ABSTRACT

TITLE OF THE THESIS:
Pharmacological evaluation of some potential Hepatoprotective plants.

BACKGROUND:
Liver is the main organ responsible for the major metabolic and secretory functions in the body. The occurrence of drug-induced liver injury (DILI) is due to indiscriminate consumption of NSAIDs, alcohol, antitubercular, anticancer and anticonvulsant drugs. It results clinically as jaundice, cirrhosis, hepatitis, steatosis, fibrosis, liver cancer and ultimately to hepatic failure.

The treatments available for the liver diseases are Modern and Traditional medications. The modern medications include anti-inflammatory, immune-modulatory, antiviral drugs, corticosteroids, S-adenosyl-L-methionine, α-lipoic acid and dipeptide caspase inhibitor. The modern therapeutic strategies are not efficient enough for complete removal of liver hazard, without provoking adverse effects. The traditional medications include antioxidant therapy, use of several plant drugs like Silymarin alone and in-combination as multi-ingredients herbal formulations. Indian System of Medicine (ISM) has recommended more than eighty seven hepatoprotective plants and many of them are used clinically. Recently, the use of herbal natural product has gained interest among the world population. But, these are however, not backed by well documented scientific data.

_Tecomella undulate_ Seem is reported to be useful for liver and spleen disorders. It is constituted into various herbal hepatoprotective formulations: Livo-plus, Exol, Liv-52, Herboliv, Amylecure etc. _Ziziphus jujube_ Lam. fruits are reported to have antioxidant potential against liver injury. The species belonging to _Ziziphus_ are reported to inhibit cyclooxygenase-induced prostaglandin synthesis. _Plumbago zeylanica_ Linn. roots are reported as hepatoprotective against paracetamol-induced acute liver injury activities. _Zingiber officinale_ Rosc rhizome is reported to act as hepatoprotective agent. Moreover, it is reported to inhibit NO, peroxynitrite, cyclooxygenase and lipoxygenases, production of cytokines.

Most of the plant drugs have been evaluated using a single animal model of liver injury in only one or two doses. Very high doses of hepatoprotective drugs have been used without any reference to LD₅₀ data. Moreover, the mechanism of action of the plant products is at best, only partially explored.
AIMS AND OBJECTIVES:
The present study was designed to investigate the possible hepatoprotective effects, at therapeutic and safe doses, of bark of *Tecomella undulate*, root of *Plumbago zeylanica*, rhizome of *Zinziber officinale*, and fruit of *Ziziphus jujube*; and their mechanism of action in three animal models of hepatotoxicity: paracetamol, carbon tetra chloride and alcohol-induced hepatocellular damage in rat.

MATERIALS AND METHODS:

**Plant materials and extraction:** Plant materials were collected, duly authentified from National Botanical Research Institute (NBRI), Lucknow, India and extracted with 95% methanol using a Soxhlet extractor. The bark of *T. undulate* was extracted using 90% ethanol and fractionated into butanolic and water fractions. The qualitative phytochemical screenings of each plant extract for the presence of alkaloids, flavonoids, glycosides, tannins, phenols and steroids were also done.

**Animals and chemicals:** Wistar albino rats (180-240 g) of either sex were used in the present study. Experiments were conducted in approval with IAEC and CPCSEA guidelines for the use and care of experimental animals. All other chemicals and biochemical reagents of analytical grade were used as freshly.

**Acute toxicity study:** Acute oral toxicity was performed as per OECD-423 guidelines.

**High performance liquid chromatographic (HPLC) study:** The plant extracts were studied for the presence of plumbagin in *P. zeylanica*, and gingerol in *Z. officinale* using Reverse Phase HPLC. Moreover, the presence of gallic acid (GA) was also ensured in each plant extract.

**Experimental hepatotoxicity:** Wistar albino rats (n=8) were divided into different groups. Each plant extract and silymarin as standard were given orally for 7 days. Paracetamol (3g/kg, *p.o.*) was given on 3rd and 5th days; and CCl₄ (1ml/kg, *s.c.*) diluted in olive oil (1:1) was given on 4th and 5th days, 2 hrs after drug administration to induce acute hepatic damage. Alcohol (15g/kg, *p.o.*) was given for 4 weeks to induce chronic hepatic injury. Each plant extract and silymarin were given for 4 weeks, two hr before the ethanol administration.

The blood was collected, serum separated for biochemical estimations. Animals were sacrificed and liver was dissected out. A 10% liver homogenate was prepared for tissue biochemical estimations.
Pharmacological assessment: The body weight and relative wt. of liver were estimated. The serum ALT, AST, ALP and total bilirubin were estimated using biochemical enzymatic kits. Tissue TBARS, GSH, SOD, nitrite/nitrate, Na⁺K⁺ATPase and hydroxyproline levels and liver histology were done. Results were expressed as mean ± Standard deviation, analyzed by one-way ANOVA followed by Bonferroni’s multiple comparison analysis as post-hoc test. p <0.05 was considered to be statistically significant.

RESULTS:

Acute toxicity study: Each plant extract: 5, 50, 300 and even at highest dose 2000 mg/kg p.o. caused no toxic symptoms up to 14 days.

Qualitative phytochemical screening: The phytochemical screening of plant extracts showed the presence of phenolics, flavonoids and glycosides. *T.undulate* showed positive tests for tannins, steroid and glycosides (iridoid).

HPLC study: The extracts of *P.zeylanica* and *Z.officinale* were found to contain plumbagin and gingerol up to 0.367 and 0.0823 % w/w respectively. The presence of gallic acid in *T.undulate*, *P.zeylanica* and *Z.officinale* extracts was found to be 0.073, 0.009 and 0.085 % w/w respectively.

Pharmacological Assessment:

Effect of Test drugs on body wt. and liver wt.: Administration of hepatotoxicants caused significant (p<0.05) decrease in body weight and increase in liver wt. as compared to control. Pretreatment with each plant extract (except TU:WF 200 mg/kg in PCM model) 100, 200 and 400 mg/kg significantly (p<0.05) reversed the toxic effects of hepatotoxicant in a dose-dependent manner. The effect of highest dose of each plant extract was not significantly different from that of the standard drug silymarin 50 mg/kg.

Effect of Test drugs on Serum Biochemical Estimations: A significant (p<0.05) increase in serum ALT, AST, ALP and bilirubin was observed in rats treated with hepatotoxicants as compared to control. Pretreatment with all the plant extracts produced significant decrease in these serum markers as compared to hepatotoxicant control. Each plant extracts at 400 mg/kg and SILY50 showed comparable, significant protection.

Effect of Test drugs on Tissue Biochemical Estimations: All the hepatotoxicants produced significant increase in tissue lipid peroxidation (TBARS), nitrite/nitrate,
hydroxyproline; and decrease in GSH, SOD and Na⁺K⁺ATPase levels. Pretreatment with each plant extract significantly (p<0.05) reversed these toxic effects.

**Effect of test drugs on Histological characteristics:** PCM or CCl₄ or alcohol control groups revealed marked central vein enlargement, sinusoidal dilation; fatty degeneration and vacuolization, steatosis, and mild infiltration as compared to control. A significant preservation of normal liver histology was observed in groups pretreated with each plant extract at 100, 200 and 400 mg/kg dose dependently, and silymarin.

**DISCUSSION:**

The present study provides *in-vivo* evidences for the pharmacological potential of extracts of *Tecomella undulate*, *Ziziphus jujube*, *Plumbago zeylanica* and *Zingiber officinale*, at 100, 200 and 400 mg/kg doses, in three experimental models of paracetamol, CCl₄ and alcohol-induced hepatocellular injuries in Wistar rat. The present study, therefore, is the first, in which, a battery of experimental models to document therapeutic potential in man, based on pharmacological activity has been used.

Paracetamol, CCl₄ and alcohol induced hepatic injuries are commonly used models for the screening of hepatoprotective agents and characterized by a decrease in body weight and increase in liver weight as also observed in present study. These effects were significantly prevented by pretreatment with each plant extract, in the doses employed, as a consequence of prevention of masangial expansion, fibrosis and hypertrophy of liver. In the present study, a significant increase in ALT, AST, ALP and bilirubin levels, confirming the severity of hepatocellular damage due to hepatotoxicants. The attenuation of these hepatotoxic effects on pretreatment with all the plant extracts may be due to their abilities to act as a radical scavenger.

The ethanolic extract of stem bark of *T.undulate* at 200, 500 and 1000 mg/kg is reported to attenuate thioacetamide-induced acute liver damage. The present study, has extended it further and demonstrated the protective effect of butanolic fraction of the ethanolic ext. of *T.undulate*, at lower dosage range *i.e.* 100, 200 and 400 mg/kg, in all the three experimental models and this may be due to increased concentration of phytoconstituents present in butanolic fraction in pure form.

The ethanolic extract of fruits of *Z.jujube*, at dose range 100-500 mg/kg has been reported as hepatoprotective against experimental liver damage. The present
study has extended the evidence for the hepatoprotective activity of lower doses of *Z. jujube* fruits especially against alcoholic hepatic injury.

The petroleum ether extract of root of *P. zeylanica*, at 300 mg/kg, has been recently reported to be protective against PCM-induced hepatotoxicity. *P. zeylanica* has been reported to contain plumbagin (chief constituent) and flavonoids as also confirmed in present study which may be responsible for its therapeutic potential. The present investigation, for the first time provide the *in-vivo* evidences for the effectiveness of ethanolic extract of roots of *P. zeylanica* at 100, 200 and 400 mg/kg, in a dose-dependent manner, against three models of experimental hepatic damage.

The *Zingiber officinale*, at 200 and 400 mg/kg, is reported to prevent PCM and CCl₄ induced acute hepatic injury. Presence of gingerol (a major active component) was confirmed by HPLC in present study. The present study evidenced that the *Z. officinale* acts as hepatoprotective in DILI and ALD. This study also provides evidence of increased potency of *Z. officinale*, as compared to the earlier published reports and recommends the use of lower doses of the extract alone or in combination with other hepatoprotective agents.

Gallic acid (GA) is reported to act as antioxidant and diminishes cellular proliferation and pro-inflammatory gene expressions. In present study, the HPLC profiling revealed for the first time the presence of GA in *T. undulate, P. zeylanica* and *Z. officinale* extracts and it may be contributing to their therapeutic potential.

The toxic reactive metabolites directly cause high free radical production, increased lipid peroxidation, nitric oxide (NO), impair the antioxidant homeostasis and leads to oxidative and inflammatory hepatic damage. In the present study, prevention of these tissue changes with each plant extract may be due to their ability to act as a radical scavenger against oxidative and nitrosative stress induced necrotic and apoptotic damage observed. Moreover, Na⁺K⁺ATPase regulates cell physiology and homeostasis. In the present study, pretreatment with each plant extract, maintained ATP level, membrane functions, and thereby restored mitochondrial dysfunctioning against hepatotoxicity. Inflammation, commonly precedes hepatic fibrogenesis as increased collagen. In the present study, each plant extract was noted to prevent fibrogenic events. The protective effects of each plant extracts was further confirmed by histopathological examination, showing marked protection against liver...
tissue damage and maintenance of normal lobular architecture as compared to respective controls.

Silymarin (*Silybum marianum*) contains flavonoids and exhibits hepatoprotective activity at dose range from 25-200 mg/kg. In the present study, the highest dose of all the four plant extracts used, was as effective a hepatoprotectant as silymarin (a highly purified compound). It is thus possible that isolation of the active principle from these extracts, may yield a compound or compounds, that are more effective than silymarin.

In present study, the phytochemical screening of each plant extract showed the presence of phytoconstituents like flavonoid, phenolics, tannins and glycosides (Iridoids in *T.undulate*) which may be responsible for their hepatoprotective effects.

**CONCLUSION:**

It may be concluded that the *Tecomella undulate, Plumbago zeylanica, Zingiber officinale* and *Ziziphus jujube* have marked hepatoprotective potential against PCM, CCl₄ and alcohol induced liver damage in rat. The efficacy of each plant extracts in doses 200 and 400 mg/kg was comparable with the standard drug silymarin.

The hepatoprotective effect of these plant extracts may be due to the involvement of anti-oxidative, anti-inflammatory, anti-fibrotic and membrane stabilizing cascades for the prevention of progression of liver injury. These results lend support to the traditional and folklore uses of these plant drugs in the treatment of liver illnesses.