

## Some Biochemical Effect of Intraperitoneal Administration of *Phyllanthus amarus* Aqueous Extracts on Normoglycemic Albino Rats

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**Abstract:** The effect of aqueous extract of the whole plant of *Phyllanthus amarus* was assessed for some biochemical effect in albino rats. Different doses (50, 100 and 200mg/kg bodyweight) of crude extract was administered Intraperitoneal for 14 consecutive days. Glucose tolerance test shows that there was significant ( $p < 0.05$ ) reduction in the serum blood glucose from 30minutes up to 120minutes for animals treated with 50mg and 200mg body weight while from 120minutes up to 180minutes there was no significant ( $p > 0.05$ ) change for all the treated animals. The level of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) of animals treated with 50mg and 100mg kg body weight was significantly ( $p < 0.05$ ) lower when compared with that of the control. Creatinine concentration was significantly ( $p < 0.05$ ) lower at 200mg body weight while no significant ( $p > 0.05$ ) change observed in urea concentration for the treated animals. There was no significant ( $p > 0.05$ ) change in packed cell volume (PCV) and hemoglobin (Hb) observed for all the treated animals compared with control. Significant ( $p > 0.05$ ) reduction in the cholesterol concentration for the animals treated with 50mg and 200mg compared with the control was observed. There was no significant ( $p > 0.05$ ) difference in the triglyceride concentration for the animals treated with 50mg and 100mg kg body weight. The low density cholesterol was significantly ( $p < 0.05$ ) lower for all the treated animals (100mg and 200mg), while there were no significant ( $p > 0.05$ ) change in the level of high density cholesterol for animals treated 100mg and 200mg body weight of the extract.

**Key words:** Aqueous extract, glucose tolerance, intraperitoneal, lipid profiles, *Phyllanthus amarus* and transaminases

### INTRODUCTION

*Phyllanthus amarus* is a broad spectrum medicinal plant that has received world wide recognition (Srividiya *et al.*, 1995). This plant has recently attained the status of a miracle plant because of its ability to cure several ailments as claimed by its proponents. It is used for the treatment of malaria, jaundice and diabetes. It also induces abortion. Whole plant of *P. amarus* is usually soaked in hot water or cooked in locally brewed alcohol and drunk as tea. Some people take it as an enema depending on what is being treated.

In clinical research over the years, the plant has demonstrated liver protective, anti-inflammatory, antioxidant, chemoprotective, hypolipidaemic, analgesic, hypotensive, antispasmodic, antimutagenic and hypoglycemic activities (Roa and Alice, 2001). The contraceptive effect of the herb has also been reported by Rao and Alice (2001). The active ingredients in *Phyllanthus* include the lignans phyllanthine, phyllanthanol, phylochrysin, phylltetralin and hypophyllanthine (Thyagarajan *et al.*, 1998). Bioflavonoids, quercetin, quercertol, quercitrin, rutin and the alkaloids, glycosides, saponins and catechins are also found in *Phyllanthus* (Khanna and Srivastava, 2002).

The objective of this study is to establish the dose dependent and non-dose dependent effects of the

intraperitoneal administration of the whole plant aqueous crude extract of *Phyllanthus 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 100g of powdered plant material, 500mls portion of distilled water was added and then stirred in a conical flask. It was then left to stand for twenty four (24)hrs. After the set time, suspension was 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.

**Animal Grouping:** Healthy wistar albino rats of both sexes weighing between 150-200g 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 free access to water and feed diet (Vital Agricultural feeds Nigeria Limited) throughout the period of the experiment. 32 rats were allowed to fast for 14 hours (overnight). They were divided into 4 groups each group containing 8 rats each.

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

**Glucose Determination:** The fasting blood glucose of animals fasted overnight was determined using commercial glucose strips (Life scan, One Touch Ultra, Melitas, CA). Glucose tolerance was tested for all the rats. Group 1 glucose was orally administered at 2g/kg body weight and the blood glucose concentration was then taken for a period of 3 hrs at 30min intervals. For extract treated groups (i.e 2, 3 and 4) their respective doses of the extracts was intraperitoneally administered to them, 30min before glucose was intragastrically administered to them at 2g/kg body weight and the blood glucose concentration was then taken for a period of 3 hrs at 30min intervals.

**Sub chronic studies:** The extract 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 hematological parameters. The remaining blood was allowed to clot and serum separated using a Pasteur pipette into clean and labeled sample bottles for determination of some biochemical parameters. Serum transaminase (ALT and AST) was determined by method of Reitman-Frankel (1957), Serum urea by Natelson (1951), Serum creatinine by Jelliffe (1971).

Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triacylglycerol (TG) 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 (Fig. 1) shows that serum glucose of treated animal significantly ( $p < 0.05$ ) increased

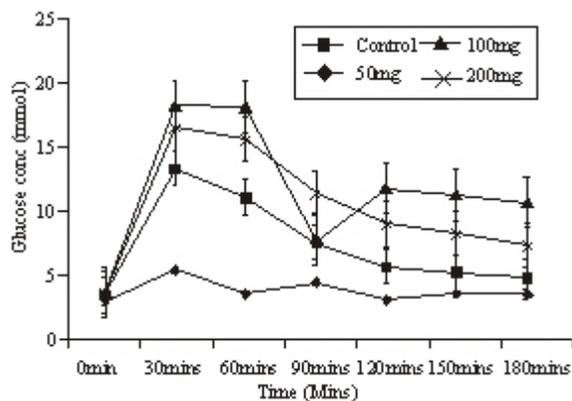


Fig. 1: Effect of aqueous extract of *Phyllanthus amarus* on glucose tolerance level in rats

at 30 minutes the serum glucose concentration was higher than at zero time but decreased significantly from 30 minutes to 120 minutes (Fig. 1) for all the animals treated 50mg and 200mg body weight, there was no significant difference from 120 minutes to 180 minutes. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) of animal treated with 50mg and 100mg body weight showed significant ( $p < 0.05$ ) reductions when compared with animals in control group. Urea of treated animal did not show any significant ( $p > 0.05$ ) difference. Significant ( $p > 0.05$ ) reduction was observed with animal treated 200mg/kg body weight for creatinine (Table 1). The extract did not produce significant ( $p > 0.05$ ) effect on hematological parameters (Table 2)

Significantly ( $p < 0.05$ ) lower level of cholesterol was observed for all the animals treated 50mg and 200mg body weight compared with control. There was no significant ( $p > 0.05$ ) difference in triacylglycerol (TAG) for all the animals treated with 50mg and 100mg compared with animals in control group. Low-density cholesterol was significantly ( $p < 0.05$ ) lower for all the treated animals, while there was no significant ( $p > 0.05$ ) change in the level of high-density cholesterol for animals treated 100mg body weight of the extract (Table 3).

The present study was designed to evaluate the Intraperitoneal effect of aqueous extract of *Phyllanthus 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 *Phyllanthus 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;

Table 1: Effect of aqueous extract of *Phyllanthus amarus* on plasma levels of some liver and kidney parameters

Parameter	Control	50mg	100mg	200mg
AST (IU/L)	170.33±23.50 <sup>a</sup>	144.67±1.29 <sup>b</sup>	120.67±9.87 <sup>b</sup>	133.67±66.49 <sup>ab</sup>
ALT (IU/L)	67.00±10.00 <sup>a</sup>	51.67±18.50 <sup>b</sup>	32.33±11.59 <sup>b</sup>	49.33±30.66 <sup>ab</sup>
Urea (mmo/L)	7.80±1.80 <sup>a</sup>	8.23±0.75 <sup>ab</sup>	6.60±2.42 <sup>b</sup>	9.10±2.60 <sup>a</sup>
Creatinine (µmo/L)	94.10±23.00 <sup>a</sup>	86.33±13.28 <sup>ab</sup>	82.33±4.50 <sup>ab</sup>	78.33±27.14 <sup>b</sup>

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 hematological parameters of normal albino rats

Parameter	Control	50mg	100mg	200mg
PCV (%)	41.57±11.75 <sup>a</sup>	39.17±5.64 <sup>a</sup>	42.50±6.37 <sup>a</sup>	36.25±5.50 <sup>a</sup>
Hb (g/dl)	13.86±3.92 <sup>a</sup>	13.05±1.88 <sup>a</sup>	14.17±2.1 <sup>a</sup>	12.09±1.83 <sup>a</sup>

Values are means of four determinations± SD

Values with different superscript in the row differ significantly (P<0.05)

Table 3: Effect of aqueous extract of *Phyllanthus amarus* on some lipid profile of normal albino rats

Parameters (mm o/L)	Control	50mg	100mg	200mg
Total Cholesterol	1.95±0.05 <sup>a</sup>	1.47±0.15 <sup>b</sup>	1.53±0.32 <sup>ab</sup>	1.37±0.12 <sup>b</sup>
Triacylglycerol	1.00±0.01 <sup>b</sup>	1.03±0.12 <sup>ab</sup>	1.80±1.39 <sup>ab</sup>	1.17±0.12 <sup>a</sup>
High density lipoprotein	0.67±0.06 <sup>a</sup>	0.37±0.12 <sup>b</sup>	0.57±0.06 <sup>a</sup>	0.43±0.12 <sup>b</sup>
Low density lipoprotein	1.32±0.40 <sup>a</sup>	0.69±0.34 <sup>ab</sup>	0.43±0.31 <sup>b</sup>	0.71±0.03 <sup>b</sup>

Values are means of four determinations± SD

Values with different superscript in the row differ significantly (P<0.05)

Bonner-wein *et al.*, 1989). However this effect was not significant for the treated animals as from 120minutes up to 180minutes, this may be as a result of the effect of route of administration, significant (p<0.05) reduction up to 180minutes has been reported for oral administration (James *et al.*, 2009), when a drug is taken by oral route food and other drugs in the digestive tract may affect how much of and how fast the drug is absorbed.

The serum level of both ALT and AST showed significant (p<0.05) reduction at 50mg and 100mg/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) and James *et al.* (2009) that the plant *Phyllanthus 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 marker of liver damage caused by exposure to toxic substances (Ranjna, 1999).

There was no significant (p>0.05) difference in the serum level of urea and creatinine except at 200mg/kg body weight treated animal, which showed significant (p<0.05) reduction in the level of creatinine 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, is 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 hemoglobin so its concentration in red blood cells is greater than in the plasma. Renal diseases which diminish the glomerular filtration leads to urea retention and decrease in urea is seen in severe liver disease with destruction of cells leading to impairment of the urea cycle (Ranjna, 1999).

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. This study shows that the extract has no effect on the liver and kidney of treated animals. The non-significant (P>0.05) changes seen in the hematological parameters indicate relative safety of the extract at the doses used in this study on haemopoetic system.

Result of the present study showed that aqueous extract of *Phyllanthus amarus* has significant (p<0.05) serum – lipid-lowering effect on the level of total cholesterol and low-density lipoprotein. The observed low cholesterol effect of and LDL – cholesterol effect may be attributed to the gut intra – luminal 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 – lumina 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 *Phyllanthus amarus* and this saponin may explain the antilipidemic effect observed in this study. The significantly (p<0.05) lower cholesterol and LDL has also been reported with animal given the extract orally (James *et al.*, 2009).

The observed non-significant (p<0.05) reduction in HDL – cholesterol concentration upon administration of aqueous extract (100mg) its significant (p<0.05) reduction at 200mg has also being reported (James *et al.*, 2009) and this indicates that the extract at 100mg /kg body weight does not have HDL – C boosting or lowering effect while at 200mg/kg body weight extract have HDL – C lowering effect.

On the other hand the extract even at the lowest dose used reduced LDL – cholesterol concentration. LDL – cholesterol transport cholesterol to the arteries were they could be retained in arterial proteoglycans starting the formation of plaques. LDL – cholesterol poses a risk of cardiovascular disease when it invades endothelium and become oxidized since the oxidized form is more easily retained 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.

### CONCLUSION

In conclusion, aqueous extract of *Phyllanthus amarus* administered intraperitoneally as low as 50mg/kg promote glucose tolerance has cholesterol and LDL – cholesterol lowering effect, which may be chemically beneficial to individuals at risk of cardiovascular disease and toxicologically safe because of its low level of serum transaminase, and its non- significant ( $p>0.05$ ) effect on creatinine and urea

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