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EFFECT OF ETHANOIC LEAF EXTRACT OF PHYLLANTUS AMARUS ON FASTING BLOOD GLUCOSE, BILIRUBIN, ALBUMIN AND TOTAL PROTEIN ON DIABETIC INDUCED ALBINO WISTAR RATS IN COLLEGE OF HEALTH SCIENCES, 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 plasma glucose and serum total protein (TP), Albumin (ALB) and Bilirubin 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. 0.05mg/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 fluoride oxalate and plain containers for estimation of glucose and other biochemical parameters respectively. Plasma glucose and serum TP, ALB and Bilirubin were analyzed using standard methods. There was a significant decrease in the mean plasma glucose (96.10±5.20Vs193.70±54.03; p=0.000) and serum Direct bilirubin (2.52±0.11 Vs 2.62±0.06; p=0.038) whereas serum level of total bilirubin was significantly raised after PA administration (9.95±0.35 Vs 9.84±0.94; p=0.003). However, serum TP and Albumin levels did not differ significantly between pre and post– PA administration. This study revealed hypoglycaemic and hepatoprotective effect of PA use. 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). Therefore, PA use could be of importance in prevention and management of diabetes mellitus and liver disorders.

Keywords: Phyllantus amarus, total protein, albumin, total bilirubin, direct bilirubin.

Introduction

The use of plants as source of remedies for the treatment of diseases can be traced back to the prehistoric times(Lawrence and Bennett, 1995; Evans, 2009; Ankita *et al.*, 2012) and medicinal herbs are being increasingly studied by pharmacological researchers (Sinclair, 1998). Indian Ayureda medicine used herbs as early as 1900BC describing about 700 medicinal plants (Aggarwal *et al.*, 2007). Herbal medicine was also important from early days in Europe. Dioscroides, who became popular with natural remedies about 60AD, described over 600 plants and plants extracts (Carr, 1997). It was not until recently that more attention was drawn to these practices. According to the World Health Organization (WHO, 2002; 2013) more than 80% of the world's populations, rely on traditional medicine for their primary health care, majority of which use plants or their active principles (Gupta *et al*., 2005). The use of plant resource mainly for herbal medicine, food forage etc in Nigeria represent a long history of human interaction with the environment and their invitro and invivo properties to microbial pathogens have been widely reported (Hashish and Gomaa, 2003; Iwalokun *et al*., 2004). A wide range of medicinal plant parts like roots, stem, flower, fruit, twig exudates and modified plants organs has been used for extraction of raw drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005). These chemicals are termed as phyto-chemicals. Phyto-chemicals are bioactive non-nutrients plants compounds that have protective or disease preventive property. They confer plants with oder (terpenoids), pigmentation (tannins and quinines) and flavor (capsacin) (Mallikharjuna *et al.*, 2007) and are a part of palnts naturally defense system. These bioactive components said to be responsible for the antimicrobial effect of palnt extract invitro. They are grouped as flavonoids, glycocides, saponins , tannins, terpenoids, carbohydrate, and sterols. Many of these plants are used in African for the treatment of different disease of man and animals such as coccidiosis (Usman *et al*., 2011), diarrhea (Sodipo *et al*., 2005), tuberculosis (Ofukwu *et al*., 2008), Skin disease (Harsha *et al*., 2003), hyrperlipidaemia (La cour *et al.*, 1995), Salmonellosis (Geidam *et al*., 2007), Fever (Devi *et al*., 2003), dysentery (Hernandez *et al.*, 2003). Reports on various plants used in the treatment of diseases have been documented in Nigeria (Sofowora, 2008). Increased attention on ethnoveterinary medicine (EVM) is justified because it is accessible, easy to prepare and administered on little or no cost all (Jabbar *et al.*, 2005). These practices may be the only option in areas where conventional services are economically unavailable or cannot be effectively reached (Mathias and McCorkle, 2004). Herbal medicines are known to broad spectrums and therefore may be a future answer to pathogen resistance to conventional drugs (Mwale *et al.*, 2005). These have necessitated a search for new anti-microbial substances from other sources including plants (Erdogrul, 2002) Phyllanthus amarus (family: Euphorbiaceae) is widely distributed in all tropical and subtropical regions of the planet (Edeoga *et al.*, 2006) and Paleobotanical studies have not found the exact geographic origin of this plant. It is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993). In tropical Africa it occurs in most countries. In Nigeria, it is called "Oyomokeisoamank edem" in Efik, "Iyin Olobe" in Yoruba and "Ebebenizo" in Bini (Etta, 2008) P. amarus has various groups of compounds such as alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols. This plant is traditionally used around the world in the treatment of liver ailments and kidney stones as derived from the Spanish name 'chanca piedra' means "stone breaker or shatter stone." The alkaloid extract demonstrated smooth muscle relaxation specific to the urinary and biliary tract which the researchers surmised facilitates the expulsion of kidney and bladder calculi (Miller, 1998; Calixto, 1984). Again, 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 glucose, total protein, albumin and bilirubin levels 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±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 Phyllantus 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 0.05mg/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 glucose, Albumin, Total protein and Bilirubin by using standard

methods as described by (Bergmeyer and Bernt , 1974; Ryan and Chopra, 1976; Tietz,1987; Shogo *et al.*, 1988) 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. Level of significance was set at $p<0.05$.

Result

The serum levels of all parameters studied, (FBS, Albumin, Total protein and Direct Bilirubin) were statistically significant at $p<0.05$ respectively using ANOVA table. In this study, the serum levels of FBS, TB,DB and Albumin were statistically significant when compared between alloxan induced diabetic rats with Phyllanthus amarus treatment and those without Phyllanthus treatment at $p<0.05$ but serum levels of Albumin and total protein were statistically insignificant (Table 1).

When the alloxan induced diabetic rats with Phyllanthus amarus treatments were compared with the control group, all the parameters (FBS,Albumin, Total Protein, Total Bilirubin) except direct bilirubin showed a statistically significant difference (Table 1).

In the present study, the serum levels of FBS, TP and Direct Bilirubin were statistically significant when the alloxan induced diabetic rats without treatment were compared with the control group in contrast to the mean serum levels of Albumin and Total Bilirubin which were statistically insignificant at $p<0.05$ at (Figure 1).

Table 1: Mean ±SD of serum levels of fasting blood, Albumin, Total protein , Total bilirubin and Direct bilirubin in alloxan induced diabetic rats with phyllantus treatment (A), without Phyllanthus amarus treatment (B) and in control group (C).

Group	FBS	Albumin	Total protein	Total bilirubin	Direct bilirubin	Weight (kg)
A (n = 10)	96.10±5.20	27.300.61	52.02±0.65	9.95±0.35	2.52±0.11	98.80±1.03
B (n=10)	193.70±54.03	24.54±5.53	43.85±8.08	9.84±0.94	2.62±0.06	119.40±1.17
C (n= 10)	100.10±2.64	21.30±3.17	41.71±5.56	11.12±1.04	2.44±0.22	100.60±0.84
F (p) value	30.989 (0.000)	6.593 (0.005)	9.187. (0.003)	7.187 (0.003)	3.685 (0.038)	150.000 (0.000)
A v B	< 0.05	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05
A v C	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	< 0.05
B v C	< 0.05	> 0.05	< 0.05	> 0.05	< 0.05	< 0.05

Key:

F (p) –Value= Mean± SD of parameter compared among group A, B and C (using ANOVA).

A v B p-value = Mean ± SD of parameter compared between group A and B (using t-test).

A v C p- value = Mean ±SD of parameter compared between group A and C (using t-test)

B v C p-value = Mean ± SD of parameter compared between B and C using t –test)

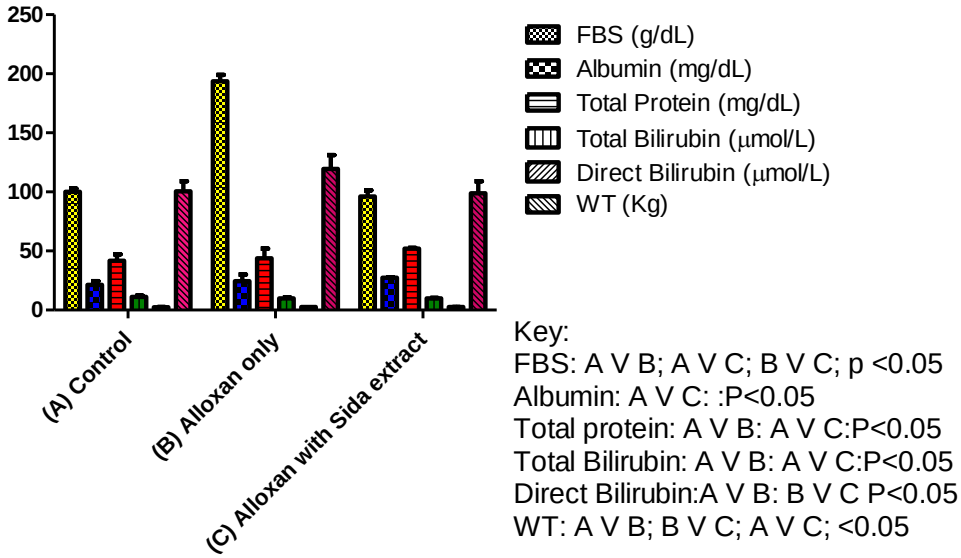

Fig 1: A multiple Bar chart showing mean ± SD serum levels of parameters studied in groups.

Discussion

The plants of Phyllanthus family seem to be a promising plant with great medicinal values in the management of diabetes, hyperlipidemia and related conditions (Igwe *et al.*, 2007; Calixto *et al*, 1998). In this study, Phyllanthus amarus adiminstration significantly reduced the serum glucose concentration in the Alloxan induced diabetic rats with Phyllantus treatment. This result confirms the report of Shetti *et al.,* (2012) in they investigated the antidiabetic effect of ethanolic leaf extract of P. amarus in alloxan induced diabetic mice and found that there was a significant decrease in mean serum glucose concentration in those mice treated with Phyllantus extract. Other similar studies in rats also did show the reducing effect of P amarus on blood glucose level (Shyamjith and Rao, 2013). In addition, the antihyperglycemic effect of P. amarus may also be due to the presence of hypoglycemic saponin and alkaloid (Sparg 2004; Sahu *et al.,* 2008). The decrease level of fasting blood sugar in the alloxan induced diabetic rats with P amarus treatment maybe as a result of its stimulation of insulin release from the pancreatic –beta-cells (Davis *et al* ., 2002; Nolte *et al* ; 2004).

The present study shows a significant increase in the mean serum levels of albumin, total protein and total bilirubin where as the mean serum level of direct bilirubin was significantly decreased following the treatment of the alloxan induced diabetic rats with Phyllanthus amarus leaf extract. This result is in concordance with the study of De peters and Omeodu, (2015)who

studied the effect of ethanolic leave extract of Phyllanthus amarus on carbon tetrachloride induced hepatotoxicity in albino rats . The study of Igwe *et al.,(* 2007) on the assessment of the hepatic effects, phytochemical and proximate compositions of P amarus also agrees with the findings therein. This may be due to its ability to enhance the conjugation function of the liver.

Conclusion

From the current study, Phyllantus amarus has a significant antihyperglycemic and hepatoprotective effects. Therefore, Phyllantus amarus can useful, at least as an adjunct, in the therapy of diabetes and liver disorders.

Recommendations

Based on our findings we recommend that Phyllantus amarus may be useful in the management of diabetes mellitus as well as liver diseases. However, further research should be carried out to unravel the full benefit and potential of this plant.

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AUTHORS' CONTRIBUTIONS

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