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Research article

SOME BIOCHEMICAL CHANGES IN SERUM OF MALE ALBINO RATS TREATED WITH AQUEOUS LEAF EXTRACT OF *PHYLLANTHUS AMARUS*

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ABSTRACT : Biochemical Changes in serum of male albino rats treated with aqueous leaf extract of *Phyllanthus amarus* were examined. 50mg/kg and 100mg/kg of the extracts were administered orally and once daily to group I and group II of test animals respectively for 28 days. The third group which served as control received distilled water only. On the 29th day, the rats which had been fasted overnight were dissected under chloroform anaesthesia and their blood collected directly by cardiac puncture. The blood was allowed to clot and centrifuged to obtain serum which was kept in a refrigerator at - 20^0C until its usage for the analysis of the following parameters; alanine and aspartate transaminases, alkaline and acid phosphatases, haemoglobin concentration, high and low density lipoproteins. The result indicates significant ($P<0.05$) increases in AST, ALT and ACP levels in tests groups compared to the control animals. There were non-significant elevation in the concentration of ALP, HDL, LDL and haemoglobin. These results suggest that *P. amarus* may be toxic if abused but when properly administered can be of use for its medicinal values.

Keywords: *Phylanthus amarus*, liver enzymes, lipoproteins, Rats.

INTRODUCTION

The plant *Phyllanthus amarus* belongs to the family Euphorbiaceae, which is a family otherwise, called the spurge family. It is a large family of about 300 genera and 600 species (Heywood, 1978). Most members are trees, shrubs and few are herbs; of rainforest and xereophylactic habitats (Burkill, 1994). Although plants of this family have important economic uses as foodstuffs, medicinal and industrials (particularly as sources of rubber and timber), most members are poisonous (Adedapo, 2002).

Phyllanthus amarus presently is considered as one of the best herbs for treating disorders such as hepatitis B, diabetes mellitus, skin ulcer (Oluwafemi and Debiri, 2008; Joseph and Raj, 2011). The plant has diuretic and purgative action and is also known to remedy inflammation of the respiratory tract and for asthma. It has a special reputation for causing bronchial relaxation (Odetola and Akojenu, 2000; Raphael and Kuttan, 2003; Kuttan, 2004, Adeneye et al, 2006). The main constituents of the plant include lignins, alkaloids, flavonoids, phenols and terpenes (Gotto, 1998; Joseph and Raj, 2011).

This study was aimed at investigating the effect of *Phyllanthus amarus* on some liver enzymes and lipoproteins of albino rats so as to adequately explore its toxicity at therapeutic doses.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of *P. amarus* collected within Abia State University and were taken to the department of Biochemistry. The plant was identified by a taxonomist in the department of Plant Science and Biotechnology of same University. The leaves were sorted to remove the dead ones, washed without squeezing to remove debris and dust particles. Large quantities of the leaves were collected and sun-dried for 5 days.

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The dried leaves were milled to get a coarse powder used for the extraction. Exactly 50g of the powder were macerated in a percolator with 250ml of distilled water. The mixture was allowed to stand for 24hours after which it was filtered. The filtrate was then placed in an oven of 55 ^{o}C to evaporate and the solid residue (5.0g) referred to as extract. 5.0g of the residue was added to 250ml (2.5% solution w/v) of water and administered to rats orally.

Experimental Animals

Twenty four white male albino rats (*Rattus novergicus*) weighing between 105 to 120g were obtained from small animal breeding unit of the Department of Biochemistry Abia State University Uturu. The rats had free access to water and standard palletized feed and were kept in the care of experienced animal technicians. They were randomly divided into three (3) groups with each group containing eight (8) rats.

Administration of Extract

Rats in group I were fed 100 mg/kg of the *P. amarus* extract, Rats in group II were fed 50 mg/kg of same extract orally once daily for 28 days, while the control received distilled water only in place of the extract. Animals were thereafter exposed to feed and water *ad libitum*.

Collection of Blood: On the 29th day, blood was collected directly from the heart of the overnight fasted rats through cardiac puncture under chloroform anaesthesia. The collected blood was allowed to clot and centrifuged to obtain the serum. The serum was kept in a refrigerator at 0-20^{0}C until further use.

Estimation of Biomolecules

Appropriate commercial Kits (Randox Laboratories, U.K) were used to determine the concentrations of alanine and aspartate transaminases (ALT and AST), alkaline and acid phosphatases (ALP and ACP), cholesterol and haemoglobin.

ALT and AST were estimated using the method of Reitman and Frankel, 1957 based on the principle that ALT involves the monitoring of the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine while AST involved monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine.

Alkaline phosphatase activity of the serum was determined as described by Bessey *et al.,* (1946), based on the principle that serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein that result in phosphoric acid and phenolphthalein at alkaline pH values. Pink coloured product is measured colorimetrically at 550nm.

Total cholesterol: This was carried out by the enzymatic colorimetric chod-PAP method.

High density lipoprotein (HDL)-Cholesterol**:** HDL separated from chylomicrons. Very low density lipoprotein (VLDL) and Low density lipoproteins (LDL) are separated by the addition of a phosphotungstic and magnesium chloride (precipitating reagent) to the serum **(**Burstein et al., 1970). After centrifugation, the cholesterol content was determined by the enzymatic colorimetric method.

Haemoglobin was determined as described by Dacie and Lewis, 1990.

Statistical Analysis

All analysis was performed in triplicates. Results were expressed in mean±S.D. Statistical significance was established using Analysis of variance (ANOVA). Means were Separated according to Duncans multiple range analysis ($P<0.05$).

RESULTS

Table 1 shows the body weight gain and haemoglobin concentration in rats of different groups during entire experimental period. The weight of the group I and group II rats did not differ significantly ($P<0.05$) as compared to the control, whereas the haemoglobin concentration was found to be maximum in group I animals as compared to the other group.

The result in Table 2 shows a significantly ($P<0.05$) increased activities of AST and ALP observed in Group I and II animals as compared to the control animals. ALT were less than 20.00 IU/L for all the test and control animals and its activity was significantly high in treated groups.

ACP has the highest value (16.16) in group II animals compared to (12.18) in Group I and in control animals (9.11). HDL-C for group I, group II and control animals were 26.90, 29.10 and 28.00 respectively and animals of group II had higher values as compared to control and group I. The LDL level in group II (33.10±6.60 mg/L) was higher as compared to control (32.00±0.10 mg/L) and group I (33.40±0.70 mg/L) animals.

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Table 1. Effects of *Phythanthus Annus* on Body Weight and Haemoglobin Concentration of Male Abino Rats.

Parameters	Group I (administered) 1.5ml of the extract	Group 2 administered 1.0ml of the extract	Control
Body weight (g)	108.00±2.00[a]	110.80±3.10[a]	111.50±2.50[a]
Haemoglobin concentration (mg/dL)	34.60±0.30 [b]	31.20±0.10[a]	29.80±0.40[a]

Figures are mean ± S.D figures bearing different alphabets on the row differ significantly (P<0.05) according to Duncan's multiple range analysis N = 8

Table 2 Effects of *Phythanthus Amarus* on some Serum Biochemical Parameters of Male Abino Rats.

Parameters	Group I (administered) 1.5ml of the extract	Group 2 administered 10ml of the extract	Control
AST (IU/L)	46.30±3.00[c]	33.70±2.40 [b]	29.6±2.20[a]
ALT (IU/L)	18.80±2.00[b]	18.40±1.00 [b]	16.80±2.10[a]
ALP (IU/L)	46.06±1.24[b]	46.16±3.40 [b]	43.68±2.00[a]
ACP (IU/L)	14.18±0.46[a]	16.16±0.79[a]	12.11±0.45[a]
HDL-C(mg/L)	26.90±0.90[a]	29.10±0.6 [b]	28.00±0.01[a]
LDL (mg/L)	33.40±0.70 [b]	33.10±6.60[ab]	32.00±0.10[a]

Figures are mean ± S.D figures bearing different alphabets on the row differ significantly (P<0.05) according to Duncan's multiple range analysis, N = 8.

DISCUSSION

All the animals gained weights though the weights gained were not significant (P<0.05) compared to the control.

Haemoglobin functions physiologically in transport of oxygen from the lungs to the peripheral tissues and also in transportation of carbon dioxide from the tissues to the lungs (Tietz, 1999). Significant increase (P<0.05) in haemoglobin concentration observed in group 1 (Table I) which occurred as the concentration of the extract increased may result in the lowering of oxidative stress (Gupta *et al;* 2002).

AST is an enzyme that is present in high concentration in the cytoplasm and mitochondria of liver, heart, skeletal muscle, kidney and brain (Benjamin, 1978; Ringler and Dabrich, 1999). Elevation in the activity of AST maybe associated with cell necrosis of many tissues. For example, pathology involving the skeletal or cardiac muscle and hepatic parenchyma allows for the leakage of large, amounts of this enzyme into the blood (Kaneko, 1980; Duncan *et al*., 1994). The elevation in AST produced by this plant is an indication of tissue damage.

ALT, a cytosolic enzyme is useful in measuring hepatic necrosis, especially in small animals (Bush, 1991). Since it is one of the specific liver enzymes its elevated level in this study may indicate hepatic damage by this plant extracts.

ALP is reported to be present in large number of cells but is only in a few cells in the activity sufficient to be of clinical importance. It is found largely in liver cells and is also associated with osteoblastic activity of bone cells (saini and saini, 1978). The significantly high (P<0.05) ALT obtained in this study compared to the control values is an indicator of the toxicity of the *P. amarus* extracts.

ACP is found throughout the body but primarily in the prostate gland (Muller *et al*., 2000). The aqueous extract of *Phyllanthus amarus* had no effect on the activity of ACP and may thus be non-toxic to the prostate gland cells.

The HDL major function is to remove unesterified cholesterol where it may have accumulated in cell membranes and plasma lipoproteins and transport it to the liver where after degradation is utilized for among other things, synthesis of the bile acids (Guyton, 1981). However LDL transports cholesterol to tissues where it may be needed for membrane structure or conversion into various metabolites such as steroid hormones (Gurr and Harwood, 1991). There were slight increases in HDL and LDL in tests animals compared to the control animals but these increases were not significant (P>0.05). This indicates that *P. amarus* has no negative impact on HDL and LDL levels in the test animals at this level of administration.

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CONCLUSION

Aqueous extract of *Phyllanthus amarus* significantly (P<0.05) increased in AST, ALP and ALT in test animals compared to controls while other serum biochemical parameters were non-significantly altered at lower doses in male albino rats. The results of the present study suggest that higher doses of the plant extract may produce damaging effects in different tissues.

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