved lines using efficient and robust DNA markers such as SSR is valuable in this regard.

Anatomical investigation is an integral part of herbal science. Microscopic analysis of a drug is the ultimate method to check the identity when the materials are in crude fragments. In the present work, a set of anatomical characters is provided which may be considered as a protocol of diagnostic features.

The anatomical features selected include leaf epidermal characters, anatomy of the midrib, histology of the lamina and vascular pattern of the petiole. In addition to these features, calcium oxalate crystals were found to be specific both in habits and localization. So the presence of calcium oxalate in sub epidermal ground cells of the midrib may be considered for botanical identity of the drug in the present investigation. Earlier pharmacognosists have largely relied upon the crystal morphology for standardization of crude phytodrugs (Wallis, 1997).
The determination of the paw volume in the experimental groups has been used for evaluating the degree of inflammation and the therapeutic effects of the drug. The extracts of *Blumea mollis* (test drug) along with the standard drug ibuprofen showed significant reduction in volume when compared with control rats. The time course of oedema development of carrageenan induced paw oedema methods in rats generally involves 3 district phases of mediators release, including, histamine and sertatonin in first phase, kinins in the second phase and prostaglandins in the third phase, (Singh 1999).

The effect of the extracts on carrageenan induced paw edema was more pronounced in the third hour of the inflammatory response, which corresponds to the phase of prostaglandin release. Also the carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitions and has been used to evaluate the effect of NSAID's which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. (Phadke. K, 1988) Based on these reports it can be concluded that the inhibitory effects of the extracts on inflammation could be due to inhibition of prostaglandin. The exact mechanism of action of the extracts requires further investigation.

In the present investigation of antipyretic study, the extracts of *Blumea mollis* (D.Don) Merr exhibited significant antipyretic activity when compared with the standard drug, paracetamol. The active principle of the extract, which has the antipyretic activity, may be confirmed on further investigation in future.
Hyperthermic body temperature of rat (160-180 g) was measured after Dandiya and Collumbine (1959). The sting extract was considered as crude venom, which possessed lethality in mice (LD50 = 9.3 mg/kg, iv), significantly decreased the normothermic body temperature of mice but had no effect on hyperthermic body temperature of rat. (D. Muhuri, et al., 2004).

The extracts of *Blumea mollis* (D.Don) Merr has very good antibacterial activity against gram positive bacteria like *Staphylococcus aureus* (multiple drug resistant strain and non-resistant strain) It has also indicated very good activity against gram negative bacteria like *E-Coli, Klebsiella pneumonia* and *Proteus vulgaris*. The *Blumea mollis* extract is comparable up to 20 mg/ml concentration with the standard antibiotic like chloramphenical (100 mg/ml).

Aleurites moluccana has activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and traditionally used for wound healing (Locher et al., 1995). The medicinal plant *Rhus glabra* (Saxena et al., (1994), plants from Nigeria like Emilia coccinea, Grewia Carpinifolia (Olukoya et al., 1993) were reported for their antimicrobial activity. Hexane and ethyl acetate extracts of *Uvaria narum* and *U. hookeri* showed significant antibacterial, antifungal and antihelminthic properties (Padmaja et al., 1993).

Antimicrobial activity of xanthones from *Calophyllum* sp. was reported against meticilin-resistant *Staphylococcus aureus* (MRSA) (Dharmaratne et al., 1996). The antibacterial activity was also reproted from the seed powder of Mirabilis jalapa (KUsamba et al., 1991), stem bark of Kigelia pinnata
(Dorothy et al., 1991), resinous exudates from twigs and leaves of Eupatorium saliva (Urzua et al., 1998) aerial part of Scrophularia frustescens and S. sanbucifolia (Fernandez, et al. 1996) and the floral petals of many angiosperm plant (Darokar et al., 1998).

More often, extracts of plants with various organic solvents yield antibacterial principles with different spectrum and activity. This is evident from the works of Dhawan et al. (1977), wherein the ethanol extract of C. quadrangularis was effective against most bacterial, yeast and fungal pathogens at the concentration of 25 μg/ml.

Different extracts (like methanol, ethanol, water, hexane, chloroform etc. of the different part of the plants like acalypha wilkesiana (Alade et al, 1993) Adhatoda vasica, Cardiospermum halicacabum (Elsamma Thomas 1999), Ixora coccinea (Latha et al., 1995) were reported to possess medicinal properties.

The Blumea mollis extract has been found to exhibit good inhibitory effect on the fungus Candida albicans. Of all the extracts used the petroleum ether and methanol extract exhibited a broad spectrum activity against Candida albicans. The Blumea mollis extract can be exploited for the treatment of various bacterial and fungal infections.

In the method of TLC Bioautography, antibacterial zones appear as clearspots against a background of bacterial colonies, zones were visualized more clearly by the use of p-iodonitrotetrazoliumchloride (INT) which indicate
bacterial dehydrogenase activity. This solution when sprayed on the face of medium, zones of inhibition (and therefore antimicrobial compounds) appear as clear zones against a purple background.

Zones of inhibition can be compared with previously developed TLC plate, so that the fractions may be further subjected to isolation of active metabolites. The present investigation is to optimize the protocol for TLC Bioautography of the *Blumea mollis* extract and further investigations may be done in future to isolate the active principle (Compound) which is responsible for the antibacterial activity of the *Blumea mollis* extracts.

Paracetamol (N-acetyl p-amino phenol, acetamino phen) a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses. It is mainly metabolized in the liver to excretable glucuronide and sulphate conjugates. However, hepatotoxicity of paracetamol has been attributed to formation of toxic metabolites when a part of paracetamol is activated by hepatic Cyt-p 450 (Savides MC & Ochma FW (1983) to a highly reactive metabolite N-acetyl-p-benzoquinoneimine (Vermeulin *et al.*, 1992 and Dehlin. O. *et al.*, 1984) which is normally conjugated with GSH and excreted in the urine as conjugates. Overdose of paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis.

Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman RB & Lawhorn
GT, 1978). Damage to liver cells cause leakage of cellular enzyme into serum. A significant rise in SGOT, SGPT could be taken as an index of liver damage. The reversal of increased serum transaminases returns to normal by *Blumea mollis* supplementation with healing of hepatic parenchyma and regeneration of hepatocytes.

ALT concentration has been used to evaluate chemically induced hepatic injury. More than 90% of ALT activity has been found to be elevated in serum of common laboratory animals used in toxicity studies. *Blumea mollis* prevented the paracetamol effect on ALT activity in serum. It is reasonable to suggest that *Blumea mollis* limited the severity of liver injury. Stabilization of serum bilirubin levels through the administration of *Blumea mollis* is further a clear indication of the improvement of the functions of the liver cells.

The present results support that the recovery of hepatic glycogen content was observed in the pretreatment of *Blumea mollis* treatment, while signs of histological and biochemical recuperation were present in the liver of rats treated with *Blumea mollis*.

GSH in the cytosolic pool consists of 85% hepato cellular GSH and 15% mitochondrial GSH. Hepatic GSH depletion or even extra hepatic GSH depletion can provide useful information on the protective role of GSH against toxic foreign compounds.

Thus GSH, be regarded as an endogenous protective agent against drugs In the present study decreased level of reduced GSH in liver was decrease in
paracetamol induced animals, while pretreatment of *Blumea mollis* clearly enhanced the GSH levels. GST is a soluble protein located in cytosol, which plays an important role in the detoxification of excretion of xenobiotics. It increases the solubility of hydrophobic substances and metabolises toxic compounds to non-toxic ones, which mean they have an increasing protective activity of the liver.

The increased hepatic GST activity induced by *Blumea mollis* can, therefore, reduce the paracetamol hepatotoxicity. There was a decrease in GPX activity in animals administered with paracetamol, which could be due to the higher production of toxicity. In presence of *Blumea mollis*, GPX levels were restored back to control levels. The increase in hepatic GSH-R activities were shown in *Blumea mollis* supplemented rats as compared with the liver of paraceta mol-induced rats.

These results suggest the hepatoprotective action of *Blumea mollis* which protect hepatic cells from paracetamol induced damage and the degree of hepatoprotection improved with increasing dosage. Further these data provide information regarding the possible use of *Blumea mollis* a hepatoprotectant in Indian systems of medicine.

The present study provide an overview of the pathological changes in the liver, kidney and spleen.

The liver assumed to play a vital role in the defense system. However reports have shown that liver also has a scavenging role. In the present
investigation it was proved that by the oral administration of *Blumea mollis* extract for 10 days the liver cirrhosis caused by paracetamol was cured. Koller *et al.* (2001) observed increased spontaneous development and progression of histopathological lesions such as mononuclear cuffing of hepatic bile ducts, progression of granulomas in renal glomeruli vessels in mice due to exposure to toxic oils and metals.

In the present investigation the renal tissue showed tubular degeneration and loss of cells in interstitium. Hemorrhagic necrosis of tubular interstitial cells of the kidney and sloughed tubular lumen were recorded by Horne *et al.*, (1977). The *Blumea mollis* extract exhibited significant curative effect.

The spleen is believed to be involved in the clearance of macromolecule (scavenging), antigen degradation and processing and the production of antibody. The spleen is mainly composed of blood cells, endothelial cells, reticular macrophage, melono macrophage cells (Press *et al.*, 1995).

In the present study the splenic tissue showed only slight changes which is not significant. Due to the slight changes produced in the spleen, by the administration of the extract, it may be concluded that the extract has no toxic effect on the spleen. Chhabra *et al.*, 1990 & Gralla, *et al.*, 1979 had seen enlargement of the spleen in rats dosed with parent aromatic amines. Bus, 1983, suggested splenic weight increase in dosed rats due to deposition of damage to erythrocytes as a result of aromatic amine toxicity.
6. SUMMARY AND CONCLUSION

The Plant Kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the significance of medicinal plants. Drugs from plants are easily available, less expensive and rarely have side effects. The compounds from plants are a source of an effective substitute for chemical drugs. The WHO (World Health Organisation) has reported that around 21,000 plants have been used for medicinal purpose in the world. About 500 higher species has been thoroughly investigated as potential source of new drugs. Nearly 119 pure chemicals were extracted from 90 plant species.

The indigenous system of medicine like Ayurvedic, Siddha and Unani have been meeting the needs of 70% of our population residing in villages. There is a growing tendency all over the world to shift from synthetic to natural based products including medicinal plants. Thus medicinal plants constitute a group of industrially important crop which bring appreciable income to the country by the way of export.

Though the conventional breeding techniques have considerably increased the productivity of modern crops, the application of biotechnology could speed up further crop improvement. It overcomes the barriers in conventional vegetative propagation and fulfills the demands for large-scale
cultivation in a short period by rapid mass multiplication. It could significantly shorten the breeding cycle.

In the present study a protocol has been developed and standardized for direct and indirect regeneration of *Blumea mollis* leaf explants by using various phytohormones. For indirect regeneration through callus, leaf explants were cultured in MS medium supplemented with various phytohormones. These calli were allowed to regenerate shoots in the basal medium containing 2,4-D + kinetin + NAA.

The present investigation on DNA finger printing with ISSR primers was to standardize the protocol, but it was found that there were changes in the PCR pattern of different DNA samples of the plants taken from different habitat which may prove significant in cultivar identification and phylogenetic study of the plant.

The plant material was further subjected to pharmacognostical, and pharmacological studies to find out their correct identities, therapeutic potential and properties.

It is evident from the pharmacognostical studies that the anatomical characters of leaf lamina, petiole, midrib, trichomes, cuticle, stomata and the crystal study determines the botanical identity of drug and to establish its purity and genuineness.
By the acute anti inflammatory study and antipyretic study conducted with the *Blumea mollis* extract, it was concluded that the herbal drug is effective against inflammation and in reducing the body temperature (Anti Pyretic effect).

Results from the present investigation indicates that paracetamol causes severe histopathological alterations in liver, kidney and spleen. Supplementtion of the herbal drug, *Blumea mollis* extract, ameliorated the toxic liver damage by paracetomol and the active principle responsible for this effect needs further investigation.

It is evident from the results of the present investigation that the *Blumea mollis* plant may be used as herbal drug for the treatment of liver diseases, inflammation and killer diseases.