Toxicological Study of the Effect of Ethanol Leaf Extract of *Pterocarpus santalinus* Extract on Liver of Wister Rats

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ABSTRACT

Aim/Introduction: ***The use of medicinal plants has attained a commanding role in health system all over the world. Pterocarpus santalinus is a plant common in Asia and Africa has been used traditionally in management of several ailments. Due to the relevance of Pterocarpus santalinus in medicine, there is the need to establish the safety profile of this plant on various organs of the body. The aim of this study is to evaluate the effect of Pterocarpus santalinus on rat's kidney over a period of 28 days.*** **Method:** ***Animals of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received Pterocarpus santalinus 100, 200 and 400 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and taken immediately for hematological and chemo pathological analysis. The histological toxic potential of the plant on the liver was studied using haematotoxylin and eosin (H&E) staining technique.*** **Result:** ***There was slightly Significant (P<0.05) decrease in RBC, HGB, MCV, while there was no change in the level of neutrophiles, basophiles, eosinophiles and platelets. Pterocarpus santalinus, slightly significantly (p<0.05) increased There were also no significant (P<0.05) increase in Alkaline phosphatase, level of bilirubin. Histological features agrees with other biomarkers.*** **Conclusion:** ***The result of the study showed that the Pterocarpus santalinus may be safe for human consumption, though with caution particularly at higher dose.***

Keyword: ***Pterocarpus santalinus, rat, blood, liver.***

INTRODUCTION

It would be difficult to overestimate the importance of the liver to the healthy functioning of the human body1. It is a remarkable organ. The liver acts as a processing plant, a battery, a filter, a warehouse and a distribution centre all in one1. The immune system, digestive tract, kidney, brain and cardiovascular system all depend on a healthy and well-functioning liver. This is why liver diseases such as hepatitis C can have such varied symptoms1. Because a diseased liver can potentially affect all the body's major systems and organs, it is very important to understand how it works and how to look after it. In most developing countries, the indigenous modes of herbal treatment are a part of the culture and the dominant method of healing therapy2. These remedies, with a considerable extent of effectiveness, are socially accepted, economically viable and, mostly, are the only available source3. Plants used in traditional medicine, therefore, have a critical role in the maintenance of health all over the world. The drugs of herbal, herbo-mineral, and animal origin have been used by the traditional healers to maintain health and treat diseases since antiquity. Such medicines are widely used in Africa and Asia, including India and China3,4. Due to the adverse side-effects, and also the development of resistance against synthetic drugs, the uses of plant-derived drugs are becoming popular in developed countries also5. The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug induced hepatotoxicity is a major cause of iatrogenic diseases, accounting for one in 600 to one in 3500 of all hospital admissions6.

Medicinal plants or their extracts have been used by humans since time immemorial for different ailments and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin)7. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders8.

Pterocarpus santalinus is a light-demanding small tree, growing to 8 metres (26 ft) tall with a trunk 50–150 cm diameter. It is fast-growing when young, reaching 5 metres (16 ft) tall in three years, even on degraded soils. It is not frost tolerant, being killed by temperatures of −1 °C9. The leaves are alternate, 3–9 cm long, trifoliate with three leaflets. The flowers are produced in short racemes. The fruit is a pod 6–9 cm long containing one or two seeds9. Pterocarpus santalinus is used in traditional herbal medicine as an antipyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, anti-hyperglycaemic and diaphoretic. Pterocarpus santalinus (red sandalwood) is one of the medicinal plants used in traditional medicine, and is rich in flavonoids and phenols10.Many previous studies found that different plant extracts have significant antidiabetic effects8,9,10. The aim of this study is to evaluate the effect of Pterocarpus santalinus on rat's liver over a period of 28 days.

MATERIALS AND METHOD

Animals: A total of twenty four (24) male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

Plant collection: Leaves of Pterocarpus santalinus were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State Nigeria.

Plant extraction: The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40 C in vacuum using rotary evaporator. The ethanol extract was stored at -4 C until used.

Animal study: Twenty four (24) rats of either sex (average weight of 240g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg) while the rats in groups 2, 3 and 4 were giving 100, 200, and 400 mg/kg of extract respectively. The weights of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as D0 while the day of sacrifice was designated as D29.

Haematological study: The rats were sacrificed on the 29th day of experiment. Blood samples were collected via cardiac puncture. One portion of the blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

Biochemical analysis: A Portion of the blood was collected used to estimate biochemical parameters including liver enzymes: alanine amino transaminase(ALT), aspartate amino transaminase (AST), alkaline phosphatase (ALP),albumin (ALB), total protein (TP), conjugated bilirubin (BILD), unconjugated bilirubin(BILT) using a photoelectric method.

Histopathology: Tissues collected were preserved in 10% formal saline solution. Small block of the tissues were taken from liver and fixed in Bouin's fluid for 16 to 24hours. Tissue were slices and processed according to the method described by (Lison,1960) and stained with haemotoxylin and eosin.

Statistical analysis: Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

RESULT

Effect of oral administration of Pterocarpus santalinus on hematological parametersin rats. Pterocarpus santalinus caused slightly significant ($p<0.05$) decrease in the level of

Table 1: Effect of oral administration of Pterocarpus santalinus on hematological parameters in wistar rats.

Hematological parameters	Treatment (mg/kg)			
	DW(10ml/kg)	100	200	400
WBC (×109/L)	8.21±0.772	6.74±1.32	7.71±0.71*	7.23±1.85
RBC (×1012/L)	8.30±0.34	6.65±0.66*	8.11±0.57	7.78±0.56
HGB (g/dL)	15.95±0.56	11.29±0.66*	14.33±0.96	14.62±0.11
HCT (g/dL)	60.26±2.03	56.60±3.74	34.67±3.18	53.40±1.81
MCV	66.62±0.93	60.40±1.44	57.17±0.31	69.60±1.72
MCH	19.17±0.17	17.80±1.02	18.83±0.37	18.80±0.20
MCHC (g/dL)	35.71±0.23	27.40±1.12	32.65±0.32	34.43±0.71
PLT (×109/L)	683.83±40.35	471.00±23.12*	652.31±12.20	677.34±52.32
LYM (%)	92.11±4.56	89.20±4.11	89.83±6.19	86.11±1.25
NEUT (×109/L)	12.14±3.67	11.99±3.54	13.14±5.66	11.56±5.32
EOSI (×109/L)	2.67±0.35	2.41±0.66	1.96±0.14	1.90±0.27
BASO (×109/L)	1.88±0.28	2.00±0.59	2.13±1.70	2.31±2.11

Data presented as Mean ± SEM: n = 6, (WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

red blood cell, hemoglobin, platelet etc. and significantly ($p<0.05$) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 100 mg/kg compared to the control. The level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly ($p<0.05$) affected.

Effect oral administration of Pterocarpus santalinus on hepatic indices in rats.

At 100 mg/kg dose level, Ocimum canum produced significant ($p<0.05$) decrease in BILD concentration in the treated rats while at 100 mg/kg dose no significant ($p<0.05$) increase was obtained in ALP levels, BILD and BILT concentrations when compared to the control (Table 2).

Effect of oral administration of ethanol leaf extract of Pterocarpus santalinus on histology Liver of rats.

The liver showed slight vascular congestion, slight hepatic necrosis and lymphocyte hyperplasia at 100 mg/kg and 200 mg/kg. There was slight Sinusoidal congestion observed at 400 mg/kg. However, there was no sign of damage to the liver of the rats in control group (Plate 1).

Table 2: Effect of sub-acute oral administration of Pterocarpus santalinus on hepatic indices in wistar rats.

Hepatic indices	Treatment (mg/kg) DW(10ml/kg)	100	200	400
ALB (g/L)	43.62±1.23	43.21±0.15	45.11±1.12	41.71±2.20
ALP (IU/L)	113.12±6.43	132.00±3.29	170.10±43.23	128.50±6.74
ALT (IU/L) S	65.25±3.01	67.34±7.12	81.40±12.19	85.22±27.17
AST (IU/L)	300.30±79.90	299.20±57.65	278.21±35.18	253.00±11.75
BILD (µmol/L)	0.28±0.17	0.16±0.12*	0.57±0.19*	0.25±0.33
BILT (µmol/L)	2.65±0.51	2.66±0.22	3.46±0.76*	2.45±0.11
TP (g/L)	79.13±2.11	76.14±2.65	71.35±5.17	81.13±2.65

Data presented as Mean ± SEM: n = 6, *significantly different from the distilled water (DW) control at $p<0.05$.
DW = distilled water (ALB = albumin, ALP = alanine phosphatase, ALT = alanine transaminase,
BILD = unconjugated bilirubin, BILT = conjugated bilirubin, TP = total protein).

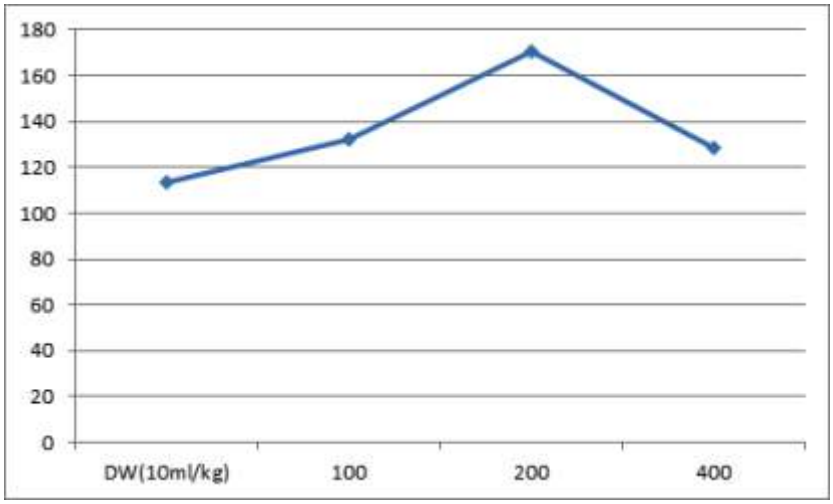

Fig 1: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum ALP level.

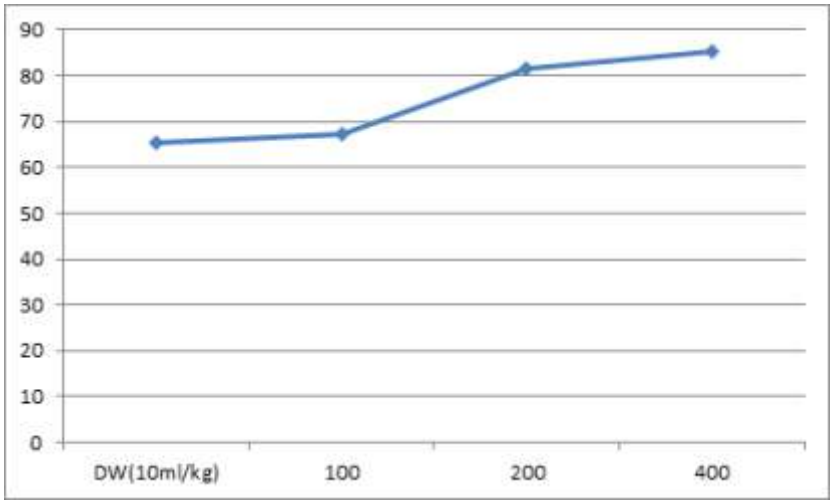

Fig 2: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum ALT level.

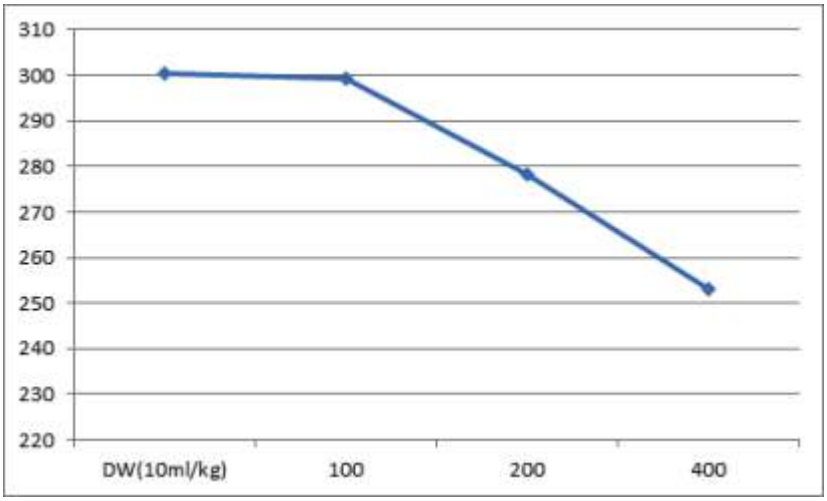

Fig 3: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum AST level.

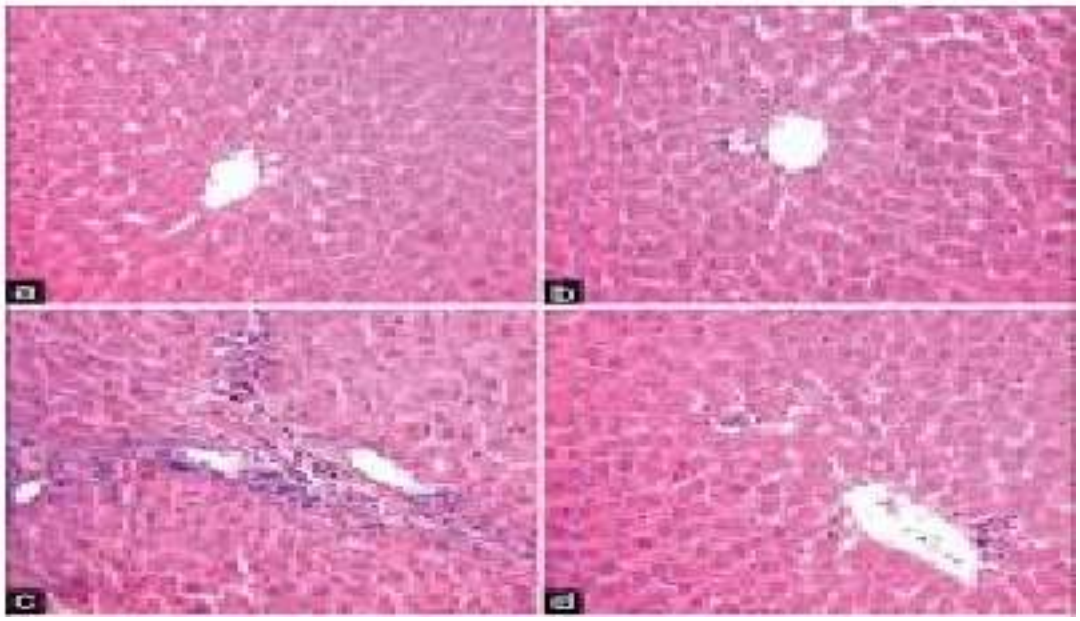

Plate 1:. figure of the liver (a) Control group, shows normal hepatocyte (H). (b) Pterocarpus santalinus 100 mg/kg (c) Pterocarpus santalinus 200 mg/kg,. d) 400 mg/kg Pterocarpus santalinsus

DISCUSSION

Herbal medicines proved to be the major remedy in traditional system of medicine. They have been used extensively in medical practices since ancient times11. There have been reports of accidental medicinal plant poisoning and over dose. In most cases this traditionally formulated drugs are consumed without appropriately establishing the dose that is safe for use. This has resulted into many untoward after effect12,13. Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems14,15,16. They can also be used to explain blood relating functions of chemical compound/plant extract15.

The hemoglobin concentrations and heamatocrit are values revealing the degree of anemia while the MCHC is a useful index of the average haemoglobin concentrations of the red cells17. Generally, low readings for RBC, Hb and hematocrit indicate anemia. At 200 and 400mg/kg dose all parameters studied were not significantly affected by Pterocarpus santalinus compared to the control group. Significant decrease in RBC, HGB, PLT and MCV at 100 mg/kg dose level indicate that Pterocarpus santalinus interferes with the normal production of haemoglobin and its concentration within RBCs and may thus possess the potential to cause anaemia at this dose level18. In addition, the significant ($p<0.05$) decrease in hemoglobin and hematocrit levels at 100 mg/kg body weight dose could be the optimal concentration of the product which may cause effect on the red blood cells indices. Some phytochemicals have been found to have effect on hematocrit. Saponins have been found to be cytolytic and can produce anemia19,20. Therefore, low red cells indices including hematocrit and hemoglobin observed may be attributed to presence of saponins found in some of the active ingredients in the product.

Chemicals produce a wide variety of clinical and pathological hepatic injury. Biochemical markers (e.g. alanine transferase, alkaline phosphatase and bilirubin) are often used to indicate liver damage21. Liver injury is

defined as a rise in either (a) ALT level more than three times of upper limit of normal (ULN), (b) ALP level more than twice ULN, or (c) total bilirubin level more than twice ULN when associated with increased ALT or ALP21,22,23. Liver damage is further characterized into hepatocellular (predominantly initial alanine transferase elevation) andcholestatic (initial alkaline phosphatase rise) types.However they are not mutually exclusive and mixed types of injuries are often encountered18,24.

The biochemical indices monitored in the liver is a useful 'markers' for assessment of tissue damage. The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis25, assault on the organs/tissues and to a reasonable extent the toxicity of the drug26. Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques27. Alkaline phosphatase, a 'marker' enzyme for plasma and endoplasmic reticulum16,24,28, is often employed to assess the integrity of plasma membrane29. In this study there ethanol extract of Pterocarpus santalinus did not cause significant change in most of liver function test values. This indicates that though the plant is use regularly by locals in different countries to exploit it medicinal benefits, it may be safe for consumption. Histological evaluation cellular and tissue parameter also agrees with chemical-pathology evaluation.

CONCLUSION

Result from the study suggests that at the doses administered ethanol leaf extract of Pterocarpus santalinus may not affect the functionality and integrity of liver, because most biomarkers accessed were relatively not negatively affected. This may prove useful to traditional people that use it regularly in the management of different conditions.

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