3. Objective & Plan of work

3.1 Objective of the work

The overall objective of the present study was to examine the hepatoprotective activity of catechin, lecithin, L-Ornithine-L-Aspartate, L-Ornithin and silymarin with respect to mitochondrial pathway. To address this objective, we formulated the following questions:

1) What are the normal levels of Bax, Bcl-2 and P53 expression in the hepatocytes?
2) Does Liver toxicant’s alter the amount of Bax, Bcl-2 and P53 expression in the hepatocytes?
3) Does catechin, lecithin, L-ornithine-L-aspartate, L-ornithin and silymarin are able to prevent the over/ under expression of Bax, Bcl-2 and P53 levels?
4) What is the effect of toxicant on the mitochondrial membrane potential?
5) Does catechin, lecithin, L-ornithine-L-aspartate, L-ornithin and silymarin are able to maintain the mitochondrial membrane potential?

3.2 Plan of work

We divided our work into two parts. In vitro studies we used human normal Chang liver cells. For in vivo studies we used colony bred Wistar strain adult albino rats (150g-200g) of either sex. We tested all the selected drugs for their hepatoprotective activity against different toxicants viz., D Galactosamine (DGalN), alcohol, carbon tetrachloride (CCl₄), paracetamol and anti TB drugs isoniazid: rifampicin: pyrazinamide (INH: RIF: PYZ) induced hepatotoxicity individually.

3.2.1 In vitro studies

a. In vitro Antioxidant activity of selected drugs by
   i. ABTS
   ii. NBT
b. In vitro cytotoxicity studies in normal chang liver cells by
   i. MTT assay and SRB assay
c. In vitro hepatoprotective activity of
   i. Selected non toxic concentrations of drugs on toxicity induced by toxicants.
Objective and plan of work

d. In vitro hepatoprotective studies using freshly rat primary hepatocytes
   i. Isolation of rat primary hepatocytes.
   ii. Studies of the effect of selected drugs against each toxicant by estimating the biochemical parameters using hepatocytes.
e. Nuclear morphological studies using Chang liver cells
   i. Studies using nuclear staining dyes to compare the effect of selected drugs against each toxicant.
f. Isolated mitochondrial studies
   i. Isolation of mitochondrial fraction and to study the effect of toxicants and drugs on the mitochondrial membrane potential.
g. Gene expression studied using Chang liver cells
   i. Studies on the expression of Bax, Bcl-2 and P53 mRNA levels in normal cells, toxicant treated and drug pretreated cells using RT PCR technique.
h. Flow cytometry analysis
   i. Studies on the effect of toxicants and drugs on Chang liver cells and to compare the percentage of apoptotic cells, before and after toxicant challenge and drug pretreatment.

3.2.2 In vivo studies

i. Determination of hepatic SOD and CAT levels
   i. Studies of selected drugs against each toxicant on the SOD and CAT levels in rats.
j. Studies on the biochemical parameters of liver
   i. Estimation of important liver biochemical parameters like ASAT, ALAT, ALP and LDH using rat models with drugs pretreatment and toxicant challenge.
k. Histopathology study of liver
   i. Histopathology studies of rats pretreated with selected drugs and challenged with toxicants.
l. DNA fragmentation studies
   i. Studies on the effect of drug pretreatment and toxicant challenge on DNA.