REVIEW OF LITERATURE
The present research investigation deals with induced chemical (EMS and dES) mutagenesis in *Andrographis paniculata*. Morphological, anatomical, palynological, cytological (meiotic chromosome analysis), biochemical (quantitative and qualitative estimation of seed protein, assessment of total andrographolide as well estimation of andrographolide by HPTLC) and molecular (RAPD analysis) assessments are made in the species (germplasm under investigation). Further, cytomorphological, genetical, biochemical and molecular aspects of the induced mutants are also evaluated. The objective of the present work is to enhance genetic diversity in the existing germplasm and to raise desirable ‘plant type’ mutants. The review of literature is conducted on the related aspects on which present study has been performed.

**A. Reproductive biology**

*A. paniculata* is hermaphrodite, self compatible and a habitual inbreeder. Both stigma and anthers are in intimate proximity showing synchronization of anther dehiscence and stigma receptivity respectively, thereby providing autonomous selfing in the species (Lattoo *et al.* 2006). Lattoo *et al.* (2006) also studied seed set percentage on open pollination (80.36 ± 0.44) and on self pollination (82.42 ± 1.92), seed germinability percentage (88.0% in petriplates and 34.0% in field), 100 seed weight (117.04 mg ± 0.44), seed moisture content (11.55%), number of pollen grains per flower (3927.12 ± 129.62), pollen fertility (73.68%), pollen size (48.26μm to 75.8 μm, mean 55.93 μm) number of ovules/flower(12), number of seeds/fruit (12 to 16), seed protein content (0.065%) and leaf protein content (0.0076%), and suggested that these parameters may be useful for breeding endeavour.
B. Cytological aspects

The somatic chromosome number in *A. paniculata* has been reported to be $2n = 50$ (Raghavan 1957, Ellis 1962, Mitra and Datta 1967, Fedorov 1969, Datta and Maiti 1970, Govindarajan and Subramanian, 1983, Roy and Datta 1988). Roy and Datta (1988) performed karyological assessment of different biotypes (collected from India and Bangladesh) of the species and suggested variations (chromosome length 0.50 $\mu$m – 1.75 $\mu$m to 1.00 $\mu$m – 2.00 $\mu$m; total diploid chromosome length – 51.66 $\mu$m to 70.00 $\mu$m) among the biotypes, which were attributed to minute structural aberrations like translocations, deletions, etc. Chromosomes with secondary constrictions among the biotypes ranged from 4 to 8.

C. Cytogenetic study

Lattoo *et al.* (2006) induced genetic male sterility (6.0 to 14.0%) in *A. paniculata* at M$_1$ (no seed set on spontaneous selfing) following 20kR gamma irradiations and the male sterile gene was found to be monogenic recessive to normal. Male sterility was of sporogenous-type, which released microspores from quartets and also induced tapetal degeneration simultaneously. The male sterile gene acted upon the tapetal layer and also affected non sporogenous tissue within the anther locule resulting in encroachment of the locule and thereby, significantly reducing the pollen production and enhancing the formation of abortive pollen. Microspore abortion was characterized by mild exine differentiation, abortive and shrunken pollen and cytoplasmic nuclear degeneration. Pollen-ovule ratio reduced significantly in the mutant. Female fertility in the mutant remained unimpaired and fully intact.
D. Identification, purification and determination of selected phytochemicals from *Andrographis paniculata* Nees.

Gorter *et al.* (1911) reported that bitter substance in the leaves of *Andrographis* is a lactone ‘andrographolide’. Cava *et al.* (1965) indicated the structure of andrographolide to be a bicyclic diterpenoid lactone.

The first bitter principle obtained as intensely bitter yellow crystals and it does not respond to any test for alkaloids and glycosides; while, second bitter substance was obtained in an amorphous form named as ‘kalmeghin’. Four diterpenes such as andrographolide, neoandrographolide, deoxyandrographolide and andrographiside were isolated from the n-butanol fraction of alcoholic extract of the plant (Gupta *et al.* 1990).

Sharma *et al.* (1992) determined the major active constituent of andrographolide by HPLC analysis and found that the leaves of the herb were with highest amount (2.39% w/w) of andrographolide; while, the seeds contain the lowest. The technique used was found to be much more sensitive and simple than the known spectrophotometric method.

Saxena *et al.* (2000) reported a rapid and simple HPTLC method for the simultaneous quantitative estimation of the biologically active diterpenoids, 14-deoxy-11, 12-didehydroandrographolide, andrographolide, neoandrographolide and andrographiside in leaves of *A. paniculata*. Methanol was considered to be the most appropriate solvent for the exhaustive extraction of andrographolide derivatives.

Chauhan *et al.* (2000) documented a simple and reproducible reverse phase high pressure liquid chromatography (HPLC) method for the determination of andrographolide in *A. paniculata*. The sensitivity of the method was found to be 0.25 mg and the linearity range of 0.25 to 2.50 mg. The andrographolide content was observed 2.12% w/w in test sample.

Rajani *et al.* (2000) developed a simple and rapid method for isolation of andrographolide from leaves of *A. paniculata*. The identity of the compound was confirmed through IR, UV,
mass and melting point, and co-chromatography with a reference standard on TLC. The purity of the compound was confirmed by TLC, UV absorption spectrum, HPLC and differential scanning calorimetry.

Further, an HPLC method for the extraction and determination of diterpenoids in leaves of *A. paniculata* was suggested by Jain *et al.* (2000) where the detection limits for andrographolide, neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide were 0.2, 0.25 and 0.1 µg/injection respectively.

Cheung *et al.* (2001) developed a method for determining bioactive diterpenoids from *A. paniculata* by micellar electrokinetic chromatography. The method was validated with near 100% accuracy as projected in correlation coefficients (0.9986-0.9989); while, relative standard deviation of migration time was between 1.14 and 2.42. It was concluded that this method could be used for speedy and accurate qualitative and quantitative analysis of bioactive diterpenoids in *A. paniculata* plant and its derived products.

Zhao *et al.* (2002) developed a micellar electrokinetic capillary chromatographic (MEKC) method for determining the active components (andrographolide, doxyandrographolide and neoandrographolide) in water: ethanol extract of the Chinese crude herb *A. paniculata* and its preparations.

A Reverse-phase high performance liquid chromatographic method for the determination of andrographolide in commercial *A. paniculata* product was also developed by Li *et al.* (2002). Du *et al.* (2003) used a speed counter-current chromatography for successful separation of andrographolide and neoandrographolide from the leaves kalmegh. A single 280-min separation yielded 189 mg of 99.9% andrographolide and 9.5 mg of 98.5% neoandrographolide applying water-ethyl acetate-n-hexane (2.5:2.5:4:1) solvent system. Structure confirmation was done by electrospray Ms, one-dimensional NMR experiments, circular dichroism, optical rotation dispersion and specific optical rotation [alpha] D.

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*Induced Chemical Mutagenesis (Ethyl methane sulphonate and Diethyl sulphate) In Andrographis paniculata (Burnt. F.) Nees (Family: Acanthaceae)*

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Kumaran et al. (2003) developed an HPLC method for the estimation of andrographolide in biological fluids from *A. paniculata*. The developed method for estimation of andrographolide in blood serum is reported to be sensitive, reliable and accurate.

A stability test on andrographolide shows that crystalline andrographolide was highly stable even at 70°C (75% relative humidity) over a period of 3 months. Heat-accelerated conditions revealed second-order kinetics of the decomposition with the rate constant at 25°C ($k_{25\,^\circ C}$) predicted from the Arrhenius plot of $3.8 \times 10^6/d$ (Lomlim et al., 2003).

Pholphana et al. (2004) also developed a simple and rapid method of HPLC for the extraction and simultaneous determination of the three active diterpenoids, andrographolide ($AP_1$), 14-deoxy-11, 12-didehydroandrographolide ($AP_3$) and neoandrographolide ($AP_4$), from various plant parts.

Srivastava et al. (2004) used HPTLC method for analyzing hexane, chloroform, methanol and water extracts of leaves and a computerized densitometer was applied to study the two-dimensional spectrographic image analysis of the HPTLC plates. An HPLC equipped with a photodiode array detector showed that andrographolide and neoandrographolide were absent in the hexane extract but were present in greater amounts in the methanol extract as compared with the other extracts. The chromatograms developed were reported to serve as a chemical fingerprint of the drug *A. paniculata* for quality control purposes and in the preparation of formulations based on the drug.

Aromdee et al. (2005) studied a spectrophotometric method for the determination of total lactones in *A. paniculata* which was established by using dinitrobenzoic acid and potassium hydroxide solution as color forming agents. The total lactones content calculated as andrographolide, determined by this method was $8.61 \pm 0.52\%$ (n=4).

Akowuah et al. (2006) described a rapid method based on HPTLC and RP-HPLC with UV-detection for quantitative determination of two major bioactive compounds in *A. paniculata*,.
andrographolide and 14-deoxy-11,12-didehydroandrographolide. The recoveries of the two compounds were between 96.5 and 99.0% by HPTLC method and 98.1 and 99.2% by HPLC assay. The relative standard deviations of the two compounds ranged between 0.89 and 0.99 (intra-day) and 0.86 and 0.98 (inter-day) for the HPTLC method and 0.86 and 1.02 (intra-day) and 0.87 and 1.12 (inter-day) for HPLC method. The methods were used for routine analyses and to obtain relative amount of the two compounds in the leaves of the plant cultivated in different locations of Malaysia. The extracts and the isolated compound exhibited lipid peroxidation inhibition and free radical activities.

Ruengsitagoon et al. (2006) proposed a simple flow injection colorimetric procedure for determination of andrographolide. It is based on the reaction between andrographolide with 3, 5-dinitrobenzoic acid, resulting in an intense purplish red complex with a suitable absorption at 536 nm. The proposed method was satisfactorily applied for determination of andrographolide in plant samples.

E. Molecular genetics

Padmesh et al. (1998) analyzed 52 accessions (displaying morphological and phytochemical variation) of A. paniculata from India, Thailand, Malaysia and Indonesia for intra-specific variability following RAPD analysis. Molecular analysis revealed moderate variation within the species. UPGMA followed by cluster analysis resulted in five major groups among/between genotypes and it was noted that AP-48 (Thailand) possesses close resemblance to AP-38 (Tamil Nadu) and AP-29 (Assam). Results also indicated that RAPD could be effectively used for assessing genetic diversity and prospective value in breeding. Lattoo et al. (2008) also used RAPD (6 highly polymorphic primers) to elucidate genetic diversity among 53 accessions (varied significantly in metric traits) of A. paniculata belonging to 5 ecogeographic regions. RAPD based UPGMA and D² cluster analysis
revealed that the accessions might originated from native places of wild abundance. The authors suggested that assessment of both morphometric traits and RAPD marker analysis were complementary approaches to make diversity analysis more explanatory and purposeful for optimum genetic amelioration and effective conservation of genotypic variability. On the contrary, Sakuanrungsirikul et al. (2008) documented that two molecular marker (ISSR-43 primers used and 391 loci detected, but monomorphic across all accessions; RAPD-41 primers used and 195 loci detected) approaches could not detect genetic variations among 44 accessions (Thailand) of the species.