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Hepatoprotective Activity of Petroleum ether Extract of *Mitragyna inermis* against Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats

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Abstract

Petroleum ether extract of *Mitragyna inermis* was studied for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride (CCl_4). Results were compared to the standard drug silymarin at a dose rate of 100mg/kg BW. It was found that the ethanolic extract of *M. inermis* at a dose of 400 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of alanine aminotransferase (ALT) or Serum Glutamate Pyruvate Transaminase (SGPT), aspartate aminotransferase (AST) or Serum Glutamate Oxaloacetate Transaminase (SGOT) to a significant extent. The lower dose (200mg/kg BW) had no effect on lowering blood serum level rather than produced toxicity at the above specified dose. The highest activity of the Petroleum ether extract of *M.inermis at* a dose of 400 mg/kg BW and the reduction of serum level of ALT, AST were 52.08% and 57.37% respectively. The hepatoprotective activity was also supported by histopathological studies of liver tissue.
Keywords: *Mitragyna inermis*, Hepatoprotective activity Carbon tetrachloride

1. Introduction

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver damage [2][3].

The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure taken in overdose can cause severe hepatotoxicity. There are not much drug available for the treatment of liver disorders [4][5]. Therefore, many folk remedies from plant origin are tested for its potential and hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts [6][7]. *Mitragyna inermis* is well known for its ornamental and medicinal purposes. Its medicinal applications, however, depends as practiced by inhabitants of different geographical areas [8]. *Mitragyna inermis* is widely known and used in traditional medicine in the West Africa to treat several diseases [8]. It is used for the treatment of fever, high blood pressure, dysentery, syphilis, wound and epilepsy. Ashes obtained from the wood are used for the treatment of edema. The roots, barks and leaves are used for the treatment of anorexia, constipation and leprosy while the root, barks and stem are used for medical illness. The leaves of *mitragyna inermis* are used for the treatment of rheumatism, cramp, syphilis, jaundice, weakness and fatigue**.** The plant is also used in association with *Cocos nuciefera* to treat jaundice in Ivory Coast.

2. Material and Methods

2.1 Animals

Thirty, male and female Wister white (*albino*) rats weighting 100-150gm were obtained from the Veterinary

Research Institute, Soba, Khartoum, where they were housed in cages and maintained in a room under standard environmental condition, controlled temperature (22 ± 2^0C), relative humidity (60%) with free access to water and food.

2.2 Plant Materials

Mitragyna inermis stem bark belong to the family of Rubiaceae were collected in November 2012 from Alnohod city. The plant was authenticated by the botanists in medicinal and aromatic plants research institute.

2.3 Preparation of Extracts

Extraction was carried out according to method described by Harborne (1984)[10]. 300g of plant sample was successively extracted by soaking in petroleum ether, ethyl acetate and 80% ethanol for about seventy two hours of each solvent with daily filtration and evaporation. Solvents were evaporated under reduced pressure to dryness using rotary evaporator apparatus and kept until use.

2.4 Experimental design

The rats were divided randomly into five groups of six rats each. The hepatoprotective activity of the plant extracts was tested using CCl_4 model. Group I (normal control) received neither the plant extract nor CCl_4 for72 hours that is they receive only food and water only; Group II (induction control) was given a single intraperitonial dose of 0.2 mg/kg BW CCl_4. Group III given the standard silymarin at a dose rate of 100mg/kg BW. Group IV given CCl_4 together with 200 mg/kg BW of the plant extract. Group V given CCl_4 together with 400 mg/kg BW of the plant extract. Clinical signs were recorded. Blood samples were obtained from the ocular vein before the start of the experimental dosing and thereafter fortnightly for hematological investigations and serum analysis. Sera were analyzed for the activities of AST, ALT, ALP and for the concentrations of metabolic indicators, total protein, urea, albumin. After 10 days the rats were dissected and liver tissues were fixed in 10% neutral buffered formalin and processed for histopathology.

2.5 Biochemical Analysis

Serum AST ALT and ALP activities were measured by a commercial kit (Randox Laboratories Ltd, U.K.) according to Reitman and Frankel and Schmidt [11][12]. Serum albumin and total bilirubin was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

2.6 Statistical analysis

The values were expressed as mean ± SD. Statistical analysis and comparison between the groups was performed by one way analysis of variance (ANOVA) using SPSS version 10.0.

3. Results

Total protein , albumin, bilirubin concentration and the levels of certain serum marker enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) are presented in table (1 & 2). Administration of CCl_4 caused significant increased in serum ALT, AST, ALP, Total proteins, ALB and bilirubin concentration in rats ($^{\circ}P<0.05$— $^{\circ\circ}P<0.01$), slightly reduced by treatment *Mitragyna Inermis* stems pet ether extract at dose 200mg/kg and 400mg/kg when compared with CCl_4 and ($^{*}P<0.05$—$^{**}P<0.01$) when compared with standard drug group C.

The mean levels of these parameters in the rats treated with pet ether extract of *Mitragyna Inermis* stems do not clearly differ statistically from the mean levels of these parameters in the rats of CCl_4 group. The level of ALT was not normalized completely but was lowered significantly by the pet ether extract of *Mitragyna inermis* stems.

Table 1: Effect of *Mitragyna inermis* stems Pet ether extract administered simultaneously with CCl_4 on Total Protein and Albumin in rats

Groups	Total Protein (Mean±S.E.)			Alb (Mean±S.E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	7.40±0.00	7.50±0.00	7.30±0.00	5.00±0.00	4.60±0.00	4.90±0.00
B	7.30±0.00	9.70±0.00	10.15±0.00	4.80±0.00	9.10±0.00	10.20±0.00$^{\circ\circ}$
C	7.20±0.00	10.20±0.00	6.90±0.00*	4.10±0.00	7.00±0.00	4.50±0.00**
D	6.70±0.42	11.40±0.99	9.25±0.64	4.55±0.21	7.55±0.78	6.25±0.07
E	6.70±0.42	11.40±0.99	9.25±0.64	4.55±0.21	7.55±0.78	6.25±0.07

Table 2: Effect of *Mitragyna inermis* stems Pet ether extract administered simultaneously with CCl_4 on ALT (GPT) and AST (GOT) in rats

Groups	ALT (GPT) (Mean±S.E.)			AST (GOT) (Mean±S.E.)		
	Day 0	Day 5	Day 10	Day 0	Day 5	Day 10
A	11.20±0.00	11.50±0.00	12.00±0.00	12.00±0.00	12.10±0.00	12.20±0.00
B	11.10±0.00	14.00±0.00	17.00±0.00$^{\circ\circ}$	11.50±0.00	13.20±0.00	15.20±0.00$^{\circ\circ}$
C	9.70±0.00	14.00±0.00	10.10±0.00*	10.20±0.00	13.20±0.00	11.30±0.00**
D	10.40±0.57	14.05±0.21	12.45±0.49	10.45±0.64	12.65±0.21	11.95±0.07
E	10.40±0.57	14.05±0.21	12.45±0.49	10.45±0.64	12.65±0.21	11.95±0.07

Histopathological changes in the livers of rats given pet ether extract of *Mitragyna Inermis* stems simultaneously with CCl_4

Fig 1: Control group showed normal histopathological appearance

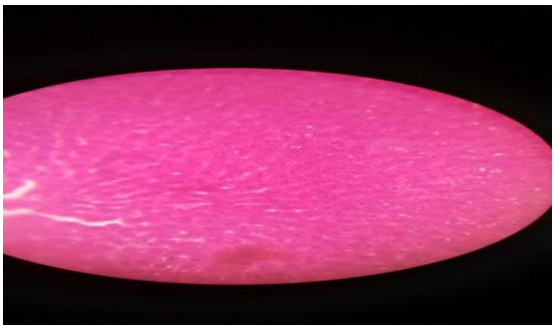


Fig 2: CCl_4 group showed hepatocellular degeration with sever fatty changes and sever koliocytosis

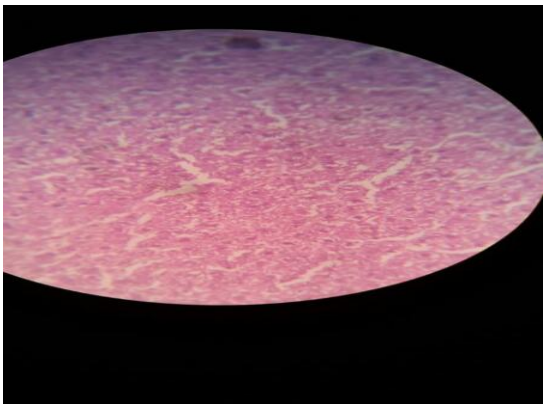
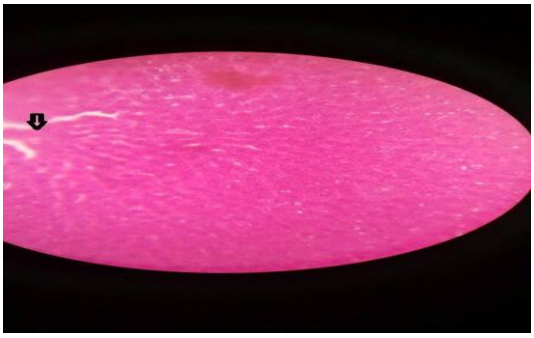


Fig 3: Group (200mg/kg) showed hepatocellular degeneration and fatty changes and koliocytosis



4. Discussion

Hepatotoxicity is the most widespread pathology world-wide, presenting up to 83% of all cases. Hepatitis, viral infection, food additives, alcohol, toxic industrial chemicals, and air and water pollutants are the major risk factors of liver toxicity. There is increasing evidence that free radicals and reactive oxygen species play crucial role in various steps that initiate and regulate the progression of liver diseases independently of the original agent.

The hepatoprotective effects of ethanolic, ethyl acetate and petroleum ether extracts of the stem of *Mitragyna inermis* at the doses of 200 and 400 mg/kg BW were studied in rats by using CCl_4 induced hepatotoxicity. Liver damage was assessed by biochemical studies (SGPT, SGOT, ALP, Albumin, Total Protein, Total and direct bilirubin) and histopathological examinations.

Our results using the model of CCl_4 – induced hepatotoxicity in rats demonstrated that *Mitragyna inermis* stems (200-400 mg/kg), caused significant elevation of ALT, AST, ALP, Total protein as well as bilirubin concentration after treatment. However, sever necrotic hepatic lesions precipitated by CCl_4 were not reduced by ethanolic, pet ether and ethyl acetate extract of plant stems.

The ethanolic, pet ether and ethyl acetate extract of *Mitragyna inermis* (200-400 mg/kg), exhibiting 15% overall reduction in CCl_4 –induced increase in the level of SGPT, SGOT, ALP, ALB and bilirubin concentration.

Since results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by CCl_4 and blood samples from the

animals treated with the ethanolic extracts of *M. inermis* showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells, the extracts of *M. inermis* could afford significant dose-dependent protection against CCl_4 induced hepatocellular injury. Our result agreed with that carried by Rajib et al (2009)[13] who studied the hepatprotective effect of different plant extracts against induced hepatotoxicity using carbon tetrachloride. He found that the liver enzymes activities were elevated after the treatment of the plants extracts which indicates the protective activity of the plants used.

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