1. **ABSTRACT:**

*Gmelina arborea* Roxb. (Verbenaceae), commonly known as Gambhari or Shevan, is highly valued in Ayurveda for treating various stomach disorders, fevers and skin problems. The plant extracts are reported to exhibit anti-inflammatory, wound healing and platelet aggregation inhibitory properties (Shirwaikar et al., 2003). Chemical constituents of *G. arborea* include lignans (Anjaneyulu et al., 1977), flavonoids (Nair and Subramanian, 1975), iridoid and phenylpropanoid glycosides (Hosny and Rossaza, 1998). Roots are described as bitter, tonic, stomachic, laxative and used in many Ayurvedic preparations like Dashmula and Chyawanprasha. It forms one of the major five roots of Dashmula and is therefore much used in a variety of diseases (Tewari, 1995). Roots have been explored for anti malarial (Simonsen et al., 2001) and cardiovascular activity (Khanna et al., 1991) but to our knowledge, hepatoprotective and antioxidant activity of roots have not been reported so far. Looking to the multiple uses of *G. arborea* in traditional system of medicine, lacuna in the field of pharmacological activities reported and phenylpropanoid rich composition of roots, the present study was designed to check and evaluate the hepatoprotective and antioxidant potentials of the plant and the constituents responsible partly or wholly for the same. In our study we have also tried to explore possible mode of action by detailed investigation with respect to its antioxidant effect in hepatic damage and correlation of both the activities.

Before undertaking pharmacological work, a detailed pharmacognostical and phytochemical evaluation was carried out for the authenticated root sample of *G. arborea*. Preliminary phytochemical analysis revealed presence of phenolics and flavonoids in roots and their estimation was carried out. Major microscopical characters include lignified cork cells, group of stone cells in cortex, abundant starch and bordered pitted xylem vessels. Crude extract (CE) was fractionated successively to prepare ethyl acetate (EAF), n-butanol (BF) and aqueous fraction (AF) and evaluated for their hepatoprotective effects on isolated rat hepatocytes by trypan blue exclusion assay. Looking to the significant protection observed with CE and EAF in *in vitro* conditions, all the
fractions (300 mg/kg, p.o.) were evaluated for protection against CCl₄ and paracetamol-induced acute hepatotoxicity in in vivo conditions and compared with silymarin that was used as standard. The hepatoprotective activity was evaluated through the measurements of aminotransferases (SGOT and SGPT), ALP and bilirubin in serum. Further, the protection against free radical generation was assessed by measuring MDA, SOD, catalase and glutathione levels in tissue homogenate. Liver sections were also studied histopathologically to observe the pathophysiological changes. Determination and evaluation of these parameters are used to assess hepatotoxicity and inhibitory effects of the test drugs on this process are indicators of hepatoprotective activity. CE and EAF (300 mg/kg) of roots of G. arborea significantly inhibited acute liver toxicity induced by high doses of CCl₄ and paracetamol in rats, as shown by a reduction of serum liver enzyme activities and hepatic lipid peroxidation, as well as the preservation of the integrity of the liver cells evident from histological study. The protective effects obtained with the EAF (300 mg/kg) were found comparable with silymarin (50 mg/kg). Amongst all the fractions, EAF was found most effective and on that basis it was evaluated for effectiveness against chronic damage induced by prolonged administration of ethanol (4g/kg, p.o., for 21 days) in rats and found to exhibit protection consistent to the above findings. To evaluate whether or not the hepatoprotective activity of EAF is mediated through the antioxidant and antilipidperoxidative effects of various phenolics and flavonoids, a detailed antioxidant activity study was carried out with all the fractions. Consistent with the results of hepatoprotective activity, EAF was found to exert significant free radical scavenging activity when checked for DPPH, nitric oxide, super oxide and reducing power assay and found to exhibit significant protection against lipid peroxidation when evaluated in different in vitro and ex vivo systems. Based on the results of pharmacological activity, ethyl acetate fraction of crude extract was subjected to isolation and characterization of possible bioactive principles by applying different chromatographic techniques. Two phenolic hydroxycinnamic acid esters of cluytyl alcohol, cluytyl ferulate and cluytyl caffeite were isolated from the active fraction (EAF) of roots of G. arborea. Phenolics have been already reported to act as free radical scavengers by virtue of their hydrogen-donating ability and
protection provided against lipid peroxidation has been associated with \( \alpha, \beta \)-unsaturated carboxyl ester moiety. Taking into account the fact that hydroxycinnamate derivatives have demonstrated hepatoprotective activity, their presence in the ethyl acetate fraction of *G. arborea* could explain the protective effects observed in present study. Further, quantitative methods were developed using silica gel HPTLC plates, automated band-wise sample application, detection with specific reagent solutions for each component and automated densitometric determination for a variety of constituents present in ethyl acetate extract and hydrolyzed ethyl acetate extract roots of *G. arborea*. The method for estimation of isolated compounds and quercetin, \( \beta \)-sitosterol, lupeol was validated for specificity, linearity, accuracy and precision. Cluytyl ferulate and cluytyl caffeite both resolved in a single run, in a single solvent system by the developed HPTLC method. Quercetin and \( \beta \)-sitosterol were found to be present and were estimated in both the extracts while lupeol was found to be present only in hydrolyzed ethyl acetate extract. These constituents from roots of *G. arborea* can be assigned as possible active compounds, which may results in synergism for antioxidant activity and thereby provide good hepatoprotection.

In conclusion, our data suggest that ethyl acetate fraction of crude extract of roots of *G. arborea* possesses significant hepatoprotective activity against three well known toxicants- induced liver damage. Further, potent antioxidant activity suggests a good correlation between antioxidant and hepatoprotective activity. This is the first report on hepatoprotective and antioxidant activity of roots of *G. arborea*, as well as isolation, characterization and estimation of possible active constituents from the active fraction of roots of *G. arborea*. Further, the developed HPTLC method is the first attempt to establish qualitative and quantitative analysis that can aids in the standardization of roots of *G. arborea*, which is highly valued in traditional system of medicine for its multiple uses.