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Safety Evaluation of the Stem Bark Aqueous Extract of African Kino Tree (*Pterocarpus erinaceus*)

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Abstract

Medicinal plants play a vital role in the treatment of illnesses. Among these plants, *Pterocarpus erinaceus* (African kino tree) is used as blood tonic to treat haematological problems. However, there is dearth of information on the safety of the plant, which necessitates scientific studies. This study evaluated the toxicity of the stem bark aqueous extract of *P. erinaceus* on albino rats (*Rattus norvegicus*). Fifteen rats were divided into three groups, comprising 5 rats each. Group 1 was made the control and received normal feed and water. Groups 2 and 3 received 250mg/kg bw and 500mg/kg bw of the extracts, respectively. After 28 days of administration, the liver, kidney and haematological function parameters of the rats were evaluated.The results of the liver function analysis revealed a significant increase ($P<0.05$) inbilirubin (0.83±0.03) and alanine transaminase (23.33±5.18) at 250mg/kg bw and 500mg/kg bw respectively compared to control (0.77±0.09 and 17.33±0.88). Furthermore, 5000mg/kg bw and 250mg/kg bw of the extract showed a significant increase ($P<0.05$) in kidney function parameters including serum concentration of urea (5.83±2.13) and creatinine (36.33±5.51) compared to control (3.03±0.32 and 29.3±1.15). It also showed a significant ($P<0.05$) reduction in the concentration of potassium (3.87±0.55) compared to control (4.27±0.27). There was a significant increase ($P<0.05$) in haematological parameters (white blood cells, red blood cells, platelet, hemoglobin and hematocrit) in a concentration dependent manner compared to control. It can be inferred from these results that extract of the plant boosted haematological parameters and had no toxic effects on liver and kidney function parameters.

Keywords: Haematological parameters, Medicinal plants, Kidney, Liver, *Pterocarpus erinaceus*

1.0 Introduction

Medicinal plants have been in use in traditional medicinesince long time ago. These plants synthesize hundreds of chemical compounds for various functions, including defense and protection against diseases [1]. According to fossil records, humans make use of plants for medicine at least 60,000 years back [2]. Medicinal plants including *Pterocapus erinaceus* (Madobiya) contributes significantly to the development of traditional medicine. Traditional medicine serves as the other choice of medication for any diseases compared to chemicals drug [3]. Medicinal plants contain bioactive compounds such as flavonoids, alkaloids, carotenoids, phenolic acids, lignans, saponins, indoles, isothiocyanates and other chemicals that possess health benefits [4]. However, the consumptionof medicinal plant without proper dosage contributes to many conditions such as liver, kidney and blood diseases [5]. *Pterocarpus erinaceus* is a medicinal plant with many health benefits. It is often used as blood tonic however without proper dosage and purification, which may lead to various negative effects. Thus, there is a need to assess its safety. Toxicity of plants are often tested on liver and kidneys. Liver is the major metabolic organ found in vertebrate animals

and responsible for many important biological functions such as detoxification, synthesis of proteins and biochemical reactions necessary for digestion and growth [6]. The bilirubin, aspartate aminotransferase (ALT), alanine aminotransferase (AST), and alkaline phosphate (ALP) are used as biomarkers to predict possible liver toxicity. Elevation in serum activities of all transaminases suggested damage to liver cells [7]. Kidneys play a very important role in maintaining chemical composition of body fluids by acidification of urine and some execrable substances are released into the urine by active transport in the renal tubules [8]. These substances include Sodium (Na), Chloride ion (Cl^+), Potassium ion (K^+) creatinine and urea [9]. Increase in urea and creatinine may attribute to in ability of the organ to remove these waste products as a result of decrease in glomerular filtration rate [10]. High levels of Na, Cl, K^+ ions, creatinine and urea are significant marker of kidney dysfunction indicating an inactivity in glomerular filtration rate [11]. This study aims to assess the safety of stem bark aqueous extract of *Pterocarpus erinaceus* on wistar albino rats (*Rattus norvegicus*).

2.0 Materials and Methods

2.1 Experimental Animals

Wistar albino rats (*Rattus norvegicus*) (120 to 130g) of both sexes were obtained from Pharmacology Department, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, in October, 2023. They were housed in metallic cages, and given standard laboratory diet produced by Vital Feeds Limited, Ibadan, and water*ad libitum*.

2.2 Plant Sample Collection, Identification and Authentication

The stem bark of *Pterocarpus erinaceus* was collected in the month of August, 2023 at Gajanga village in Akuyam District, Misau Local Government Area of Bauchi State, Nigeria. The plant was identified and authenticated in Biological Science Department of Sa'adu Zungur University, Bauchi. The plant was given a voucher specimen number 00062.

2.3 Preparation of Stem Bark Aqueous Extract of *Pterocarpus erinaceus*

The stem bark aqueous extract of *Pterocarpus erinaceus* was washed and dried at room temperature, then grinded to powder using pestle and mortar. Hundred grams (100g) of the powder was macerated in 4°C water (500mL) for 24 hours with constant shaking using shaker. The extract was filtered using Whatman filter paper number 1. The filtrate was then concentrated to dryness on a water bath at 50ºC, the crude extract was kept for use.

2.4 Extract Administration and Blood Sample Collection

The aqueous stem bark extract of *Pterocarpus erinaceus* was dissolved in distilled water. The experimental animals were divided into three groups of five (5) rats each: Group 1 (control) was administered with water, Group 2 was administered with 250mg/kg bw of the extract, Group 3 was administered with 500mg/kg bw of the extract. All administrations were done orally and continued daily for 28 days. All the rats were sacrificed under a mild chloroform anaesthesia at the end of the experiment. Two types of tubes were used for the blood collection: One with anti-coagulant ethylenediamine-tetraacetate (EDTA) was used to immediately analyse haematological parameters. The other portion collected with no anti-coagulant was centrifuged at 3000 rpm for 15 minutes to obtain serum, which was used to analyse biochemical parameters (liver and renal indices).

2.5 Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Liver Function Indices

Randox kits were used in determining liver function parameters which includes bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) as described by the manufacturer [12]. Exactly 100 µL of sulphaninlic acid was added to the blank and test, 100 µL of nitrite was added to the blank, 25 µL of nitrite was also added to the test. 100 µL of normal saline was added to blank and standard. 100 µL of serum was added to the test. Mixed and incubated at room temperature for 10 minutes. Absorbance was taken at 540nm.

2.6 Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Renal Function Indices

Randox kits were used in determining the renal function parameters which includes sodium, creatinine, urea potassium and chloride

concentrations as described by the manufacturer [13]. Exactly 0.1mL Randox standard creatinine solution was added to 1.0 mL of Randox Alkaline picrate reagent in a cuvette followed by mixing of the contents and absorbance was read after half a minute, the absorbance was read again at 492nm using a spectrophotometer after two (2) minutes again. Distilled water serve as blank.

2.7 Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus*on Haematological Parameters

Standard haematologicsl methods were used in determining haemoglobin (Hb), white blood cells (WBC), red blood cell (RBC), platelet (PLT) and percentage of haematocrit (HCT%) [14]. The analyzer works according to two principles: volumetric impedance and optical detection. There are 4 independent measurements: leukocyte counting and differentiation performed by optical principle (diffraction light usage), leukocyte counting performed by impedance principle in the current canal (measurement of single cell electrical resistance), erythrocyte and platelet counting performed in the independent canal and haemoglobin measurement performed by spectrophotometer. White blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), and platelets (PLT) are measured directly, but other parameters (HTC, MCV, MCH, MCHC, RDW, and PDW) are calculated automatically from the measured parameter data. Haemoglobin is measured by spectrophotometer according to cyanmethemoglobin method at wavelength of 540 nm.

2.8 Statistical Analysis

Standard statistical software Package for Social Science (SPSS) version 17.0 was used to analyse the data, which are displayed as Mean ± standard error of mean (SEM). Dunett's Posthoc test was used to assess differences between two and multiple means. Analysis of variance (one-way ANOVA) was used for data analysis at $P < 0.05$ significant level.

3.0 Results

3.1 Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Liver Function Indices

Table 1 shows the effect of stem bark aqueous extract of *Pterocarpus erinaceus* on liver indices. The extract was able to increased Bilirubin (0.80±0.11), AST (25.00±3.79) at 500mg/kg bw and increase ALT (26.67±5.46), ALP (115.33±4.18) at 250mg/kg bw. There are no significant different between treatments groups and the control group at $P < 0.05$ significant level.

Table 1: Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Liver Function Indices

Treatments	Billurubin (mg/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	AST/ALT RATIO
250mg/kg bw	0.83^{d}±0.03	23.33^{c}±5.18	26.67^{c}±5.46	115.33^{b}±4.18	0.23
500mg/kg bw	0.80^{d}±0.11	25.00^{c}±3.79	16.67^{c}±4.71	109.33^{b}±3.93	0.15
Control	0.77^{d}±0.09	17.33^{c}±0.88	24.67^{c}±2.34	107.67^{b}±4.18	0.23

Values refer to means and standard error. Means that do not share the same letter (between treatment and control) are significantly different at 95% confidence level. N= 5 in each group.

3.2 Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Renal Function Indices

Table 2 shows the effect of stem bark aqueous extract of *Pterocarpus erinaceus* on renal indices. The extract was able to increased serum urea (5.83±2.13), creatinine (98.33±19.69), at 500 and 250 mg/kg bw, and potassium (4.07±0.51) at 250 mg/kg bw. The extract was also able to increase chloride (96.33±2.61), and decrease sodium (141.67±3.18) at 500 and 250 mg/kg bw respectively. All indices have no significant different with control group at $P < 0.05$ significant level except creatinine (98.33±19.69) that shows slight elevation at 250 mg/kg bw.

Table 2: Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus*on Renal Function Indices

Treatments	Urea (mg/dl)	Cretinine (mg/dl)	Chloride (mg/dl)	Potasium (meg/l)	Sodium (meg/l)
250mg/kg bw	3.20^{d}±0.72	98.33^{b}±19.69	88.67^{b}±2.97	4.07^{d}±0.51	141.67^{a}±3.18
500mg/kg bw	5.83^{d}±2.13	36.33^{c}±5.51	96.33^{b}±2.61	3.87^{d}±0.55	145.00^{a}±3.22
Control	3.03^{d}±0.32	29.3^{c}±1.15	95.33^{b}±1.33	4.27^{d}±0.27	143.0^{a}±2.89

Values refer to means and standard error. Means that do not share the same letter (between treatment and control) are significantly different at 95% confidence level. N= 5 in each group.

3.3 Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Haematological Parameters

Table 3 shows the effect of stem bark aqueous extract of *Pterocarpus erinaceus* on haematological parameters. The extract increased the level of WBC (7.00±0.36), RBC (5.20±0.25), PLT (345.67±41.96), HGB (11.93±1.04) and HCT (38.67^{b}±2.19) compared to control at P <0.05 significant level.

Table 3: Effect of Stem Bark Aqueous Extract of *Pterocarpus erinaceus* on Some Haematological Parameters

Treatments	WBC($10^{\wedge}9$/L)	RBC ($10^{\wedge}12$/L)	PLT ($10^{\wedge}9$/L)	HGB (g/dL)	HCT (%)
250mg/kg bw	6.60^{b}±0.42	3.77^{b}±0.57	388.33^{a}±144.05	11.13^{b}±1.19	34.33^{b}±2.73
500mg/kg bw	7.00^{b}±0.36	5.20^{b}±0.25	345.67^{a}±41.96	11.93^{b}±1.04	38.67^{b}±2.19
Control	5.96^{b}±1.62	4.36^{b}±0.03	138.00^{a}±17.41	11.80^{b}±1.65	34.67^{b}±2.19

Values refer to means and standard error. Means that do not share the same letter (between treatment and control) are significantly different at 95% confidence level. N= 5 in each group.

4.0 Discussion

The prepared stem bark aqueous extract of African kino tree (*Pterocarpus erinaceus*) was resulted with 20% extract (%yield). This result is in consistent with that of Seidu*et al*. [15], who reported that aqueous extract of *Pterocarpus erinaceus* resulted with 20%yield. The results from this study revealed that stem bark aqueous extract of *Pterocarpus erinaceus* decrease the activities with concentration of extract for AST at 500mg/kg bw and increase at 250kg bw. The result is in line with the result reported by Elisabeth*et al*., [16], who reported that aqueous extract of *Pterocarpus erinaceus* lead to decrease the concentration of AST. The results also shown increase in activities of bilirubin, ALT and ALP with the concentration of extract. This finding is consistent with other earlier studies [17,18], which revealed that aqueous extract of *Pterocarpus erinaceus* increased the concentration of bilirubin, ALT and ALP. The values of the test groups for bilirubin, ALT, ALP and AST at 250mg/kg bw were slightly higher than that of the control group but there are no significance differences between them. This implies that the extract may not have toxic effect on liver cells. Higher value of the bilirubin, ALT, AST and ALP was probably due to the present of component in the extract that stimulate the activities of bilirubin, ALT, AST, and ALP.

The results of the effect of administration of stem bark aqueous extract of *Pterocarpus erinaceus* on renal indices of albino rats indicated an increase in the values of serum urea and creatinine. Atchou *et al*. [19], reported similar result. The results also shown increase in chloride and sodium in the test groups at 500 mg/kg bw and decrease at 250 mg/kg bw each compared to control group. The increase in urea and creatinine across the groups indicated that the extract has component which compromised the integrity of the kidney. The increase in sodium and potassium compared to control group suggested that the stem bark aqueous extract of *Pterocarpus*

erinaceus may contribute to sodium potassium levels in addition to compromise renal function. There is no significance difference between all the test groups and control group which indicated that the extract may not decrease glomerular filtration rate or cause kidney dysfunction.These results tallied with other documented findings [20-22], which revealed that administration of aqueous extract of *Pterocarpus erinaceus* increased serum urea, creatinine, chloride and sodium.

The effect of stem bark aqueous extract of *Pterocarpus erinaceus* on haematological parameters shown increase in RBC, HGB and HCT at 500mg/kg bw and decrease at 250mg/kg body weigh each. Rachmaini*et al* [23], also reported that stem bark aqueous extract of *Pterocarpus erinaceus* lead to increase the level of WBC, RBC, and HCT. Increase in platelet count recorded suggested that administration of stem bark aqueous extract of *Pterocarpus erinaceus* stimulate the oxygen carrying capacity of the blood. The increase in RBC count suggested that the extract may have stimulate the erythropoietin in rats, the increase in WBC has been recorded. These findings were supported with other similar reported results [24-26].

5.0 Conclusion

Stem bark aqueous extract of African kino tree doesn't cause toxicity to the liver and kidney. The extract was found to boost blood level by increasing the concentration of some haematological parameters. These effects may be related to the phytochemical components of the extract.

6.0 Recommendation

From the result of this research further studies required to investigate the fractionation of stem bark aqueous extract of African kinotree with different solvent of different polarity and subjecting it to see whether it is more active.

7.0 Acknowledment

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8.0 Conflict of Interest

There is no conflict of interest regarding the publication of this article.

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