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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *BARRINGTONIA ASIATICA L.* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Barringtonia asiatica is used in folklore medicine in hepatoprotective. There was no scientific evidence justifying the use of bark of *Barringtonia asiatica*, therefore the present study was investigate the hepatoprotective activity of the plant. To investigate the hepatoprotective activity and acute oral toxicity of extract of bark of *Barringtonia asiatica L.* (MEBA) in male wistar albino rats by using CCl_4 induced hepatotoxicity. The MEBA at doses of 250 and 500mg/kg, p.o and the standard drug Silymarin (100mg/kg, p.o) were administered three times at 12h intervals and then CCl_4 (1ml/kg) was administered to all the groups except normal control for 2 days. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, ALP, γ-GT, TP and total bilirubin along with histopathological studies were observed after 36h of CCl_4 treatment. The MEBA at the doses of 250 and 500mg/kg inhibited CCl_4 induced liver toxicity in Wistar albino rats as assessed by the biochemical changes and histopathological studies. The methanol extract of bark of *Barringtonia asiatica L.* afforded significant protection against CCl_4 induced hepatocellular injury.

Keywords: *Barringtonia asiatica L.*, Hepatoprotective, CCl_4, Silymarin.

INTRODUCTION

Every year about 20,000 deaths are found due to liver disorders. Thus to maintain a healthy liver is a crucial factor for overall health and well beings [1]. Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies [2]. *Barringtonia asiatica* (L.) Kurz (Family – Barringtoniaceae) is a tree to 25 m tall with glossy alternate, petiolate, entire bark, obovate, 12-40 cm long, 10-20 cm broad. Flowers are large and showy, petals white, calyx green, with pinkish filaments with yellow anthers. Fruit a large fibrous drupe (up to 12 cm long), shiny green, quadrangular (square in cross section), containing a large single seed. This tree usually forms large spreading branches as well as a large, spreading buttress root system. It is common along the sea shore, edges of mangroves, lowland river margins and coastal forests. It is widespread throughout the tropical Pacific and Indian Oceans and widely cultivated in tropical areas. Gallic acid, saponins (including barrinin A1), hydrocyanic acid, monosaccharides, triterpenoids (bartogenic acid, 19-epibartogenic acid, and anhydrobartogenic acid) [3]. Traditional used In the Cook Islands, the seed is grated, mixed with coconut cream and rubbed onto burns and wounds. In Fiji, a decoction of the leaves is used to treat hernia. A decoction of the bark is used to treat constipation and epilepsy. In Samoa, the fruit or bark is used to treat yaws, seed to treat ringworm and the bark is used in treating tuberculosis. In Solomon Islands and Samoa it is used to stun fish [4,5]. Therefore we attempt to investigate the hepatoprotective activity of this plant against CCl_4-induced liver damage in rats to support the claim. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant material

The bark of *Barringtonia asiatica L.* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. *Madhava Chetty, K.*, Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected bark was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with methanol using soxhlet apparatus. The extraction was carried out until the extractive becomes

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colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 10.5%w/w.

Animals Used

Albino rats (185–210 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study

The procedure was followed according to the OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per group. Depending on the mortality and or moribund status of the animals, on an average 2-4 steps may be necessary to allow judgment on the acute toxicity of the testing substance. According to this procedure minimum number of animals were to be used for acceptable data band scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemical which causes acute toxicity.

Adult female wistar rats were used for this study. The starting dose of bark of *Barringtonia asiatica L.* extract was 2000 mg/kg body weight, as most of the crude extracts possess LD_{50} value more than 2000 mg/kg body weight. The dose was administered to overnight fasted rats and food was withheld for a further 3-4 hours after administration of the drug and observed for signs of toxicity.

Body weight of the rats before and after treatment were noted and any changes in skin, eye, and mucous membranes, salivation, nasal discharge, urination and behavioral (sedation, depression), neuromuscular (tremors, convulsions), cardiovascular, lethargy, sleep and coma were noted. The onset of toxicity was also noted. The animals were kept under observation for 14 days.

The acute toxicity of methanol extract of *Barringtonia asiatica L.* bark was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, $1/8^{th}$ (250mg/kg) and $1/4^{th}$ (500mg/kg) of this dose were selected for further study [15].

Carbon tetrachloride induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride (CCl_4) model described by *Rao and Mishra* [16]. Wistar albino rats (150-200g) were divided into five groups and were subjected to the following treatments; group-I served as normal control; received vehicle only. Group-II served as untreated group; received only CCl_4, to assist assessing the severity of toxicity produced by carbon tetrachloride administration. Groups III-V served as treated groups; received MEBA at the dose of 250 and 500mg/kg, p.o. and standard drug Silymarin at a dose of 100mg/kg, p.o. were administered orally to rats of the respective groups three times at 12h intervals. Carbon tetrachloride diluted with liquid paraffin (1:1) was administered in dose of 1ml/kg, p.o. for 2 days to all animal groups except for normal control. After 36h of carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital sinus. Serum was separated by centrifugation at 2500rpm at 37^0C for 15min and analyzed for various biochemical parameters.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by *Reitman and Frankel* method [17], alkaline phosphatase (ALP) by *Kind and King* method [17], and bilirubin by *Malloy and Evelyn* method [19].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

The body weight of the rats before and after administrations were noted that there is slightly increased the body weight. But there are no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also not there. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant is safe for consumption and for medicinal uses. In the acute toxicity study, the animals treated with the MEBA at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of MEBA on CCl_4 – induced hepatotoxicity

The results of MEBA on Carbon tetrachloride-induced hepatotoxicity were represented in Table 2. The animals treated only with CCl_4 exhibited a significant

increase (P<0.001) the levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as decrease in the levels of TP when compared to the normal control group after 36h of CCl_4 treatment, indicating hepatocellular damage. The MEBA at tested doses (group-III & IV) produced a significant reduction (P<0.001) in the CCl_4 induced elevated levels of SGOT, SGPT, ALP, γ-GT and total bilirubin as well as increases the TP when compared to the animals treated only with CCl_4 (group-II) after 36h of CCl_4 treatment. Overall, MEBA at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.

Table 1. Effects of MEBA on alternation of hepatic enzyme and serum bilirubin in rat after 36h. of CCl_4 treatment

Groups (n=6)	Biochemical Parameters					
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	γ-GT (IU/L)	TP (gm/dl)	Total Bilurubin (mg/dl)
Group-I (Normal Control)	36.24 ± 1.45***	27.34 ± 1.37***	188.28 ± 3.64***	53.22 ± 1.16***	11.49 ± 1.39***	0.92 ± 0.04***
Group-II (CCl_4: 1ml/kg)	72.18 ± 1.27	42.42 ± 1.38	422.12 ± 1.43	105.31 ± 1.25	2.52 ± 0.43	3.47 ± 0.05
Group-III (MEBA: 250mg/kg)	47.64 ± 1.29***	35.22 ± 1.19***	245.64 ± 3.14***	63.33 ± 1.47***	3.74 ± 0.76***	1.46 ± 0.05***
Group-IV (MEBA: 500mg/kg)	41.39 ±1.42***	24.13 ± 1.71***	222.45 ±3.24***	59.27 ± 1.42***	5.12 ± 0.54***	0.86 ± 0.04***
Group-V (Silymarin: 100mg/kg)	31.62 ± 1.21***	22.33 ± 1.46***	192.46 ±2.73***	51.24 ±1.35***	7.62 ± 0.34***	0.76 ± 0.05***

Values are expressed as mean ± SEM of 6 rats in each group. *** p<0.001, as compared to CCl_4-treated group. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate tranaminase, ALP = Alkaline phosphatase, γ-GT = Gamma glutamyl transpeptidase, TP = Total proteins.

DISCUSSION AND CONCLUSION

Liver is the vital organ of metabolism and excretion. It produces and secretes bile; it also produces fibrinogen, prothrombin, heparin and sulfuric acid ester. The liver converts sugar into glycogen [20]. Any changes in anatomy or functions of liver are characterized by cirrhosis, jaundice, tumors, liver cell necrosis and hepatitis, metabolic and degenerative lesion etc. The management of hepatic diseases is still a challenge to the modern medicines [21]. Herbal medicines play a major role in the treatment of liver disorders. A number of medicinal plants and their formulations are widely used for the treatment of these disorders [22,23]. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of the plants from Indian flora is *Barringtonia asiatica L.* The present studies were performed to investigate the hepatoprotective activity of methanol extract of bark *Barringtonia asiatica L.* in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver diseases.

Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases [24]. CCl_4 is potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to trichloromethyl free radicals (CCl_3.) by liver cytochrome P-450 dependent monooxygenases. This CCl_3 free radical combined with cellular lipids and proteins in the presence of oxygen to produce lipid peroxides [23]. Thus, antioxidant or free radical generation inhibition is important in protection against CCl_4 induced liver lesion [24]. The flavonoids constituents possess free radical scavenging properties [26].

In general, the extent of liver damage is assessed by histopathological evaluation and levels of hepatic enzymes such as ALP, SGOT, SGPT and also Bilurubin release in circulation [27]. The estimation of gamma glutamyl transpeptidase (γ-GT) is an important screening test with a high negative predictive value for hepatic disease [28].

Administration of hepatotoxins CCl_4 elevated the serum levels of SGOT, SGPT, ALP, γ-GT and bilurubin as well as decreases total serum proteins (TP) significantly [29,30]. The rise in serum enzymes level and bilurubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [31].

In our investigation, the biochemical changes were observed after 36h. of CCl_4 treatment. Thereby, it was found that the animal groups which are pretreated with MEBA at the dose of 250 and 500mg/kg (groups-III and IV) as well as silymarin at the dose of 100mg/kg (group-V) for three times at 12h. intervals, resulted in significantly decreases the hepatic enzymes such as SGOT, SGPT, ALP and γ-GT and also total bilurubin; as well as increases the total serum proteins (TP) as compared to animals treated only with CCl_4 (group-II). These results give us the suggestion that, the animals which are pretreated with

MEBA as well as silymarin, showed a protection against the injurious effects of CCl_4 that may results from the interference with cytochrome P-450. These biochemical restoration may be due to the inhibitory effects on cytochrome P-450 or/and promotion of its glucuronidation [32,33]. Silymarin is a known hepatoprotective drug. It is reported to have a protective effect on the plasma membrane of hepatocytes [34].

In histopathological assessment, it was found that the normal liver architecture was disturbed by CCl_4 intoxication. In the liver section of rats treated with MEBA showed the ability of MEBA to prevent hepatocellular necrosis, thereby further confirming the significant hepatoprotective effect of bark of *Barringtonia asiatica L.*

It is well documented that the phytoconstituents comes under the category of flavonoids, alkaloids, glycosides, carotenoids, phenols, coumarins, lignans, essential oil, lipids, monoterpenes, xanthenes and organic acids are reported to have hepatoprotective activity [35]. Literature review revealed that various chemical investigations were carried out with this plant. *William Carey Mamidipalli et al*., have been reported the preliminary phytochemical screening of the methanol extract of *Barringtonia asiatica L.* revealed that presence of steroids, flavonoids, tannins, alkaloids and glycosides [36]. The hepatoprotective activity of *Barringtonia asiatica L.* may be attributed due to presence of these constituents. This study supports the traditional claims and the MEBA could be added in traditional preparations for the various liver diseases.

It is concluded from the data, that the methanol extract of bark of *Barringtonia asiatica L.* possesses significant hepatoprotective activity and may prove to be effective for the treatment of liver disorders. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent hepatoprotective drug.

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