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Pre-Treatment and Post-Treatment of Aqueous Extract of *Pterocarpus erinaceus* and *Bauhinia rufescens*: Status of AST, ALT and ALP against CCl₄-Induced Liver Damage in Rats

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ABSTRACT

Background: Medicinal plants are by far the crucial source of drugs to protect the body against an insult by toxic compounds. The present study is aimed at assessing the hepatoprotective effects of aqueous extract of *Pterocarpus erinaceus* (PE) and *Bauhinia rufescens* (BR) on carbon tetrachloride (CCl₄) induced liver damage in albino rats for a period of two weeks.

Materials and methods: The study was carried out on a total of thirty (30) albino rats which were divided into six (6) groups of five (5) replicates. Group I was used as controls, group II received CCl₄ in groundnut oil (1 ml/kg) by subcutaneous injection; group III and IV received CCl₄ in groundnut oil (1 ml/kg) by subcutaneous injection followed by 250 mg/kg of PE and BR on day 12, 13 and 14 for group III and IV, respectively. Group V and VI received 250 mg/kg of PE and BR daily for 12 days followed by CCl₄ (1 ml/kg) on day 12, respectively. The rats were sacrificed 24 h after the last administration and blood samples were collected and the sera obtained were tested for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities/levels. Results obtained were statistically analyzed using One-Way ANOVA followed by LSD for parameters found to be statistically significant at $\alpha=0.05$.

Results: Mean serum activities of AST, ALT and ALP of group II, III, IV, V and VI were found to differ significantly ($p<0.05$) higher when compared with control except AST level of group V and VI that was resulted to differ slightly ($P>0.05$) higher than that of control. In the same vein, mean serum levels of AST, ALT and ALP of group III, IV, V and VI were found to differ significantly ($p<0.05$) lower when compared with Group II.

Conclusion: Taken together, the results of this study showed that 250 mg/Kg of PE or BR extract was found effective as hepatoprotective agents, as evidenced by the abovementioned biochemical parameters against CCl₄-induced liver damage in rats.

Keywords: Carbon tetrachloride, *Bauhinia rufescens*, *Pterocarpus erinaceus*, Hepatotoxicity

INTRODUCTION

Currently, drug-induced liver damage is responsible for bringing 5% of all hospital admissions and 50% of all acute liver injuries about [1]. Proper understanding of the mechanism of action of CCl₄-induced hepatic damage in rats would reveal the potency and efficacy of certain medicinal plants claimed to have hepatoprotective effects. Hepatitis simply refers to inflammation of the liver which is usually caused by viruses but not limited to alcohol and chemicals such as CCl₄ (carbon tetrachloride) and acetaminophen, among others [2]. Chemicals such as those used in laboratories (e.g. CCl₄ and acetaminophen), industries (e.g. lead and arsenic) and natural chemicals (e.g. microcystins and aflatoxins) which bring liver damage about; are called hepatotoxins [1]. Five viruses have been so far identified (A, B, C, D and E) as causes of infection that primarily targets the liver [1,2]. The liver has a limited number of ways of responding to injury. Acute injury to the liver may be asymptomatic but often presents as jaundice [1,2]. The

two major acute liver diseases are acute hepatitis and cholestasis. The long duration between infection and illness represents an opportunity for the individual to seek alternative or complementary treatments, and to adopt lifestyle and dietary changes which will enhance liver performance and overall health status [3]. *Pterocarpus erinaceus*, also known as rosewood is a good medicinal plant for the treatment of hepatitis; the stem bark is the major part that gives it the medicinal property responsible for treating hepatitis. The tree is a bitter herb which contributes to its ability to treat various liver ailments [4]. *Bauhinia rufescens* is a safe and reliable herb for all liver disturbances including hepatitis. It also helps lower cholesterol made in the liver and thus assists in weight reduction [4]. Several studies on medicinal plants with hepatoprotective effects against CCl₄-induced hepatic damage in rats are being carried out in many countries in order to assess the status of the problem and thus to offer suitable solutions. This research is aimed at identifying the effect of aqueous extract of *P. erinaceus* and *B. rufescens* as well as their efficacy against CCl₄-induced hepatic damage in rats for a period of two (2) weeks.

MATERIALS AND METHOD

Study area

This research was carried out at the Department of Biological Sciences' Laboratory, Federal University Kashere, Gombe State – Nigeria. The study was approved by the ethical committee of the University and the informed consent was obtained prior to experimentation.

Chemicals

All chemicals were of the highest commercially analytical grade and were obtained from Sigma-Aldrich Co.

Collection and preparation of plant materials

B. rufescens root and *P. erinaceus* stem bark were purchased at Tsohon Kasuwa Gombe State-Nigeria. *B. rufescens* root and *P. erinaceus* stem bark were grinded making them into powdered forms. 100 g each of the *B. rufescens* and *P. erinaceus* powdered forms was soaked in 1 L distilled water, shaken for three minutes and then allowed to stay for 72 h. The mixtures were filtered with Whatman No. 1 filter paper (24 cm). The filtrates were evaporated to dryness using water bath evaporator at 40°C-50°C in order to obtain the crude extract and the crude extract was reconstituted up to 200 ml distilled water [5].

Laboratory animals

Thirty (30) Wister albino rats (*Rattus norvegicus*) weighing (74-95 g) were obtained from Vom Plateu State, Nigeria. They were acclimatized for a period of fourteen (14) days in well-ventilated room and housed in a well-ventilated plastic cage maintained under standard laboratory conditions (12 h light/dark cycle; 25-32°C) and a gain in weight (160-200 g) has been observed prior to experimentation. They were fed with commercial rat chow (Vital Feeds LMT) and sachet water and handled according to standard protocol.

Induction of liver damage by carbon tetrachloride (CCl₄)

4 ml of CCl₄ from the stock was dissolved up to 50 ml of groundnut oil. 1 ml/Kg was administered subcutaneously to experimental groups.

Experimental design

A total of thirty (30) experimental albino rats were randomly divided into six (6) groups consisting of three (3) replicates are thus as follows:

- ✓ Control group: Groundnut oil (1 ml/kg) twice per week for a period of 2 weeks;
- ✓ Group II: CCl₄ (1 ml/kg) twice per week for a period of 2 weeks;
- ✓ Group III: CCl₄ (1 ml/kg) twice per week for a period of 2 weeks followed by (250 mg/kg) of *Bauhinia rufescens* on day 12, 13 and 14, respectively;
- ✓ Group IV: CCl₄ (1 ml/kg) twice per week for a period of 2 weeks followed by (250 mg/kg) of *Pterocarpus erinaceus* on day 12, 13 and 14, respectively;
- ✓ Group V: (250 mg/kg) *Pterocarpus erinaceus* daily for 12 days followed by CCl₄ (1 ml/kg) on day 12;

✓ Group VI: (250 mg/kg) *Bauhinia rufescens* daily for 12 days followed by CCl₄ (1 ml/kg) on day 12.

Biochemical analysis

The rats were sacrificed 24 h after last administration of the extract and blood samples were collected inside centrifugation tubes and allowed to clot at room temperature. The samples were later centrifuged and the sera obtained were tested for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities/levels by the method of Reitman and Frankel [6], serum alkaline phosphatase (ALP) activity by the method of Rec [7].

Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by LSD at 5% level of significance.

RESULTS

Table 1: Effects of CCl₄ subcutaneous injection in ALT, AST and ALP levels

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I	Control	10.94 ± 0.50	27.07 ± 0.85	14.57 ± 0.92
Group II	CCl ₄ (1 m/kg)	43.48 ± 2.54 ^a	78.34 ± 0.72 ^a	53.21 ± 1.66 ^a

Values are expressed as means ± standard error of 5 replicates; a=significant difference at P<0.05 when compared with control

Table 1 shows the mean serum activities of AST, ALT and ALP of group I and group II. When mean serum levels of AST, ALT and ALP of group II is compared with control, the difference is statistically significant (P<0.05). Subcutaneous injection of CCl₄ (group II) induced significant elevation (P<0.05) in ALT, AST and ALP levels when compared with control Table 1.

Table 2: Effects of aqueous extract of *B. rufescens* and *P. erinaceus* in ALT, AST and ALP activities

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I	Control	10.94 ± 0.50	27.07 ± 0.85	14.57 ± 0.92
Group II	CCl ₄ (1 m/kg)	43.48 ± 2.54 ^a	78.34 ± 0.72 ^a	53.21 ± 1.66 ^a
Group III	CCl ₄ (1 m/kg)+R (250 mg/kg)	26.85 ± 0.33 ^{a,b}	47.41 ± 0.21 ^{a,b}	43.23 ± 4.98 ^{a,b}
Group IV	CCl ₄ (1 m/kg)+PE (250 mg/kg)	22.47 ± 0.44 ^{a,b}	40.03 ± 0.66 ^{a,b}	23.62 ± 0.32 ^{a,b}

Values are expressed as means ± standard error of 5 replicates; a=significant difference at P<0.05 when compared with control; b=significant difference at p<0.05 when compared with CCl₄ treated rats; BR: *B. rufescens*; PE: *P. erinaceus*

Table 2 reveals the mean serum activities of AST, ALT and ALP of group I, group II, group III and group IV. When mean serum levels of AST, ALT and ALP of group III and group IV are compared with control, the difference is statistically significant (P<0.05) and also when they are compared with group II the difference is statistically significant (P<0.05). Posttreatment of aqueous extract of PE or BR resulted in a significant reduction (P<0.05) of the abovementioned parameters when compared with CCl₄-treated group (group II).

Table 3: Effects of aqueous extract of *B. rufescens* and *P. erinaceus* in ALT, AST and ALP levels

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I	Control	10.94 ± 0.50	27.07 ± 0.85	14.57 ± 0.92
Group II	CCl ₄ (1 m/kg)	43.48 ± 2.54 ^a	78.34 ± 0.72 ^a	53.21 ± 1.66 ^a
Group V	PE (250 mg/kg)+CCl ₄ (1 m/kg)	13.27 ± 0.31 ^b	29.83 ± 0.28 ^{a,b}	29.62 ± 1.05 ^{a,b}
Group VI	BR (250 mg/kg)+CCl ₄ (1 m/kg)	15.62 ± 0.48 ^b	34.92 ± 0.36 ^{a,b}	19.98 ± 0.33 ^{a,b}

Values are expressed as means ± standard error of 5 replicates; a=significant difference at P<0.05 when compared with control; b=significant difference at p<0.05 when compared with CCl₄ treated rats; BR: *B. rufescens*; PE: *P. erinaceus*

Table 3 shows the mean serum activities of AST, ALT and ALP of Group I, II, V and VI. When mean serum levels of ALT and ALP of Group V and VI are compared with control, the difference is statistically significant ($P < 0.05$) and also when they are compared with group II the difference is statistically significant ($P < 0.05$). Mean serum levels of AST of Group V and VI are compared with Group II, the difference is statistically significant ($P < 0.05$). However, when mean serum levels of AST of Group V and VI are compared with Control, the difference statistically insignificant ($P > 0.05$). Pretreatment of aqueous extract of PE or BR induced significant reduction ($P < 0.05$) of the abovementioned parameters when compared with CCl_4 -treated group (group II).

DISCUSSION

AST, ALT and ALP are non-plasma specific enzymes; however, when their levels in the plasma are high they indicate damage to the organs or tissues producing them [2,8]. The result of this study revealed that CCl_4 administration brought a marked increase in ALT, AST and ALP levels about Table 1. These findings were corroborated with the findings of previous studies on CCl_4 -induced hepatic damage in rats by Mohammad et al. [9], Adekeye et al. [10], Al-Malki et al. [11] and Saba et al. [12]. It has also been observed that AST, ALT and ALP levels increased significantly ($P < 0.05$) with ALT levels $>$ AST levels Table 1. This observation was confirmed by Burtis et al. [2] and Vasudevan et al. [8]. The result of Table 2 indicated that post-treatment of aqueous extract of PE or BR antagonized the increase in ALT, AST and ALP activities caused by CCl_4 administration statistically significant ($P < 0.05$). These findings as well were tallied with the works of Veena et al. [13] and Manjunatha [14]. However, when mean serum levels of AST of Group V and VI are compared with Control, the difference statistically insignificant ($P > 0.05$). The result of Table 3 also revealed that pretreatment of aqueous extract of PE or BR was found to cause significant decrease in ALT, AST and ALP levels and thus abrogated the elevation in ALT, AST and ALP activities induced by CCl_4 administration statistically significant ($P < 0.05$). Our findings also were in conformity with the works of Veena et al. [13] and Manjunatha [14]. However, when mean serum levels of AST of Group V and VI are compared with Control, the difference statistically insignificant ($P > 0.05$) (Table 3).

CONCLUSION

According to this study, liver damage at 4 ml CCl_4 dissolved up to 50 ml was apparent. Pretreatment and post treatment of CCl_4 treated group with *P. erinaceus* and *B. rufescens* orally administered at a dose of (250 mg/kg) were efficacious.

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