International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Effect of Phyllanthus Amarus Leaf Extract on the Serum Lipid Profile of Alloxan-Induced Diabetic Albino Wistar Rats in College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

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ABSTRACT

This study was designed to investigate the effect of *Phyllanthus amarus* (PA) leaf extract on serum lipid profile in alloxan induced diabetic albino wistar rats. A total of 30 albino wistar rats each weighing 100g were assembled and divided into 3 groups (A-C) consisting of 10 rats. Group A received PA treatment, B was without PA treatment while group C served as the control group. 400mg/kg of aqueous extract of PA leaf was administered orally to the rats in group A but not in group B while group C received only water for 7 days. Blood samples were collected into plain containers for estimation of biochemical parameters (TC, TG, LDL and HDL) respectively. Serum TC, TG, LDL and HDL were analyzed using standard methods respectively. There was a significant decrease in the mean serum level of TC (2.10±0.11 vs 2.6±0.16; p=0.000), TG (0.55±0.03 vs 0.61±0.04; p=0.000) and LDL (1.07±0.03 vs 1.53±0.06; p=0.000) whereas the mean serum level of HDL was significantly raised after PA administration (0.77±0.09 vs 0.72±0.06; p=0.000). Again, the result shows a significant decrease in the mean weight of the subjects after PA administration (98.80±1.03 s 119.40±1.17; p= 0.000). This study revealed hypolipidemic as well as anti-obesity effects of PA use. Therefore, PA use could be of importance in prevention and management of cardiovascular diseases and obesity in patients.

Keywords: *Phyllanthus amarus*, Low density lipoprotein, High density lipoprotein, Triglyceride, Total cholesterol, wistar albino rat.

INTRODUCTION

Medicinal plants have been used for the treatment of several human diseases over the century and have been very important in the health care delivery of every nation at one stage or the other

(Oluma *et al.*, 2004). Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries (Aiyegoro *et al.*, 2007). Plant derived medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases (Mohana *et al.*, 2008:Ghaleb *et al.*, 2009; Ajayi and Akintola, 2010). Plant parts such as the roots, leaves, shoots, barks, fruit peels, immature and unripe fruits have been used in most herbal preparations. According to George and Pamplona-Roger (1998), the therapeutic value of some common plants such as *Anthocleista vogelii Planch, Morinda lucida, Triplochiton scleroxylon, Alchornea cordifolia, Cassia sieberiana, Mangifera indica, Anacardium occidentale, Nauclea latifolia, Daniela oliveri,Citrus paradise, Ananas sativus and Carica papaya* have been used in the treatment of various ailments including enteric fever, diarrhoea, dysentery, malaria, common cold, convulsion, yellow fever, jaundice, dental caries, intestinal parasites, gastroenteritis, bacterial, viral and protozoan diseases. Antiseptic, diuretic, antibacterial and anti-inflammatory properties have equally been reported (Alanis *et al.*, 2005).

Phyllanthus amarus Schum. & Thonn., belonging to the family Euphorbiaceae, is a medicinal plant that has been used in traditional Thai medicine for treatment of fever, jaundice, ascites, hemorrhoid and diabetes (Pongboonrod, 1976). The plant is indigenous to tropical Americas, the Philippines and India (Tirimana, 1987; Cabieses, 1993; Chevallier, 2000). Plants in the genus Phyllanthus are found around all tropical regions of the world; from Africa to Asia, South America and the West Indies. The genus contains about 550-750 species in 10-11 subgenera (Unander, 1995).Some related species with medicinal importance include *P. epiphyllanthus, P. niruri, P. urinaria, P. acuminatus and P. emblica* (Tirimana, 1987). The plant can be found along roads, valleys, on riverbanks and near lakes. *P. amarus* is sometimes mistaken and wrongly identified with the closely related *P. niruri* L. in appearance, phytochemical structure and history of use. *P. niruri* also reaches a height of 60 cm with larger fruits and dark brown and warty seeds than that of *P. amarus* (Morton, 1981). Several pharmacological activities of *P. amarus* have been reported including anti-amnesic, antibacterial, anti-fungal, anti-viral, anti-cancer, anti-diarrheal, gastro-protective, antiulcer, analgesic, anti-inflammatory, antioxidant, diuretic, anti-plasmodial, aphrodisiac, contraceptive, antihypertensive, hypoglycemic, hypo-cholesterolemic, immunomodulatory, nephroprotective, radioprotective and spasmolytic activities (Houghton *et al.*, 1996; Adeneye *et al*., 2006a ; Patel *et al.*,2011), hepatoprotective against ethanol-, paracetamol-and carbon-tetrachloride-toxicity (Pramyothin *et al.*, 2007, Wongnawa *et al.*, 2006, Krithika and Verma 2009). The secondary metabolites present in *P. amarus* are alkaloids, flavonoids, hydrolysable tannins (ellagitannins), major lignans, polyphenols, triterpines, sterols and volatile oil (Patel *et al.*, 2011).

A lipoprotein is a biochemical assembly if protein and lipids, created in the intestinum tenue and the liver, which transports triaclglycerols and cholesterol in the blood between all the tissues of the body (Carl *et al.*, 2008); the most outstanding ones being the liver and the adipocytes of the adipose tissue. In addition to providing a soluble means for transporting cholesterol through the blood, lipoproteins have cell-targeting signals that direct the lipids they carry to certain tissues (Olson, 1998). The several types of lipoprotein in the blood are donated in order of their increasing density as chylomicron, very-low density lipoprotein (VLDL), intermediate-density lipoprotein, low-density lipoprotein (LDL) and high density lipoprotein (HDL) (Nelson and Cox, 2000); the density being dependent on the proportion of lipid to protein in the lipoprotein. Elevated levels of

the lipoprotein fraction, LDL, HDL, VLDL are regarded as atherogenic (Rahilly and Catherine, 2011), hence levels of these fractions, rather than the total cholesterol level, correlate with the extent and progress of atherosclerosis. The different lipoproteins contain apoproteins, which serve as ligands for specific receptors on the cell membranes.

HDL is the smallest and densest of the lipoprotein particles because it contains the highest proportion of protein to cholesterol. Its most abundant apolipoprotein are apo A-I and apo A- II (Toth and Peter, 2005) and increases in size as it circulates through the bloodstream and incorporates more cholesterol and phospholipid molecules from cells and other lipoproteins (Kwiterovich *et al*., 2000). HDL particles remove fats and cholesterol from cells, including within artery wall atheroma, and transport it back to the liver for excretion or re-utilization; in a process known as reverse cholesterol transport (RCT) (Merrill and Sandhoff, 2002), thus the cholesterol carried within HDL particles (HDL-C) is sometimes called "good cholesterol". Individuals with higher levels of HDL-C tend to have fewer problems with cardiovascular diseases, while those with low HDL-C cholesterol levels (especially less than 40 mg/dl or about 1 mmol/L) have increased rates for heart disease (Toth and Peter, 2005).

LDL molecules, also referred as "bad cholesterol", are the major carriers of cholesterol in blood, and each contains approximately 1500 molecules of cholesterol ester and one molecule of apolipoprotein B-100 in the shell (Segrest *et al.,* 2001). LDL particles are formed as very low density lipoproteins, lose triglyceride through the action of lipoprotein lipase and become smaller and denser, containing a higher proportion of cholesterol esters (Warnick *et al.,* 1990). Blood tests typically report LDL-cholesterol which is commonly used to estimate how much low density lipoproteins are driving progressions of atherosclerosis (Carl *et al.,* 2008).

The liver clears chylomicron from the blood; incorporates the triglycerides into lipoprotein and release them back into the bloodstream as very low density lipoprotein (VLDL) molecules (Kwiterovich et al., 2000) which contains apolipoprotein B-100 and apolipoprotein E in their shells. VLDL transports endogenous triglycerides, phospholipids, cholesterol and cholesteryl esters, whereas chylomicrons transport exogenous (dietary) products (Carl *et al.,* 2008). The degradation of VLDL yields intermediate density lipoproteins (IDL) which are not in the blood (Nelson and Cox, 2000).

A triglyceride (TG, triacylglycerol, TAG, or triacyglyceride) is an ester derived from glycerol and three fatty acids (Nelson and Cox, 2000). As a blood lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver; they are the main constitutes of vegetable oil (more unsaturated), animal fats (more saturated) and human skin oil (Sidhu and Naugler, 2012).

Total cholesterol is defined as the sum of HDL, LDL, and VLDL. Cholesterol is an organic molecule formed predominantly in the liver and later excreted in the non-esterified form via bile into the digestive tract from where it is reabsorbed by the small intestine into the bloodstream (Hanukoglu *et al.,* 1992). It is an essential structural component of animal cell membranes required to establish proper membrane permeability and fluidity as well as serve as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D (Olson, 1998). Since cholesterol is essential for all animal life, each cell synthesizes from simpler molecules, a 37-step process which starts with the intracellular protein enzyme HMG-CoA reductase (Lecerf and De Lergoril, 2011). However, high levels of cholesterol in the blood circulation, depending on its mode of transportation within lipoproteins, are strongly associated with progression of atherosclerosis. Dietary cholesterol has little if any, effect on total body cholesterol

content or concentration of cholesterol in the blood because most ingested cholesterol is of the esterified form which is poorly absorbed; the body compensates for any absorption of additional cholesterol by reducing cholesterol synthesis (Lecerf and De Lergoril, 2011).

Traditionally, *P. amarus* is used in the treatment of liver ailments and kidney stones. Base on its Spanish name 'chanca piedra' meaning "stone breaker or shatter stone. " The plant is a popular medicinal herb used as a remedy around the world for influenza, dropsy, diabetes and jaundice (Foo, 1993).Whole plant is employed in some genitourinary infections. The young tender shoots are used in chronic dysentery and the juice of the stem is mixed with oil in ophthalmia for eye treatments. According to Foo and Wong (1992), the aerial part of *Phyllanthus amarus* is highly valued in traditional medicine for its healing properties. Fresh leaf juice of the plant can be applied externally for the treatment of cuts and bruises. It is also good for treating Arthritis and Asthma in patients (Adebisi, 1999). Chevallier (2000) reported that *P. amarus* has been used traditionally in India to treat cardiovascular problems. In the light of the above findings and the paradigm shift from the use of synthetic chemicals in food and its detrimental effects which necessitates the search of plants for their therapeutic roles in combating symptoms and diseases with safety, efficacy and dependability as compared to costly synthetic drugs, many of which has adverse effects, it became important to investigate the effects of *Phyllanthus amarus* leaf extract on the lipid profile of alloxan induced diabetic wistar rats in College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

MATERIALS AND METHODS:

Study Location

The study was carried out at The Human Biochemistry Laboratory, Nnamdi Azikiwe University. It is located in the suburb of Nnewi - a popular town in Anambra State, Nigeria.

Collection and identification of plant

The *Phyllanthus amarus* plant was collected from Okofia, College of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi campus, Anambra State, Nigeria, in the month of January, 2016 and identified by Mrs. Aziagba B.O., Department of Botany, Nnamdi Azikiwe University, Akwa.

Animals

Wistar albino rats (100g) of both male and female were obtained from the Institute Animal House and maintained at 25±2 ºC temperature and relative humidity 45-55% under 12:12 h light:dark cycle. Rats were fed with standard rat chow and water *ad-libitum*.

Preparation of the plant extract

The method used is based on the method described by Kalita *et al; (*2013), although with some modification. About 150 g of dried leaves of *Phyllanthus amarus* were taken in a 1000 mL of the round bottom flask and extracted for 72 h by a continuous hot percolation process using the solvent ethanol as solvent. The extracts were filtered through the Whatmann filter paper to remove impurities. The extracts were then concentrated by vacuum distillation, cooled and placed in desiccators to remove the excessive moisture.

Alloxan *induced hyperglycemia*

Animals were divided into three groups, each consisting of ten rats. Rats in the first group (A) received 400mg/kg *Phyllanthus amarus* dissolved in ethanol while the second group of rats (B) received ethanol. Rats in groups 3 were normal rats and served as the control groups (C). All the animals received their respective assigned treatment daily for a period of seven days. Rats were daily fasted over night before *Phyllanthus amarus* treatment. On day 8, the animals were anesthetized with ether, and blood was collected using cardiac puncture. Serum was then separated for the estimation of lipid profile parameters (LDL, HDL, TC, TG) respectively using standard

methods as described by (Assman *et al*., 1984; Burstein *et al.,* 1980; Roeschlau *et al.*, 1974; Rifal and Warnick, 1994) respectively.

Ethical Consideration

The protocol was approved by the Faculty of Health Sciences and Technology Ethical Committee, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria.

Inclusion and Exclusion criteria

Apparently healthy Wistar rats weighing 100g were included for the study while Unhealthy Wistar rats with weight less or above 100g were excluded from the study in order to ensure accuracy and uniformity in result interpretation.

Statistics

Statistical Package for Social Science (SPSS) version 20 was employed in the analysis of the result. The results for the parameters studied were expressed as Mean± SD and the data were analyzed for general group differences using one way ANOVA while post-HOC comparison was used to determine the inter-group differences. Correlation was done using Pearson correlation and Level of significance was set at $p<0.05$.

RESULT

The mean serum levels of all the parameters studies were statically significant at $p<0.05$ respectively using ANOVA table. In this study, the mean serum level of high density lipoprotein was significantly increased (0.77±0.09 vs 0.72±0.06; $p<0.05$) when the alloxan induced diabetic rats with *Phyllanthus amarus* treatment were compared with those rats without *Phyllanthus amarus* treatment. However, the mean serum levels of total cholesterol, triglyceride as well as low density lipoprotein were significantly decreased (2.10±0.11 vs 2.6±0.16; 0.55±0.03 vs 0.61±0.04; 1.07±0.03 vs 1.53±0.06; $p<0.05$) respectively when the alloxan induced diabetic rats with *Phyllanthus amarus* treatment were compared with those rats without *Phyllanthus amarus* treatment. Again, following administration of *Phyllanthus amarus,* there was significant decrease in the mean weight of the rats (98.80±1.03 vs 119.40±1.17; $p<0.05$) compared to those rats without *Phyllanthus amarus treatment* (See table 1)

However, when the subjects with *Phyllanthus amarus* treatment were compared with the control group, all the parameters differed significantly ($p<0.05$) except the mean serum level of total cholesterol ($p>0.05$) (See table 1).

Furthermore, comparing the parameters studied between the subject group without *Phyllanthus amarus* treatment and control groups indicates significant changes in the mean serum level of parameters studied ($p<0.05$). However, the mean serum level of low density lipoprotein did not differ significantly ($P>0.05$) (See table 1).

Table 1: Serum levels of lipid profile in alloxan induce diabetic rat with phyllanthus treatment (A), without phyllanthus treatment (B) and Control group (C) (Mean ± SD, n = 10).

Group	Total Cholesterol (Mmol/L)	LDL (Mmol/L)	HDL (Mmol/L)	Triglyceride (Mmol/L)	Weight (g)
A (n=10)	2.10±0.11	1.07±0.03	0.77±0.09	0.55±0.03	98.80±1.03
B (n=10)	2.6±0.16	1.53±0.06	0.72±0.06	0.61±0.04	119.40±1.17
C (n=10)	1.89±0.14	1.03±0.08	0.61±0.03	0.43±0.05	100.60±0.84
F (p)-valve	74.331 (.000)	217.840 (.000)	13.124 (.000)	50.709 (.000)	150.000 (000)
AVB	<0.05	<0.05	<0.05	<0.05	<0.05
AVC	>0.05	<0.05	<0.05	<0.05	<0.05
BV C	<0.05	<0.05	>0.05	<0.05	<0.05

*Statistically significant at p<0.05

Key: F (p)-value = mean± SD of parameter compared among groups A, B, and C (using ANOVA).
A V B p-value = mean ± SD of parameter compared between group A and B (using t-test).
AVC p-value = mean ±SD of parameter compared between group A and C (using t-test).
B VC p-value = mean ±SD of parameter compared between group B and C (using t-test).

The mean serum levels of Triglyceride and Low density lipoprotein was positively correlated in the control group (See table 2).

Table 2: Level of association between TG and LDL in control group(C) (Mean ± SD, n = 10).

Parameter	Pearson Correlation r	P-Value
TG vs LDL	.677	.032

*Statistically significant at p<0.05

However, only Low density lipoprotein and High density lipoprotein was negatively correlated in those group without treatment *Phyllanthus amarus* treatment (See table 3).

Table 3: Level of association between LDL and HDL in alloxan induced diabetic rats without treatment (B) (Mean ± SD, n = 10).

Parameter	Pearson Correlation r	P-Value
LDL vs HDL	-.660	.038

*Statistically significant at p<0.05

DISCUSSION

Several pharmacological activities of *P. amarus* have been reported including anti-amnesic, antibacterial, anti-fungal, anti-viral, anti-cancer, anti-diarrheal, gastro-protective, antiulcer, analgesic, anti-inflammatory, antioxidant, diuretic, anti-plasmodial, aphrodisiac, contraceptive, anti-hypertensive, hypoglycemic, hypocholesterolemic, immunomodulatory, nephroprotective, radioprotective and spasmolytic activities as well as hepatoprotective activities (Houghton *et al.*, 1996; Kuttan,1998; Adeneye *et al*., 2006a; Wongnawa *et al.,* 2006; Pramyothin *et al.*, 2007, Krithika and Verma 2009; Patel *et al.*,2011). This study investigated the effect of *Phyllanthus amarus* leaf extract on the lipid profile of alloxan-induced diabetic albino wistar rats in College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

The present studies show a significant decrease in the mean serum levels of TC (2.10±0.11 vs 2.6±0.16), TG (0.55±0.03 vs 0.61±0.04) and LDL (1.07±0.03 vs 1.53±0.06) respectively ($p<0.05$) whereas the mean serum level of HDL was significantly increased (0.77±0.09 vs 0.72±0.06; $p<0.05$) after the alloxan induced diabetic rats were treated with *Phyllanthus amarus* leaf extract. This is in line with the report of Nwankpa *et al.* who investigated the effects of *Phyllanthus amarus* on serum lipid in *salmonellae typhi* infested wistar rats and found that the mean serum TC, TG as well as LDL were significantly decreased while HDL was significantly increased in the subjects after *Phyllanthus amarus* treatment compared with the control group (Nwankpa *et al.*, 2012). This may suggest the protective role of *Phyllanthus amarus* against degenerative disease. The antherogenic risk predictor indices, HDL-cholesterol/Total cholesterol and LDL-cholesterol/HDL-cholesterol ratios fell within the desirable limits. This may be indicating that the ethanolic extract of *Phyllanthus amarus* has the potential to reduce the risk of development of cardiovascular diseases. More so, these observed results 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) and Nwankpa *et al. (*2015) in aqueous extract of *Phyllanthus amarus* and this saponin may explain the antilipidemic effect observed in this study. Other similar results have been reported (James *et al.*, 2009; James *et al.*, 2010).

Interestingly, there was also a significant reduction in the mean weight of the alloxan induced diabetic rats after its treatment with *Phyllanthus amarus* leaf extract. This may pint to the anti-obesity property of the plant, possibly suggesting its diuretic nature (Alanis *et al.*, 2005). However, there was a negative correlation between LDL and HDL in the alloxan induced rats.

CONCLUSION

From the present study, we conclude that *Phyllanthus amarus* have significant anti-hyperlipidemic as well as anti-obesity effects. Therefore, *P. amarus* can be useful, at least as an adjunct in the therapy of cardiovascular diseases as well as obesity. However, further study is necessary for the screening of chemical compounds and the structure elucidation of the anti-hyperlipidemic as well as its anti-obesity properties along side its extraction mechanism.

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How to cite this article: Ezeugwunne IP, Chukwuma FC, Ogbodo EC et al. Effect of phyllanthus amarus leaf extract on the serum lipid profile of alloxan-induced diabetic albino wistar rats in college of health sciences and technology, Nnamdi Azikiwe University, Nnewi campus, Anambra state, Nigeria. Int J Health Sci Res. 2018; 8(2):199-207.

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