Evaluation of protective effect of *Butea monosperma* (lam.) Taub in experimental hepatotoxicity in rats

Sir,
The liver is the second largest organ in the body, and is often seen as the most important one. Toxins, infectious agents, medications, and serum inflammatory mediators may result in a diverse range of disease processes, leading to loss of normal histological architecture, reduced cell mass, and loss of blood flow. Consequently, functional liver capacity may be lost. Efforts have been made to search for effective hepatoprotective agents. Therefore, the prevention of liver diseases has a great significance both in theory and in practice. Butea monosperma (lam.) Taub-(Fabaceae) is a medium size deciduous tree, found throughout India and traditionally used for the treatment of hepatopathy, ulcers, tumors, and diabetes. B. monosperma has been scientifically investigated for anthelmintic, anticonvulsive, antidiarrheal, antifertility, antimicrobial and antistress activities. This study was performed to evaluate the hepatoprotective potential of ethanolic extract of *B. monosperma* in carbon tetrachloride (CCl₄) intoxicated rats.

Plant material was collected from Dharmapuri district, Tamilnadu, in the month of July and was authenticated by a Botanist, Plant Anatomy Research Center, Chennai. The powdered bark material was extracted using ethanol in a soxhlet apparatus. The solvent was completely removed by using a rotary flash evaporator.

Wistar albino rats of either sex, weighing 200–250 gm, maintained under standard husbandry conditions (temperature 23±2°C, relative humidity 55±10% and 12 hr light:12 hr dark cycle) were used for all experiments. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols were approved by the institutional animal ethics committee of Periyar College of Pharmaceutical Sciences for girls, Tirchy, Tamil Nadu.

An acute toxicity study was performed for ethanolic bark extract of *B. monosperma* according to the acute toxic classic method as per OECD guidelines using female albino rats. Rats were divided into five groups of six each that is normal control, CCl₄ control, two test groups, and standard group. Normal control group : 5% CMC (10 ml/kg; oral), CCl₄ control group : CCl₄ in 50% v/v, olive oil (3 ml/kg; i.p.) Test groups : Ethanolic extract (100 mg/kg and 200 mg/kg; oral).

Standard drug treated group : Silymarin (25 mg/kg; oral)

All the doses were administered daily once for 7 days after CCl₄ administration. On eight day, the blood sample was collected from all the groups and allowed to clot for the separation of serum.

The serum was analyzed for the estimation of biochemical parameters such as glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TBL), and acid phosphatase (ACP). All the determinations were carried out using standard kits.

One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were killed, liver was removed, and fixed in a mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde, and 5 ml of glacial acetic acid for 12 h, then embedded in paraffin using conventional methods and cut into sections and stained using the hematoxylin–eosin dye. The sections were observed under a microscope for histopathological changes in the liver architecture and their photomicrographs were taken.

The results are expressed as mean ± SD of six animals from each group. The data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett’s test. A *P* value < 0.05 was considered significant.

The ethanolic extract did not cause any transience up to 2000 mg/kg and were considered safe (OECD, 1996). CCl₄ intoxication in normal rats significantly elevated the levels of SGOT, SGPT, ALP, ACP, and TBL, indicative of acute hepatocellular damage and biliary obstruction. The rats treated with ethanolic extract (100 and 200 mg/kg) and silymarin showed a significant decline in all the elevated SGOT, SGPT, ALP, ACP, and TBL levels [Table 1].

Histopathological examination of liver sections of the normal control group showed normal cellular architecture with separate hepatic cells, sinusoidal spaces, and a central vein.
Disarrangement of normal hepatic cells with intense necrosis and vacuolization of periportal vein are observed in CCl₄ intoxicated liver. The liver tissue of CCl₄ control treated animals showed hydropic changes and mild portal chronic inflammatory cell infiltrate. The liver sections of the rat intoxicated with CCl₄ and treated with ethanolic extract (100 and 200 mg/kg) showed the absence of necrosis and overall no visible changes were observed as compared to the standard group.

This study was performed to assess the hepatoprotective activity of B. monosperma in rats against CCl₄ as hepatotoxin to prove its claims in folklore practice in liver disorders. The ethanolic extract of B. monosperma shows significant \((P<0.01)\) hepatoprotective effects in the CCl₄ intoxication in rats. Physicochemical analysis on the extracts showed the presence of flavonoids in the ethanolic extract. According to these results, it may be hypothesized that flavonoids present in the ethanolic extract could be considered responsible for the hepatoprotective activity of plants.

The hepatotoxicity of CCl₄ has been reported to be due to the formation of the highly reactive trichloro free radical (CCl₃) formed by the hemolytic cleavage of CCl₄ or by an even more reactive species, chloromethylperoxy free radical (CCl₃COO⁻) formed by the reaction of ·CCl₃ with O₂ which attacks polyunsaturated fatty acids. It produces hepatotoxicity through altering liver microsomal membranes in experimental animals. From Table 1, it is discernible that the ethanolic extract of the plant was able to decrease all the elevated biochemical parameters due to the hepatotoxic level. It was observed from the liver sections that the protective effect of 200 mg/kg dose of the ethanolic extract was more than that of 100 mg/kg dose. At 200 mg/kg dose of the extract, CCl₄ intoxicated liver cells showed good protective effects as compared to the 100 mg/kg dose. On the basis of our results, it can be concluded that the ethanolic extract of B.utea monosperma possesses hepatoprotective activity against CCl₄ intoxication in rats.

### Table 1: Effect of the ethanolic bark extract of Butea monosperma on CCl₄ induced hepatotoxicity in rats

| Groups (dose)       | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | ACP (IU/L) | Total bilirubin (mg/100 ml of blood) |
|---------------------|-------------|-------------|------------|------------|-----------------------------------|
| Control             | 97.3 ± 1.18 | 35.08 ± 0.2 | 15.92 ± 0.72 | 10.5 ± 0.064 | 0.39 ± 0.04                          |
| CCl₄ control (3 ml/kg i.p.) | 186.7 ± 1.82* | 136.9 ± 1.94* | 98.3 ± 7.9* | 38.6 ± 2.9* | 0.89 ± 0.07*                          |
| Ethanolic extract (100 mg/kg) | 145.4 ± 2.85** | 75.7 ± 5.3** | 67.3 ± 5.1** | 23.7 ± 0.85** | 0.58 ± 0.08**                          |
| Ethanolic extract (200 mg/kg) | 103.8 ± 7.89** | 49.6 ± 3.7** | 42.4 ± 2.9** | 16.0 ± 0.24 | 0.44 ± 0.04**                          |
| Standard (Silymarin) (25 mg/kg) | 105.3 ± 4.3** | 49.4 ± 3.6 | 34.8 ± 2.9** | 16.2 ± 1.2** | 0.24 ± 0.03**                          |

Data are expressed as mean±SE, \(n=6\), \(^*P<0.01\), compared to control group, \(^**P<0.01\), compared to the CCl₄ treated group.

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Sir,

The ulcers that affect the gastrointestinal system are normally provoked by an imbalance between aggressive and protective factors in the stomach, which is affected by factors such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cell regeneration, prostaglandins, and epidermal growth factors.[1] Stress, smoking, nutritional deficiencies, ingestion of nonsteroidal anti-inflammatory drugs, hereditary predisposition, and infection by Helicobacter pylori are all factors that can increase the incidence of gastric ulcer.[2] Moreover, calcium plays an important role in increased production of gastric acid. Induction of hypercalcemia through intravenous administration of calcium is usually associated with increased gastric volume and acidity. The acid stimulating ability of calcium is well known, and there is extreme sensitivity to calcium in patients with Z.E. syndrome. It has been documented that C. tinctorius (Safflower) has natural calcium channel blocker activity.[3] C. tinctorius has long been used as Chinese medicine in clinics to treat cardiovascular disease, and has demonstrated anti-myocardial ischemia effects.[4] Safflower also possesses other pharmacological effects, including anti-coagulant, antioxidant, and neuroprotective. This study was planned to evaluate the effects of extract from C. tinctorius and to compare it with H$_2$-receptor antagonist cimetidine and calcium channel blocker verapamil on the volume and acidity of carbachol-induced gastric secretion.

An aerial part of C. tinctorius L. (Asteraceae) was collected in the month of April from the Hingoli district of Maharashtra, India. Identification and authentication of the C. tinctorius was done by a Botanist, Post Graduate Teaching Department of Botany, Rashtra Santa Tukadoji Maharaj Nagpur University, Nagpur (Voucher specimen no. 9715). The plant materials were cleaned, shade dried, and coarsely ground. The powdered material was soaked in 70% aqueous-methanol for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. This procedure was repeated thrice, and the combined filtrate was evaporated on a rotary evaporator under reduced pressure to a thick, semi-solid mass of dark brown color, i.e. the crude extract yielding approximately 6.1%.

Wistar albino rats of either sex weighing between 160 and 180 g were obtained from the Animal House, S.N. Institute of Pharmacy, Pusad. The animals were housed in polypropylene cages and maintained at 24 ± 2°C under 12 h light/dark cycle and were feed ad libitum with standard pellet diet and had free access to water. The study was approved by the Institute Animal Ethics Committee, and all the animal experiments were carried out as per CPCSEA guidelines.

Thirty Wistar rats were divided into six groups containing six animals and grouped as follows:

- Group I: Carbachol
- Group II: C. tinctorius 200 mg/kg
- Group III: C. tinctorius 400 mg/kg
- Group IV: Cimetidine 2.5 mg/kg + Carbachol
- Group V: Verapamil (10 mg/kg) + Carbachol

All the animals were kept fasting for 48 h with free availability of water before they were subjected to an experimental procedure. The operative procedure was the one adopted by Visscher et al.[5] Animals were anesthetized with ether, abdomen opened and pylorus was ligated with silk suture. Then the abdominal wall was closed with suture clips and intraperitoneally injections of Carbachol 600 µg/kg body weight were administered to group I, 200 mg/kg body weight of extract to group II, 400 mg/kg body weight of extract to group III, 2.5 mg/kg body weight of Cimetidine to group IV, Verapamil 10 mg/kg to group V followed by Carbachol 600 µg/kg body weight after 15 min to groups II, III, IV, and V. The rats were deprived of water for 4 h after administration of drugs. Then, the rats were killed, the thorax and abdomen were opened, esophagus was ligated, and the stomach was removed quickly. The contents of the stomach were collected. The volume of the gastric juice was measured. Then, the