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SERUM ACTIVITY OF ALANINE AMINOTRANSFERASE AND ASPARTATE AMINOTRANSFERASE IN ALBINO RATS ADMINISTERED AQUEOUS EXTRACT OF FRESH LEAVES *PTEROCARPUS SANTALINOIDS.*

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ABSTRACT

Various parts of *Pterocarpus santalinoids* are used by traditional medicine practitionersin Eastern Nigeria in management and treatment of several disorders such as heart and liver related diseases. This research was carried out to investigate the effect of aqueous extract of fresh leaves of *Pterocarpus santalinoids* on serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in albino rats. A total of twenty-five (25) adult malealbino rats were used in this study. They were randomly distributed into five groups (A, B, C, D and E), with each group contained five rats. Groups A, B, C and D were administered 200, 400, 600 and 800 mg/kg body weight respectively of the extract for seven (7) consecutive days. Group E was used as control. There was a decrease in physical activities, the rate of feed and water intake and body weight of the animal in the test groups when compared with the control. AST and ALT activitiesin the animals given the extract (200 – 600mg/kg) were significantly lower ($P<0.05$) than the control group, while those of the group administered 800mg/kg were significantly higher ($P<0.05$) than the control. This effect was found to be dose dependent. The difference between serum total protein concentrations in the tests groups and the control was not significant ($P > 0.05$). These results indicated that aqueous extract of fresh leaves *Pterocarpus santalinoids* may possess hepatoprotective potential, especially at doses not greater than 600mg/kg body weight. This may be responsible for the application of the leaves of *Pterocarpus santalinoids* in the management of liver related disorders.

Keywords: Serum activity, Alanine aminotransferase, Aspartate aminotransferase, Albino rats and *Pterocarpus santalinoids.*

INTRODUCTION

Plants have been used as medicine since prehistoric times. Many of the herbs and spices used by humans to season foods also yield useful medicinal compounds. In developing countries like Nigeria, where modern western medicine is expensive, most of the indigenes rely on indigenous plants for the treatment of various ailments. In Nigeria, different medicinal plants are implicated in some herbal recipes for the treatment of several diseases [1]. Plants like garlic, *Digitalis lanata and Lola nitida* are used to treat cardiac disease and pile. Others like *Carica papaya* is used as a remedy for hypertension, *Allium sativium* is used in the treatment of malaria and many other diseases [2].

Medicinal formulations have served man over a series of centuries on his quest to survive his world of abundant microbes capable of causing different illness/diseases as conventional pharmaceuticals are either expensive or not available [3].

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent time. Pharmacologists, microbiologists, botanists and natural product chemists are combining the search for phytochemicals and drugs that could be developed for the treatment of various diseases [4].Among the over 120 active compounds presently isolated from the higher plants and widely used in modern medicine today, over 80% show a positive correlation between their modern therapeutic and traditional use of the plant from which they are derived [5]. More than two thirds of the world's plant species at least 35,000 of which they are estimated to have medicinal values come from developing countries [6].

Pterocarpu ssantalinoides commonly called red sandal wood in English, "Uturukpa" in Igbo is used in the treatment of many diseases. It is classified under kingdom *plantae* (*Fabales*), famliy (*faboideae*), genus (*pterocarpus*) and species (*santaliniodes*). Various morphological plant of *Pterocarpus santalinoids* are used in ethno-medicine in many African countries like Nigerian to treat an array of human ailments. *Pterocarpus stantalinoids* is a climbing tree of about 8-10m found in tropics of West and Central Africa and in India. Due to its scarcity in China many indigene of India are been caught every year smuggling the tree of *Pterocarpus stantalinoids* to China. Its effect on gastro-intestinal disorders has been scientifically proven with its triglyceride and glucose lowering properties [7].

Liver is an organ of paramount importance which plays a pivotal role in regulating various biochemical, physiological and biological processes such as storage, metabolism, secretion and control in the body. It plays the role of maintenance and regulation of homeostasis of

the body system. Liver function tests are tests that are carried to critically evaluate function of the liver for example metabolism, storage, filtration and excretion[8]. AST is found in the liver, heart, skeletal muscle, kidneys brain and red blood cells.SerumAST level, serum ALT (alanine aminotransferase) levels and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health. AST catalyzes the inter-conversion of Aspartate and α-ketoglutarate to oxaloacetate and glutamate, as a prototypical transaminase, AST relies on PLP (Vitamin B6) as a co-factor to transfer the amino group from aspartate or glutamate to the corresponding keto acid. In the process, the co-factors shuttle between PLP and the pyridoxamine phosphate (PMP) form. The amino group transfer catalysed by this enzyme is crucial in both amino acid degradation and synthesis. In amino acid degradation, following the conversion of α-ketoglutarate to glutamate, glutamate undergoes subsequent oxidative deamination to form ammonium ions which are excreted as urea.

AST is similar to ALT (Alanine transaminase) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is formed predominantly in the liver, with clinically negligible quantities found in the kidneys, heart and skeletal muscle as well in the red blood cells. As a result, ALT is more specific indicator of liver inflammation than AST, as AST may be elevated also in disease affecting other organ, such as myocardial infarction, acute power arthritis, acute hemolytic anemia, severe bums, acute renal diseases, musculoskeletal disease and trauma.

AST was first defined as a biochemical marker for the diagnosis of acute myocardial infarction in 1954. However, the use of AST for such a diagnosis is now reductant and has been superseded by the cardiac troponins. AST is commonly measured clinically as a part of diagnostic liver function test; to determine the health of liver (its functionality). However, it is important to have in mind that the source of AST (and to a lesser extent ALT) in blood tests may reflect pathology in organ other than the liver. Muscle inflammation due to dematomyositis may cause AST>ALT.

ALT Alanine Aminotransferase is a transaminase enzyme. ALT is also called Alanine transaminase and was formerly called Serum Glutamate-Pyruvate Transaminase (SGPT) or Serum Glutamate-Pyruvic Transaminase. ALT was first characterized in the mid-1950s by Arthur Karmen and Colleagues. ALT is found in plasma and in various body tissues but is most common in the liver it require the coenzyme pyridoxal phosphate like other transaminase, the pyridoxal phosphate is converted into pyridexamine in the first phase of the reaction, when an amino acid is converted into a keto acid.

ALT catalyzes the transfer of amino group from L-Alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L - glutamate. The ratio of ALT level and AST level is used as a common measured clinically biomarkers for liver health and they are also part of test used as part of blood panels (AST/ALT) ratio.

ALT is commonly used as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. When used in diagnostics, it is almost always measure in international units liter.

Both ALT and AST levels are increased in liver diseases but ALT > AST. Rise in ALT levels maybe noticed several days before clinical signs such as jaundice are manifested [10].

Moderate increase of ALT maybe seen in chronic liver diseases such as cirrhosis, hepatitis C and non-alcoholic steatohepatitis. For year, the American Red Cross used ALT fasting as part of the battery of test to ensure the safety of its blood supply by determining donors with elevated level of ALT. The intent was to identify donors potentially infected with hepatitis C because no specific test for the disease was available as at that time.

However, unlike AST that its elevated level implicates cardiac or skeletal muscle injury ALT is more specific in assessing the health nature of the liver and are specifically in clinical diagnosis for liver function/diseases than AST. These two (AST and ALT) helps in monitoring the response to treatment in liver disease. Elevated ALT level is also caused by dietary choline deficiency. Obviously mean that medical problems exist. Fluctuation of ALT levels is normal ease in response to strenuous physical exercise [11].

Further, when elevated ALT levels are found in the blood, the possible underlying causes can be further move down by measuring other enzymes, for example elevated ALT levels due to hepatocyte damage can be distinguished from bile duct problems by measuring alkaline phosphatase. Also, myopathy-related elevations in ALT should be suspected when the aspartate transaminase (AST) is greater than ALTs; the possibility of muscle disease causing elevation in liver tests can be further explored by measuring muscle enzymes including creatine kinase.

AIM

The aim of this research work was to determine the effect of aqueous extract of fresh leaves of *Pterocarpus santalinoids* on the liver of albino rats by measuring the level of liver enzymes AST and ALT in albino rats treated with aqueous extract of *Pterocarpus stantalinoides* (uturukpa).

AIM/OBJECTIVE

To assess the effect of the fresh leaves aqueous extract of *Pterocarpus santalinoids* on the liver by measuring the activities of ALT and AST in albino rats treated with the extract.

MATERIALS AND METHOD

METHODS

Sample Collection

Twenty-five (25) healthy adult male albino rats were purchased from University of Nigeria Nsukka and transported in steel cages to the Department of biochemistry animal house in Ebonyi State University Abakaliki. They were housed and allowed to acclimatise for seven days.

Collection of Plant Sample

Fresh leaves of *Pterocarpus santalinoides* (Uturukpa) was collected from Izzi local government Area of Abakaliki Ebonyi State and was identified by Dr. Nnamani C. U. of the department of Applied Biology, Ebonyi State University Abakaliki.

Plant Extract Preparation

Fresh leaves of the *Pterocarpus santalinoids*, washed and air-dried under shade. About 800g were ground into paste using pestle and mortar. The ground extract was soaked in 300ml of distilled water in a beaker for one hour.The extract was concentrated to obtain a concentration of 0.13g/ml (130mg/ml).

Animal Handling and Treatment

The animals were acclimatised for 7 days (one week) during which they were allowed free access to feed grower's marsh and water. The weight of the animal (rats) was taken using standard weighing balance calibrated in grams.

Administration of Extract

Oral administration of the extract was used throughout the experiment using 2ml syringe. Groups A, B, C and D were administered 200, 400, 600 and 800 mg/kg body weights of the extracts respectively for seven (7) consecutive days. Group E was used as control.

Collection of Blood Sample

After seven (7) days of extract administration the rats were starved overnight under a mild anesthesia using chloroform, blood sample was collected via cardiac puncture. The collected bloods were put into sterile bottle.

Preparation of Serum

The collected blood samples were centrifuged in the laboratory using desktop centrifuge (Biobit UK) resulting to separation of the blood into two layers and the serum being the supernatant was pipetted into a fresh sterile plain bottle.

Determination of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

This was carried out by the method described by [12].

RESULTS

PHYSICAL OBSERVATIONS

Administration of aqueous extract of *Pterocarpus santalinoides* fresh leaves to the albino rats produced a decrease in physical activities relative to the untreated group (control). Rate of feed and water consumption by the rats in the treated groups also decreased relative to the control.

CHANGES IN AVERAGE BODY WEIGHT OF THE RATS DURING SEVEN DAYS OF EXTRACT ADMINISTRATION

The change in the average body weight of the animals is presented in table 1 below.

There was an insignificant ($P>0.05$) decrease in average body weight of the albino rats in the groups given the extract, while the increase in the control group was not significant

($P > 0.05$). This change in the body weight of the test groups varied linearly with the dose.

Table 1: Weight (g) of albino rats.

Days	Group A	Group B	Group C	Group D	Group E
1.	96.65 ± 3.78	125.21 ± 6.56	113.21 ± 4.84	146.55±5.90	120.35 ± 6.32
2.	95.05 ± 4.03	122.01 ± 3.76	110.05 ± 2.99	145.25± 5.55	118.45 ± 6.35
3.	94.25 ± 5.35	120.35 ± 4.23	110.05 ± 3.62	144.45 ± 6.02	128.35 ± 7.80
4.	92.35± 3.45	118.21 ± 3.41	108.65 ± 5.11	141.25 ± 5.37	131.25 ± 6.74
5.	92.51 ± 3.37	116.51 ± 3.55	1108.41 ± 9.41	142.25 ± 5.5	134.21 ± 6.86
6.	90.35 ± 4.21	118.35 ± 3.61	106.15 ± 2.21	140.01 ± 5.66	134.42± 6.29
7.	88.21 ± 3.62	114 ± 4.52	104.05 ± 3.50	138.25± 6.71	136.05± 6.97

Values are in the mean (X) average body weight ± standard deviation.

THE ACTIVITY OF AST, AND ALT AFTER SEVEN (7) DAYS OF TREATMENT ARE SHOWN IN TABLE 2

The activity of AST and ALT in the serum of the albino rats administered the extract were significantly lower ($P<0.05$) than in the control group.The total protein did not differ significantly ($P>0.05$) between the test and the control.

Table 2: AST and ALT activities in the rats after treatment.

Parameters	AST Enzyme Activity (U/L)	ALT Enzyme Activity (U/L)	Protein Concentration. (mg/dl)
GROUPS			
GROUP A	20.01±1.70	15.29±0.66	0.58±0.34
GROUP B	17.37±1.68	11.59±0.86	0.77±0.19
GROUP C	13.18± 4.73	7.20±0.87	0.67±0.38
GROUP D	68.65 ± 3.32	39.25±0.57	0.44±0.34
GROUP E	33.62±2.20	19.15±1.57	0.68±0.29

Values are Mean ± SD; n = 5.

DISCUSSION AND CONCLUSION

Discussion

Within the seven days of treatment, there was an obvious decrease in physical activities of the albino rats after the administration of the aqueous extract. A reduction in feed and water intake was also noticed after the administration of the extract as compared to that of the control (data not shown). The reason for this observation is yet not fully understood. However, it could be attributed to the changes in metabolic activities of the treated animals elicited by constituents of the extract. The average body weight of groups given the extract decreased throughout the period of administration, while that of the control increased (table 1). This decrease in body weight may be due to the observed decrease in feed and water intake.Similar observations have beenreported by Agbafor [13]; Agbafor et al [14].

The decrease in activity of ALT and AST produced 200 - 600mg/kg of the extract suggests hepatoprotective potential. This may be due to the chemical constituents of the leaves such as flavonoids which antioxidant property. Antioxidants scavenge free radicals and prevent lipid peroxidation of biomembranes thereby reducing the leakage of intracellular enzymes [15].

However, at a higher dose (800mg/kg) of the extract, the serum levels of the enzymes increased. This increase is an indication of toxicity. Clinical observations and experimental studies have shown that subtle changes in the membranes of hepatocytes are sufficient to allow passage of intracellular enzymes into the extracellular space. Cell damage increases membrane permeability, causing cytosolic enzymes to spill into circulation [15]. For instance, in liver disease associated with hepatic necrosis, serum ALT and AST levels are elevated even before the clinical signs and symptoms of the disease appear [15].

Several phytochemicals have been reported to exhibit hepatotoxicity. Pyrrolizidine alkaloids are metabolized in the liver to pyrroles which are very toxic to hepatocytes, causing hepatocellular death and fibrosis [16]. Nwogu et al [17] reported the hepatotoxicity of pyrogallol, a hydrolysable tannin derived from simple phenolic acids like gallic acid.

CONCLUSION

From **t**he results of the analyzed parameters, the extract may be hepatoprotective at doses not greater than 600mg/kg body weight, and may be toxic at higher doses. Thus, the leaves can be used to manage/treat liver related disorders.

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