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Full Length Research Paper

Glucose tolerance test and some biochemical effect of *Phyllanthus amarus* aquoeus extacts on normaglycemic albino rats

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Aqueous extract of the whole plant of *Phyllanthus amarus* was assessed for some biochemical effect in albino rats. Oral administration of aqueous extract at doses of 50, 100 and 200 mg/kg body weight shows that the extract promote glucose uptake. Daily administration of the extracts (50, 100 and 200 mg/kg bodyweight) for 14 days showed that the extract significantly ($p < 0.05$) reduced aspartate amino transferases (AST) and alanine amino transfarase (ALT) and urea at 100 mg/kg bodyweight when compared with other concentration doses and that of the control. However, significantly ($p < 0.05$) higher concentration of ALT was observed at 200 mg/kg body weight. Significant ($p < 0.05$) reduction for packed cell volume (PCV) and heamoglobin observed at 200 mg/kg body weight *P. amarus* was within the normal range. There was no significant ($p > 0.05$) change on the creatinine and triacyiglycerol (TAG) concentration for all the animal administered the extract compared with the control. The concentration of total cholesterol was significantly ($p < 0.05$) reduced for all the concentrations; the reduction was found to be dose dependant. The atherogenic index Log (TG/HDL-CH) of the animals showed no significant differences between tests and control. These findings may be of clinical importance to individuals at risk of cardiovascular disease.

Key words: *Phyllanthus amarus,* aqueous extract, glucose tolerance, lipid profiles, transaminases.

INTRODUCTION

Traditional medicine is the total combination of knowledge whether explicable or inexplicable use in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience handed down from generation to generation (WHO 1978). A medicinal plant is any plant used for the extraction of pure substances either for direct medicinal use or for hemi-synthesis of medicinal compounds which can be used for the therapeutic purpose or as a precursor for the synthesis of useful drugs (Sofowora, 1993). In recent years emphasis is on the development of drugs for the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world, the reason is that plant drugs could be effective and at the same time have less or no side effect.

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The plant *Phyllanthus amarus* is one of the most important medicinal plants used in traditional medicine for treatment of diabetes and excessive body weight reduction. Its hypoglycemic properties have been reported (Srividya and Perival, 1995; Raphael et al., 2002b). Several active compounds have been identified in *P. amarus* were found to be potent inhibitors of rat liver cyclic AMP–dependant protein kinases (Polya et al., 1995); phyllanthin and hypo-phyllanthin present are reported to be hepatoprotective agents and protect hepatocytes against carbon tetrachloride and galactosamine induced liver toxicity in rats (Syamasundar et al., 1985). In Nigeria, the plant is extensively used in traditional medicine to eliminate waste from the body, restore the activity of the liver and build up blood and innate defense system.

Despite the wide spread use of *P. amarus* much has not been reported about the biochemical effect in Nigeria. A recent work in Nigeria shows that the aqueous extract of *P. amarus* has hepatic cell function enhancement

(Chidi et al., 2007). This work was therefore designed to evaluate the effect of aqueous extract of *P. amarus* on the liver, kidney and normal glucose level.

MATERIAL AND METHODS

Plant materials

The plant sample under study was collected around the garden/ surroundings of Ahmadu Bello University, Samaru - Zaria, Kaduna State, Nigeria. The collected plant was then taken to the herbarium at the Department of Biological Sciences, Ahmadu Bello University, Zaria, for identification

Preparation of plant (whole plant)

The collected plant was rinsed in clean water and dried at room temperature for two weeks. The dried plant sample was ground into powder using a mortar and pestle, the powder obtained was then used to prepare the extracts.

Extractions

To 100 g of powdered plant material, 500 ml portion of distilled water was added and then stirred in a conical flask. It was then left to stand for twenty four (24) h. After the set time, suspension were filtered and the filtrates were then concentrated in a crucible using a water bath set at 40°C and the weight of sample taken. The concentrated extracts were then stored in a refrigerator until they were required for further analysis *(*Ibitoye and Ogunleye, 2003).

Animal grouping

Healthy wistar albino rats of both sexes weighing between 150 – 200 g were purchased from University of Jos, Plateau state, Nigeria and were kept in well aerated laboratory cages and acclimatized for two weeks. They were allowed to free access of water and feed diet (Vital Agricultural feeds Nigeria Limited) throughout the period of the experiment. 32 rats were allowed to fast for 14 h (overnight). They were divided in to 4 groups each group containing 8 rats each:

Group 1 Rats given water and feed only
Group 2 Rats given 50 mg/kg body weight extract
Group 3 Rats given 100 mg/kg body weight extract
Group 4 Rats given 2000 mg/kg body weight extract

Glucose determination

The fasting blood glucose of animals fasted overnight was determined using using commercial glucose strips (Life scan, One Touch Ultra, Melitas, CA).Glucose tolerance test was carried out for all the rats. For group 1, glucose was orally administered at 2 g/kg body weight and the blood glucose concentration was then taken for a period of 3 h at 30 min intervals. For extract treated groups (that is, 2, 3 and 4), their respective doses of the extracts was orally administered to them, 30 min before glucose was intragastically administered to them at 2 g/kg body weight and the blood glucose concentration was then taken for a period of 3 h at 30 min intervals.

Subchronic studies

The extracts treated groups were continued on their respective oral doses of the extract solution for 14 days, at the end of 14 days the animals were weighed anaesthetized by using chloroform and bled by cardiac puncture, and the blood samples were collected in specimen bottle. Part of the blood was used for heamatological parameters. The remaining blood was allowed to cloth and serum separated using pasture pipette into clean and labeled sample bottles for determination of some biochemical parameters. Serum transaminases (ALT and AST) were determined by method of Reitman-Frankel (1957), serum urea by Natelson et al. (1951) and serum creatinine by Jelliffe (1971).

Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triacylglycerol (TAG) were determined by enzymatic methods as described by Stein (1987). The low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald et al. (1972). The atherogenic risk predictor indices were calculated using the formulae of Dobiasova and Frohlich (2001).

Statistical analysis

Data obtained were analyzed by the use of student t-test distribution test and values for $P < 0.05$ were considered statistically significant

RESULTS AND DISCUSSION

Glucose tolerance test (Figure 1) shows that serum glucose of treated animal significantly ($p < 0.05$) increased at 30 min, the serum glucose concentration was higher than at zero time but decreased significantly from 30 to 180 min (Figure 1) for all the extracts doses administered. Serum alanine amino transfarase (ALT) and aspartate amino transfarase (AST) of animal treated with 100 mg/kg body weight showed significant ($p < 0.05$) reductions when compared with animal treated 50 and 200 mg/kg body and that of control. However significant ($p < 0.05$) elevation of AST was observed with animals treated 200 mg/kg body weight extract. Creatinine of treated animal did not show any significant ($p > 0.05$) difference. Significant ($p < 0.05$) reduction was observed with animal treated 100 mg/kg body weight for urea (Table 1). The extract did not produce significant ($p > 0.05$) effect on heamatological parameters except that the animals receiving the highest dose (200 mg/kg body weight) of the extracts had significant ($p < 0.05$) lowered PCV and Hb (Table 2). All the animals gained some weight, while for treated animal (50 and 100 mg), the weight gain are significantly ($p < 0.05$) lower compared to the control (Table 3).

Significantly ($p < 0.05$) lower level of cholesterol was observed for all the treated animals compared with control; this reduction was dose dependent. Triacylglycerol (TAG) and high density lipoprotein cholesterol (HDL-cholesterol) did not show any significant ($p > 0.05$) difference compared to control except for 200 mg/kg body weight (Table 4) that was significantly ($p < 0.05$) lower in HDL–cholesterol compared with the control. Table 5 shows the mean values of atherogenic risk predictor indices (HDL-CH/T-CH, LDL-CH/HDL-CH and Log (TG/HDL-CH) of the control and treated groups. The LDL-CH/HDL-CH were found to be significantly ($p < 0.05$)

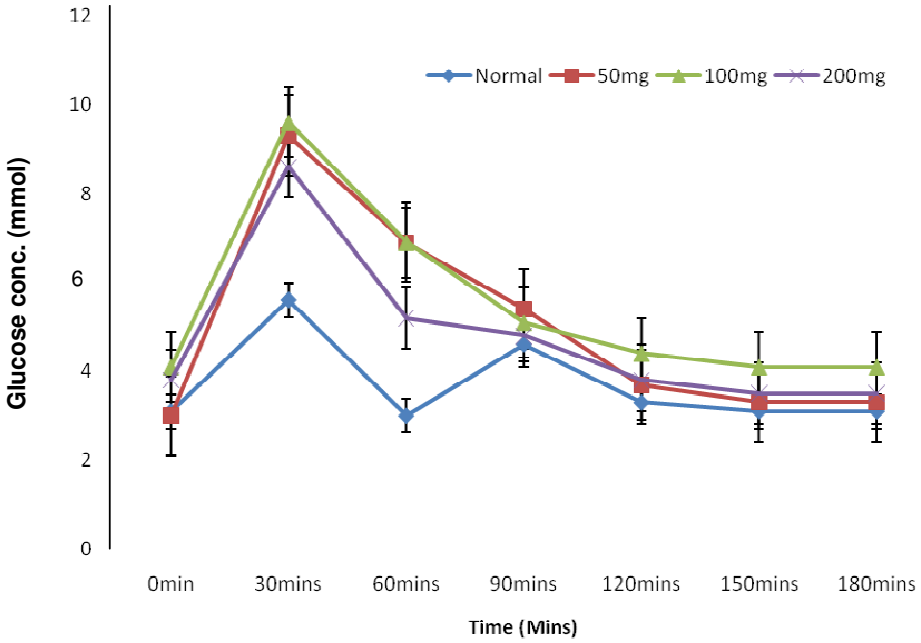

Figure 1. Effect of aqueous extract of *Phyllanthus amarus* on glucose tolerance level in normal albino rats.

Table 1. Effect of aqueous extract of *Phyllanthus amarus* on plasma levels of some liver and kidney parameters of normal albino rats.

Parameter	Normal	50 mg	100 mg	200 mg
AST (IU/L)	170.56±23.50^{ab}	150.67±7.54^{b}	121.67±2.87^{c}	182.00±5.72^{a}
ALT (IU/L)	67.00±10.00^{b}	99.33±1.89^{b}	38.67±11.09^{c}	125.00±14.70^{a}
Urea (mmo/L)	7.80±1.90^{ab}	6.13±0.47^{b}	4.33±0.94^{c}	8.00±1.04^{a}
Creatinine (µmo/L)	94.00±23.00^{a}	101.67±10.84^{a}	101.33±22.16^{a}	113.33±24.23^{a}

Values are means of four determinations±SD.
Values with different superscript in the row differ significantly (P < 0.05).

Table 2. Effect of aqueous extract of *Phyllanthus amarus* on some heamatological parameters of normal albino rats.

Parameter	Normal	50 mg	100 mg	200 mg
PCV (%)	46.25±3.59^{ab}	47.50±0.58^{a}	44.00±1.41^{b}	41.67±1.25^{c}
Hb (g/dl)	15.33±1.36^{ab}	15.75±0.17^{a}	14.67±0.47^{b}	13.89±0.42^{c}

Values are means of four determinations±SD.
Values with different superscript in the row differ significantly (P < 0.05).

reduced with non significant (P > 0.05 reduction in the Log (TG/HDL-CH) in all the treated groups.

The present study was designed to evaluate the effect of aqueous extract of *P. amarus* extract on glucose tolerance test, liver, kidney and serum lipids profile. Liver and kidney are two important organs that perform vital function for healthy survival of the body. Liver detoxify harmful substances, secretes bile into intestine, synthesizes and store important molecules. The kidney helps in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. In the oral glucose tolerance test *P. amarus* extract showed significant (p < 0.05) reduction of serum glucose level based on the hypoglycemic effect in normal rats. It was observed that the hypoglycemic mechanism involved insulin–like effect, most probably through the peripheral glucose consumption (Ozturic et al., 1996 and Bonner–wein et al., 1989).

Table 3. Effect of aqueous extract of *Phyllanthus amarus* on mean weight change of normal albino rats.

Group	Mean initial weight (g)	Mean final weight (g)	Mean weight change (g)
Normal	166.50±7.05	223.00±5.72	53.00±4.97^{a}
50 mg	167.25±5.50	194.00±2.94	28.66±4.60^{b}
100 mg	232.50±9.57	165.75±9.74	29.00±2.16^{b}
200 mg	218.75±2.5	171.33±7.93	58.75±4.03^{a}

Values are means of four determinations±SD.
Values with different superscript in the column differ significantly ($P < 0.05$).

Table 4. Effect of aqueous extract of *Phyllanthus amarus* on some lipid profile of normal albino rats.

Parameter (mmo/L)	Normal	50 mg	100 mg	200 mg
Total Cholesterol	1.95±0.09^{a}	1.67±0.05^{b}	1.47±0.38^{bc}	1.17±0.17^{c}
Triacylglycerol	1.00±0.01^{a}	1.10±0.16^{a}	0.93±0.05^{a}	0.97±0.09^{a}
High density lipoprotein	0.67±0.06^{a}	0.63±0.05^{a}	0.57±0.09^{ab}	0.53±0.05^{b}
Low density lipoprotein	1.08±0.01^{a}	0.83±0.02^{b}	0.71±0.28^{b}	0.39±0.06^{b}

Values are means of four determinations±SD.
Values with different superscript in the row differ significantly ($P < 0.05$).

Table 5. The mean values of plasma antherogenic risk predictor indices recorded after 2 weeks of treatment.

Parameter (mmo/L)	Normal	50 mg	100 mg	200 mg
HDL-CH/T-CH	0.34±0.02^{c}	0.38±0.02^{b}	0.40±0.04^{b}	0.46±0.02^{a}
LDL-CH/HDL-CH	1.61±0.14^{a}	1.32±0.12^{b}	1.22±0.30^{b}	0.74±0.05^{c}
Log(TG/HDL-CH)	0.17±0.12^{a}	0.24±0.12^{a}	0.22±0.18^{a}	0.26±0.01^{a}

Values with different superscript in the row differ significantly.
Values of HDL-CH/T-CH ratio < 0.30 are atherogenic and undesirable, values of LDL-CH/HDL-CH ratio >2.3 are atherogenic and undesirable (Ojiakor and Nwanjo, 2005).

The serum level of both ALT and AST showed significant ($p < 0.05$) reduction at 100 mg/kg body weight compared to the control groups and other treated groups. This result is in line with the work reported by Chidi et al. (2007) that the plant *P. amarus* lower serum level of transaminase and that the plant has a hepatic cell protection. The rise in levels of ALT is always accompanied by elevation in the level of AST which play a role in the conversion of amino acid to keto acid. Both AST and ALT are excellent markers of liver damage caused by exposure to toxic substances (Ranjna, 1999). AST is not specific for the liver only but is also located in other organs like the heart, brain, kidney and skeletal muscle. ALT is more liver specific enzyme for diagnostic use; when the integrity of the hepatocellular membrane is compromised, there is extrusion of the enzyme into the plasma (Moss and Henderson, 1996). The significant reduction in ALT activity that was observed in animals treated with 100 mg/kg bodyweight of the extract suggests hepatoprotective at that concentration.

There was no significant ($p > 0.05$) difference in the serum level of urea and creatinine except at 100 mg/kg body weight treated animals which showed significant ($p < 0.05$) reduction in the level of urea compared to the control. Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Some of the urea is bound to heamoglobin so its concentration in red blood cells is greater than in the plasma. Renal diseases which diminish the glomerular filteration lead to urea retention and decrease in urea is seen in severe liver disease with destruction of cells leadings to impairment of the urea cycle (Ranjna, 1999). Significant ($p < 0.05$) decrease in serum urea observed with animals treated 100 mg/kg body weight may not be as a result of liver damage or abnormal functional kidney. Since this does not correlate with increase in liver marker enzymes, it might be as a result of protein intake. From this study it was observed

that the weight gain change of the animals treated 100 mg/kg body weight of *P. amarus* extract was significant ($p < 0.05$) lower compared with control. The weight change difference is as a result of change in feed intake. The decrease in PCV and heamoglobin is the group treated with the highest dose of the extract was within the normal range and could be due to stress and not as a result of anemia (Cole, 1986).

Creatinine is a waste product formed in muscle by creatine metabolism. Creatine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment.

Result of the present study showed that aqueous extract of *P. amarus* has significant ($p < 0.05$) serum-lipid lowering effect on the level of total cholesterol and low density lipoprotein. The observed dose administration dependent cholesterol and LDL-cholesterol effect may be attributed to the gut intra-lumenal interactive effect of saponins. Saponins are known antinutritional factors which reduce the uptake of certain nutrients including glucose and lipid especially cholesterol at the gut through intra- lumena physicochemical interaction. Hence saponins have been reported to have hypocholesterolemic effect (Price et al., 1987). Presence of saponins has been reported by Chidi et al. (2007) in aqueous extract of *P. amarus* and this saponin may explain the antilipidemic effect observed in this study. The significantly ($p < 0.05$) lower cholesterol may have contributed to the observed non-significant high serum HDL-cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL. It is hypothesized that HDL-cholesterol can remove cholesterol from antheroma within arteries and transport it back to the liver for excretion or re-utilization. Thus high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000). The observed non-significant ($p > 0.05$) reduction in HDL-cholesterol concentration upon administration of aqueous extract (50 and100 mg) and its significant ($p < 0.05$) reduction at 200 mg indicates that the extract does not have HDL-C boosting effect at low dose and it does not also have significant ($p > 0.05$) lowering effect on HDL-cholesterol at those concentration. While at 200 mg/kg body weight, the extract have no HDL-C boosting effect

On the other hand, the extract even at the lowest dose used significantly ($p < 0.05$) reduced LDL-cholesterol concentration. LDL-cholesterol transport cholesterol to the arteries where they can be retained in arterial proteoglycans starting the formation of plagues. LDL-cholesterol poses a risk of cardiovascular disease when it invades endothelium and become oxidized since the oxidized form is more easily retain by the proteoglycans. Thus increased level of LDL-cholesterol is associated with atherosclerosis heart attack, stroke and peripheral vascular disease (Crowwell and Otvos, 2004). The important of this LDL-cholesterol lowering effect of the extract is that the extract may aid in the prevention or reduction of cardiovascular risk factors. The non-significant changes in the levels of atherogenic index of Log (TG/HDL-CH) of the extract treated animals may be due to the dependence of the index on the unvarying TAG. The non-significant reductions in the atherogenic indexes, nevertheless, portend a decreased risk of vascular disease since high atherogenic index of Log (TG/HDL-CH) has been positively correlated with cardiovascular risk (Igwe et al., 2007).

In conclusion, aqueous extract of *P. amarus* promotes glucose tolerance and has cholesterol and LDL-cholesterol lowering effect, which may be of benefit to individuals at risk of cardiovascular disease.

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