ABSTRACT

The Glutathione-S-transferases (GSTs) (EC.2.5.1.18) are enzymes that participate in cellular detoxification of endogenous as well as foreign electrophilic compounds, function in the cellular detoxification systems and are evolved to protect cells against reactive oxygen metabolites by conjugating the reactive molecules to the nucleophile tripeptide, glutathione (GSH, γ-glu-cys-gly). The GSTs are versatile proteins and are distributed in a cell cytoplasm, microsomes and mitochondria. Cytosolic GSTs have been grouped into seven distinct classes as: alpha (α), mu (μ), pi (π), sigma (σ), omega (ω), theta (θ) and zeta (ζ).

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes. *Hybanthus enneaspermus* is an erect shrub of Violaceae family. Because of its free radical scavenging nature it has been subjected to intensive phytochemical screening to determine its medicinal usage. The active principle isolated by HPLC showed a single peak at a RT of 2.16 minutes and the λ max at 217 nm. This compound on chemical analysis found to be 1,3-butadiene and dicarboxylic aromatic molecules.

Glutathione S-transferases were purified from mice kidney and testis using affinity chromatography and then the purified sample was analyzed by using SDS-PAGE gel to determine the protein subunit band pattern. The molecular weight of the subunits of GSTs kidney and testis of mice were found in the range that was consistent with previous studies. The enzyme activity and protein content were decreased rapidly however it produced pure GST proteins. The GSTs purified from mice kidney were resolved as two subunits i.e Yc and Yb and testis as four subunits those are Yc, Yb, Yβ and Yδ. This is also confirmed by Western Blott analysis.

During experimentation to analyze the effect of the extract of *H. enneaspermus* mice were subjected to both acrylamide and mixture of AC & HI E. This exposure significantly alter the specific activity of mice GSTs.
Polyclonal primary antibodies were produced to purified GSTs from mice kidney and testis for immunoblot analysis. These treatment studies on mice kidney and testis GSTs showed significant difference of expression in dose and time dependent manner. Therefore the present results based on treatment on mice especially kidney and testis it is calculated that HE protects these two tissues due to the regulation of the synthesis of GST proteins.

**Key Words:** Glutathione-S-transferases, mice kidney and testis GSTs, Purification, Acrylamide, *Hybanthus enneasperneaus* active principle, Western blotting.