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STUDIES OF MUSSAENDA RAIATEENSIS EXTRACTS ON HEPATOTOXICITY IN RATS

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ABSTRACT

To investigate the hepatoprotective activity and acute oral toxicity of methanolic extract of aerial parts of Mussaenda raiateensis (MEMR) in male Wistar albino rats by using CCl_4 induced hepatotoxicity. The MEMR at doses of 200 and 400mg/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 MEMR at the doses of 200 and 400mg/kg inhibited CCl_4 induced liver toxicity in Wistar albino rats as assessed by the biochemical changes and histopathological studies. The methanolic extract of aerial parts of Mussaenda raiateensis afforded significant protection against CCl_4inducd hepatocellular injury.

KEY WORDS

Mussaenda raiateensis, Hepatoprotective, CCl_4, Silymarin, Hepatotoxicity.

INTRODUCTION

Mussaenda raiateensis J.W. Moore (Family - Rubiaceae) is Shrub to small tree up to 10 m tall. Leaves are opposite, short petiolate, ovate, 8-25 cm long and hairy. Flowers are tubular, usually yellowish orange, subtended by 1 white or yellowish conspicuous leaf-like sepal, the flowers borne in dense terminal clusters. Fruit a green berry up to 20 mm long. Flowers and fruits are available throughout the year. It is common in forest clearings, secondary forests, and open ridges from sea-level to mid-montane. It is distributed indigenous and common from Vanuatu east wads to the Society Islands. Traditional used to improve fertility and to relieve vaginal pain. To treat respiratory illness, severe pain (during pregnancy), rheumatic aches, sore throat, toothache, diarrhoea, and liver trouble [11,12].

MATERIALS AND METHODS

Plant collection

The aerial parts of Mussaenda raiateensis were collected from Tirupati, Andhra Pradesh, in the month of August 2010. The plant was authenticated by Prof. P. Jayaraman, Director of National Institute of Herbal Science, W.Tambaram, Chennai. The voucher specimen of the plant was deposited at the college, for further reference.

Preparation of plant extract

The aerial parts of Mussaenda raiateensis were dried in shade and pulverized in grinder-mixer to obtain a coarse powder. It was then passed through the 40-mesh sieve. A weighed quantity (210gm) of the powder was subjected to continuous hot extraction with methanol in Soxhlet apparatus for 48h. The extraction was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to

give an extract sample. The percentage of yield of methanolic extract of *Mussaenda raiateensis* was found to be 32.91%w/w.

Animals used

Male Wistar albino rats (150-200g) were obtained from the animal house. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of **CPCSEA.**

Acute Toxicity Study

The acute toxicity methanolic extracts of Mussaenda raiateensis was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5 th (400mg/kg) of this dose were selected for further study [13].

Carbon tetrachloride induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride (CCl_4) model described by Rao and Mishra [14]. 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 MEMR at the dose of 200 and 400mg/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^{0}C 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 [15], alkaline phosphatase (ALP) by Kind and King method [16], and bilirubin by Malloy and Evelyn method [17].

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

In the acute toxicity study, the animals treated with the MEMR 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 MEMR on CCl_4 – induced hepatotoxicity

The results of MEMR on Carbon tetrachloride-induced hepatotoxicity were represented in Table 1. 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 MEMR 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.

TABLE-1: Effects of MEMR on alternation of hepatic enzyme and serum bilirubin in rat after 36h. Overall, MEMR at tested doses significantly reduced the levels of hepatic enzymes and total bilirubin.

Values are expressed as mean ± SEM of 6 rats in each group. *** p<0.001, as compared to CCl_4-treated group. SGOT = Serum

	Biochemical Parameters					
Groups (n=6)	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**	**GT (IU/L)**	**TP (gm/dl)**	**Total Bilirubin (mg/dl)**
Group-I (Normal Control)	32.52± 0.04***	17.45± 0.24***	190.2± 0.04***	49.27± 1.05***	7.72 ± 0.05***	0.74± 0.02***
Group-II (CCl_4: 1ml/kg)	61.42 ± 0.14	36.40 ± 0.24	424.37 ± 0.77	97.17 ± 0.21	2.05 ± 0.04	3.72 ± 0.02
Group-III (MEMR: 0mg/kg)	43.15± 0.12***	30.11± 0.13***	255.4± 0.05***	63.14 ± 0.13***	3.52 ± 0.10***	1.22 ± 0.13***
Group-IV (MEMR: 400mg/kg)	37.14 ±0.11***	25.14± 0.21***	225.15±0.04***	57.24 ± 0.21***	5.6 ± 0.04***	0.85 ± 0.03***
Group-V (Silymarin: 100mg/kg)	31.04± 0.04***	22.12± 0.04***	195.24 ±0.05***	50.12 ±0.15***	7.10 ± 0.05***	0.75 ± 0.02***

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 management of hepatic diseases is still a challenge to the modern medicines [10,19]. 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 [20,21]. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of the plants from Indian flora is Mussaenda raiateensis. The present studies were performed to investigate the hepatoprotective activity of methanolic extract of aerial parts Mussaenda raiateensis 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 [22]. 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].

In general, the extent of liver damage is assessed by histopathological evaluation and levels of hepatic enzymes such as ALP, SGOT, SGPT and also Bilirubin release in circulation [26,27]. The estimation of gamma

glutamyl transpeptidase (γ-GT) is a 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 bilirubin as well as decreases total serum proteins (TP) significantly [29,30]. The rise in serum enzymes level and bilirubin 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 MEMR at the dose of 200 and 400mg/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 bilirubin; 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 MEMR 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 restorations 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 MEMR showed the ability of MEMR to prevent hepatocellular necrosis, thereby further confirming the significant hepatoprotective effect of aerial parts of Mussaenda raiateensis.

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 methanolic extract of Antigononleptopus revealed that presence of steroids, flavonoids, tannins, alkaloids and glycosides, Mulabagal vanisree *et al*., have been reported that the purification of the methanolic extract yielded n-hentriacontane, ferulic acid, 4-hydroxycinnamic acid, quercetin-3-rhamnoside and kaempherol-3-glucoside; along with beta-sitosterol, beta-sitosterol-glucoside and d-manitol .The hepatoprotective activity of Mussaenda raiateensis may be attributed due to presence of these constituents. This study supports the traditional claims and the MEMR could be added in traditional preparations for the various liver diseases.

It is concluded from the data, that the methanolic extract of aerial parts of Mussaenda raiateensis 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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