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EFFECT OF PHYLLANTUS AMARUS LEAF EXTRACT ON THE SERUM LIVER ENZYMES 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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ABSRACT
This study was designed to investigate the effect of *Phyllantus amarus* (PA) leaf extract on serum liver enzymes activity 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 (ALT, AST and ALP) respectively. Serum ALP, ALT and AST were analyzed using standard methods. There was a significant decrease in the mean serum activities of ALP (72.20±2.57 vs 94.90±9.34; p=0.000) and AST (36.10±2.60 vs 40.40±3.24; p=0.004) after PA administration whereas, ALT did not differ significantly (p>0.05). 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 the hepatoprotective effect of PA use. Therefore, PA use could be of importance in prevention and management of liver diseases.

KEYWORDS: *Phyllantus amarus*, Alanine aminotransferase, Aspartate aminotransferase, Alkaline Phosphatase, Weight, Liver Disease, Albino Wistar Rats.

INTRODUCTION

Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine (Akinyemi *et al*., 2005). In many parts of the world, including Ghana, herbal medicine practitioners are still consulted as a first choice in the treatment of ailments, due to the fact that traditional medicine blends readily with the socio-cultural life of the people and the fact that orthodox medicine are more expensive to procure and some orthodox pharmaceutical preparations are many times faked (Amuse *et al*., 2011). There is a vast array of medicinal plants used singly or in combination with other medicinal plants that confer synergistic effect in the treatments of various ailments. These medicinal plants or their extracts are administered orally, topically, by inhalation of vapours or by steam bathing. *Phyllanthus amarus* is reported to have healing properties and not toxic to either the kidney or liver. The plant also contains several phytochemical elements including glycosides, flavonoids, alkaloids, phenylpropanoids, sterols, saponins, limonine among others. *P. amarus* is used for the treatment of several medical conditions including liver, kidney and bladder problems, diabetes, intestinal parasites, inflammation, prostate, influenza, dropsy and jaundice problems (Heyde, 1990; Foo, 1993). *Phyllanthus amarus* is a broad spectrum medicinal plant that has received world- wide recognition (Srividiya and Perival, 1995).

In Nigeria, it is called “Oyomokeisoamankedem” in Efik, “Iyin Olobe” in Yoruba and “Ebebenizo” in Bini (Etta, 2008).

P. amarus is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, as anti-helminthes to expel intestinal worms and act as a mild Laxative. *P. amarus* also has antiseptic, diuretic, antiviral, anti-diabetic, hypotensive and antipyretic properties and is also used in the treatment of jaundice, diarrhoea, dysentery, wound, ulcers and urogenital diseases (Calixto *et al.,* 1998; Santos *et al.*, 1995). The plants of the genus Phyllanthus are widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver disease (Liu *et al.*, 2003). Again, *Phyllantus amarus* has also been used as chemoprotective agent (Kumar and Kuttan, 2005), antimutagenic agents (Sripanidkulchai, 2002) and exhibits hypoglycaemic properties (Rephael, 2002). Its effect in excretory system is due to its antiurolithic property and is used in the treatment of kidney/gallstones, other kidney related problem, appendix inflammation and prostate problems (Sen and Batra, 2013). The flower paste of plant is applied externally as antidote against snake bite (Chandewar and Dhongade, 2013). Plants contain numerous constituents; some tend to possess some level of toxicity. Cases of this toxicity in plants have been reported (Santos *et al.*, 1995; Shaw *et al*., 1997; Kaplowitz, 1997). *P. amarus* has been classified among plants with a low potential for toxicity, with an LD_{50} averaging 2000 mg/kg/day (Krithika and Verma, 2009). The phytochemical analysis of the *P. amarus* extract confirmed the presence of tannins, saponins, flavonoids and alkaloids. The plant extract have been found to contain high levels of saponins, tannins, flavonoids and alkaloids (Fernand, 1998; Naaz, 2007; Krithika and Verma, 2009).

The liver is the most vital organ in the mammalian body and performs all important functions that impact all body systems. The liver has lobular structure and lies in the abdominal cavity below diaphragm. The circulatory system of the liver is different from that of other organs. Roughly 75% of the blood entering in liver through the portal vein is the venous blood returning back from the small intestine, stomach, pancreas and spleen. From this portal venous blood all nutrients along with drugs and other potentially harmful substances are absorbed. The remaining 25% of the arterial blood received by liver is the oxygenated blood being carried from the pulmonary system to the liver by the hepatic artery. The blood contents of the hepatic artery as well as hepatic portal vein empty into sinusoids. Sinusoidal blood moves towards the central vein of each lobule and empties its content. Hepatic veins carry deoxygenated blood from liver to the inferior vena cava (Fawcett, 1994; Malarkey *et al.,* 2005). Liver has a significant role in glucose homeostasis and acts to retain normal glucose levels during fasting and in the postprandial period. The role of liver in developing of type 2 diabetes has attracted much interest. Furthermore, it is thought that abnormal function of liver attributed to insulin-resistance syndrome may lead to development of type 2 diabetes (Marchesini *et al*., 2001). Liver function tests are assessed through using liver enzymes aspartate aminontransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Both AST and ALT are considered markers of hepatocellular health. ALT is considered the specific biomarker of liver pathology and is found mainly in liver (Lee *et al.,* 2004). Because AST and ALP can be found in other tissues, they are thought to be less specific biomarkers of liver function (Lee *et al*., 2003). However, the paradigin shift from the use of synthetic chemicals in food and its detrimental effects 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 with adverse effects. X-raying the above facts, it became important to investigate the effects of *Phyllantus amarus* extract on the serum liver enzymes of alloxan induced diabetic wistar rat in 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 *Phyllantus 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±2ºC 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 *Phyllantus amarus* were taken in a 1000 mL of the round bottom flask and extracted for 72h 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 *Phyllantus 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 *Phyllantus 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 liver enzymes (ALT, AST and ALP) respectively using standard methods as described by (Rifal and Warnick, 1994; Tietz, 1987; Shephard *et al.*, 1986) 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.

Statistical Analysis

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 $p<0.05$.

RESULTS

The mean serum activities of all the parameters studied were statically significant at $p<0.05$ respectively, using ANOVA table. In this study, the mean serum Alkaline Phosphatase activity was significantly decreased (72.20±2.57 vs 94.90±9.34; p=0.000) when the alloxan induced diabetic rats with *Phyllantus amarus* treatment were compared with those rats without *Phyllantus amarus* treatment. Again, the mean serum Aspartate aminotransferase activity was significantly decreased (36.10±2.60 vs 40.40±3.24; p=0.004) when the alloxan induced diabetic rats with *Phyllantus amarus* treatment was compared with those rats without *Phyllantus amarus* treatment. Furthermore, the mean serum Alanine aminotransferase activity decreased although statistically insignificant after the treatment with *Phyllantus amarus* ($p>0.05$). Again, following administration of *Phyllantus 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 *Phyllantus amarus* treatment (Table 1).

However, when the subjects with *Phyllantus amarus* treatment were compared with the control group, all the parameters differed significantly ($p<0.05$) except the mean serum level of Alanine aminotransferase activity increased although statistically insignificant after the treatment with *Phyllantus amarus* ($p>0.05$) (Table 1).

Furthermore, comparing the parameters studied between the subject group without *Phyllantus amarus* treatment and control groups indicates significant changes in the mean serum activities of parameters studied ($p<0.05$). However, the mean serum activty of Aspartate aminotransferase did not differ significantly ($P>0.05$) (Table 1).

Table 1: Mean serum liver enzyme activity in alloxan induced diabetic rats with *Phyllanthus* treatment (A), without *Phyllanthus* treatment (B) and in control group (C) (Mean ±SD; n=10).

Group	ALT(U/L)	ALP (U/L)	AST(U/L)	WEIGHT(g)
A (n =10)	35.30±4.85	72.20±2.57	36.10±2.60	98.80±1.03
B (n= 10)	38.80±1.40	94.90±9.34	40.40±3.24	119.40±1.17
C (n=10)	35.60±3.80	40.10±15.72	38.30±1.77	40.10±15.72
F (P) –valve	2.822 (0.077)	66.688 (0.000)	6.806 (0.004)	150.000 (0.000)
A VB	>0.05	<0.05	<0.05	<0.05
A VC	>0.05	<0.05	<0.05	<0.05
B V C	<0.05	<0.05	>0.05	<0.05

KEY

F (P)-VALUE mean± SD of parameter compared among groups A, B, and C (using ANOVA) test.

A VB (P-value) mean± SD of parameter compared between group A and B using (t-test)

B VC (P-valve) mean± SD of parameter compared between group B and C using (t-test)

A VC (P-valve) mean± SD of parameter compared between group B and C using (t-test).

A VC (P-valve) mean± SD of parameter compared between group A and C using (t-test).

DISCUSSION

The liver is an organ of paramount importance not only for its metabolism of various xenobiotics and environmental pollutants (Pulok *et al.*, 2006) but for its unique and considerable regenerative capacity, even a moderate cell injury is not reflected by measurable change in its metabolic functions. However, some of its functions are so sensitive that abnormalities start appearing depending upon the nature and degree of its initial damage (Ibrahim *et al.*, 2008). A number of medicinal plants are used in traditional system of

medicine for the management of liver disorders. Nature has given us a large number of medicinal plants, some of which are yet to be explored and validated for their medicinal value. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver diseases, carefully synergizing the strengths of traditional medicine with the modern concept of evidence based medical evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy. Several herbs are known to possess antioxidant properties and may be useful as liver protective agents (Mccord, 1985).

The present study shows a significant reduction in the mean serum activities of Alkaline Phosphatase (72.20±2.57 vs 94.90±9.34; p<0.05) and Aspartate aminotransferase (36.10±2.60 vs 40.40±3.24; p=0.004) where as Alanine aminotransferase was also decreased although not very significant statistically (35.30±4.85 vs 38.80±1.40; p=0.077). This is in line with the report of Sugunabai *et al*. who investigated the protective effect of *centella asiatica* and *phyllanthus amarus* on ethanol induced hepatotoxicity in wistar rats and found that *Phyllanthus amarus* (300mg) treated group showed 144% of reduction in AST and 19.3% in ALT (Sugunabai *et al*., 2015). Syed *et al*. reported that In-vivo methanolic and aqueous extracts of the seeds of Phyllanthus amarus (250mg/kg) were found to have protective properties in rats with CCl4 induced liver damage and caused statistically significant decrease in all the above parameters (Syed *et al*., 2012).

Interestingly, Sule and Arhoghro, (2016) had earlier demonstrated the hepatoprotective effect of *P. amarus in* their study in which methanol extract of *P. amarus* leaves caused a significant decrease in the levels of alkaline and acid phosphatases, AST and ALT in a dose dependent manner. Other similar studies also did showed the hepatoprotective effect of *P. amarus* (Marchesini *et al.,* 2001; Pourmorad *et al*., 2007; Naaz *et al.,* 2007; Chidi *et al*., 2007; Pramyothin et al., 2007; Manjrekar *et al*., 2008; James *et al*., 2009; James *et al.,* 2010). These findings may be as a result of the hepatocytes effective and efficient functional conjucative mechanisms. 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). Since increase in these enzymes is related to hepatic disorders therefore their reduction shows that the leaves of *P. amarus* have hepato protective properties (Obianime and Uche, 2008). Again, the results may be due to the presence of Phyllanthin and hypophyllanthin in the plant which are chemicals that help in carrying out liver protecting activities (Chaudhury, 2007).

Furthermore, there was a significant reduction in mean weight of the subjects after *P. amarus* administration compared with those without *P. amarus* treatment (98.80±1.03 vs 119.40±1.17; p=0.000). This may suggest the diuretic property of the plant (Alanis *et al*., 2005). This could be of clinical importance in disease conditions where weight loss is of interest.

CONCLUSION

From the present study, we conclude that *Phyllantus amarus* have significant hepatoprotective as well as anti-obesity effects. Therefore, we recommend that *Phyllantus amarus* may be useful in the management of liver diseases as well as conditions involving obesity. However, further research should be carried out to unravel the full benefit and potential of this plant.

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