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Combined Effect of Aqueous Extracts of *Phyllathus amarus* and *Vitex doniana* Stem Bark on Blood Glucose of Streptozotocin (STZ) Induced Diabetes Rats and Some Liver Biochemical Parameters

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Abstract: The effects of aqueous extract of *Phyllathus amarus* and *Vitex doniana* stem bark on blood glucose of Streptozotocin (STZ) induced diabetes rats and some liver biochemical parameters were investigated. Study was conducted with 36 albino rats (wistar strain). Thirty- six rats (30 diabetic and 6 non-diabetic) were assigned into six groups of 6 rats each. Daily administration of the extracts for 21 days was done. Group 1 were the control animals, and group two diabetic control, while those of group 3, 4 and 5 were administered (100 mg/kg body weight extracts of *Phyllanthus amarus*, *Vitex doniana* and combination of the two respectively, group 6 was injected with insulin (5 units/kg) as a standard drug. Significantly ($p<0.05$) lower fasting blood glucose relative to their initial values at the end of treatment were observed for all treated group compared to normal and diabetic control. Similarly fasting blood glucose respectively decrease by 46.53, 74.46, 37.31 and 66.6% for *Phyllanthus amarus*, *Vitex doniana*, combination of *Phyllanthus* and *Vitex doniana* and insulin groups respectively relative to the diabetic control value of 525.50±77.93 mg/dL. The extent of decrease of fasting blood glucose in the *Vitex doniana* extracts is significantly ($p<0.05$) higher than the other extracts treated groups. The extracts significantly ($p<0.05$) lowered the serum activities of marker enzymes; ALT, AST and ALP. There was positive significant ($p<0.05$) correlation between serum activities of marker enzymes and fasting serum glucose (R = 0.85 for ALT, R = 0.75 for AST and 0.68 for ALP). Result of total *bilirubin* and total protein shows that non-treated diabetic group was significantly ($p<0.05$) higher in total bilirubin compared with treated group. While non-treated diabetic group, showed significant ($p<0.05$) decreased in total protein compared with the normal and the treated groups. These results suggest that the use of aqueous extract of these plants and their combination in the treatment of diabetes produce a significant ($p<0.05$) antidiabetic and hepatoprotective effect.

Key words: Aqueous extracts, albio rats, diabetes, hepatoprotective, *Phyllathus amarus*, polytherapy, *Vitex doniana*

INTRODUCTION

Medicinal plants formed the basis of health care throughout the world (Ahmad *et al*., 2006). About 90% of the African population still relies exclusively on plants as a source of medicine (Hostettmann *et al*., 2000). The World Health Organisation (WHO) had in one of its charters in Geneva recommended further investigation into this area, particularly as it concerns chronic diseases such as diabetes mellitus (WHO, 1998).

In diabetes mellitus where little attention is given to its prevention and curation , but rather management, there is a need to focus on plants in the search for appropriate hypoglycemic/antihyperglycemic agents. The reason among others is that the plants secondary metabolic contain a variety of herbal and non herbal ingredients that are thought to act on variety of targets by various modes and mechanism given the multi-factorial pathogenicity of the disorder (Tiwari and Rao, 2002). Moreover, polyherbal therapies the combination of various types of agents from different plant sources, can be used to enhance efficacy. According to Tiwari and Rao (2002) polyherbal therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves, that study together in a dynamic way to produce therapeutic efficacy with minimum side effects.

It is in this light that this work was designed to investigate the antidiabetic efficacy of combined extracts from *Phyllanthus amarus* and *Vitex doniana* used in traditional medicine.

Phyllanthus amarus is a plant of family Euphorbiaeace found in many countries including Philippines, Cuba, it is commonly call ‘carry me seed’; wind ‘breaker’, ‘gulf flower’ or ‘gala wood’ (Chidi *et al*., 2007), use traditionally to treat many ailments such as

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bowel inflammation, constipation, diabetes, digestion stimulation, dysentery, fever, gonorrhea, jaundice, intestinal gas, kidney ailments and so on.

On the other hand *Vitex doniana* is a savanna species in wooded grassland and can also be found along forest edges. It can be found throughout tropical Africa (Ruffo *et al*., 2002), from information made available from indigenous traditional healers in Nigeria, a decoction of the chopped stem bark part of *V. doniana* is prepared and taken orally for treatment of gastroenteritis, diarrhoea and dysentery. Earlier reports have shown that the plants extracts can be used for medicinal purposes like treatment of anaemia, gonorrhea (root), dysentery, jaundice, liver disorder and leprosy (Babalola, 1993; James *et al*., 2010).

Since hyperglycemia increases the generation of free radicals by glucose auto-oxidation and this increment of free radicals usually would lead to organ damage (Kim *et al*., 2006), In this study, we evaluated the combined effect of aqueous extracts of *Phyllathus amarus* and *Vitex doniana* stem bark on blood glucose of Streptozotocin (STZ) induced diabetes rats and some liver biochemical parameters

MATERIALS AND METHODS

Plant materials: This study was conducted in September, 2010 in Biochemistry Department, Ahmadu Bello University, Samaru, Zaria. The plant sample under study was collected around the garden surrounding Ahmadu Bello University, Samaru Zaria, Kaduna State Nigeria. The collected plants was taken to the herbarium at the Department of Biological Sciences Ahmadu Bello University Zaria for identification

Animals: Albino Rats (120-200 g) of both sexes were purchased from the Animal House at Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) in Kaduna State. The animals were harboured in stainless steel cages under standard laboratory condition of 12 hours light/dark cycle and were allowed to adjust to the laboratory environment for the period of 2 weeks before the commencement of the experiment. They had access to feed (grower's mash) and water *ad libitum*

Preparation of plants: The collected plants were rinsed in clean water and dry at room temperature for two weeks. The dry plants sample was ground into powder using Laboratory Mills. 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 48 h. After the set time, suspension were filtered and filterates were then concentrated in a crucible using a water bath set at 45ºC and the weight of sample taken. The concentrated extracts were then stored in a refrigerator until required for analysis

Lethal dose at 50 (LD_{50}): Lethal dose 50 test involves the administration of a substance to a group of animals at increasing doses in order to determine the dose that kills 50% of the test subjects within a set time frame. Administration of *Phyllanthus amarus* and stem barks of *Vitex doniana* were orally. The animal used for LD_{50} was grouped into 3 phases. All the phases had 3 groups with 3 animals in each group.

Induction of diabetes: The rats were fasted for 12 h (overnight) and diabetes was induced by a single intraperitoneal administration of freshly prepared streptozotocin which was dissolved in 0.05m citrates buffer of pH 4.5.

The prepared streptozotocin (STZ) at a dose of 55 mg/kg body weight was use for the induction. Diabetes was confirmed after second day of administration by polydipsia, polyuria and by measuring fasting blood glucose concentration, using commercial glucose strip (accu-Check glucometer) only animals with fasting blood glucose level of 200 mg/dL and above was considered diabetes and used for the experiment.

Animal grouping: The animals were grouped into six groups of six animals each for the sub-chronic studies.

Group 1: Control animals given water and feed only
Group 2: Diabetic animals given water and feed on
Group 3: Diabetic animals given water, feed and 100 mg/Kg body weight of *phyllathus amarus* extract
Group 4: Diabetic animals given water, feed and 100 mg/Kg body weight of *Vitex doniana* extract
Group 5: Diabetic animals given water, feed and 100 mg/Kg body weight of both polyherbal (1:1 *phyllathus amarus* and *vitex doniana* extract)
Group 6: Diabetic animals given water, feed and 5 units/Kg body weight of rat insulin as a standard

Sub-chronic studies: The extracts groups were given oral doses of 100 mg/Kg body weight for 21days, at the end of 21 days the animals fasting blood glucose of the animals were taken, the animals were weighed, anaesthetised using chloroform and bled by cardiac puncture. The blood sample were collected in specimen bottle for determination of some biochemical parameters. Serum transaminases (ALT and AST) were determined by method of Reitman and Frankel (1957). Alkaline phosphatise was by Haussament (1977), total bilirubin

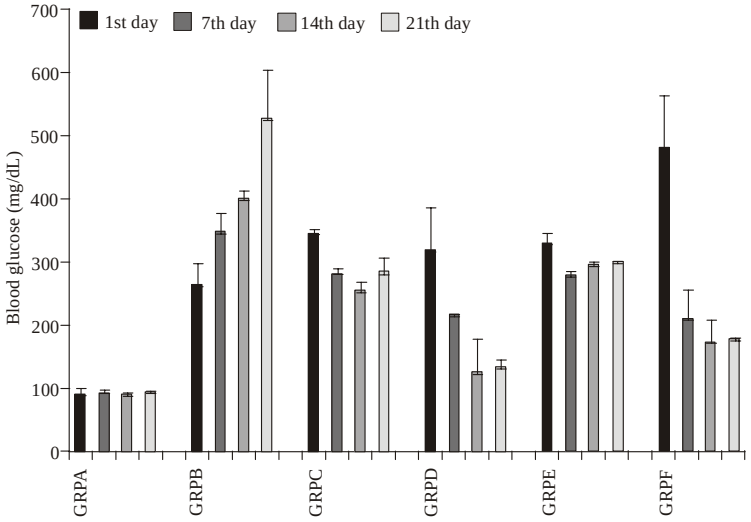

Fig. 1: Effect of aqueous extract *of Phyllanthus amarus* and *Vitex doniana* on streptozotocin induced diabetes

(TB) and direct bilirubin (DB) were by method of acid diazo method, of Doumas *et al*. (1973), indirect bilirubin was calculated by substracting the DB from TB total protein determination by Biuret method of Gornall *et al*. (1949).

RESULTS AND DISCUSSION

Results expressed in Fig. 1 revealed antidiabetic potential of the different extracts tested. All the animals in treated groups produced significant ($p<0.05$) reduction on first week, second week and third week of the experiments compared with the untreated diabetic control, the normal control group shows no significant ($p<0.05$) change in the blood glucose level for the period of 21 days.

The serum concentration of the marker enzymes ALT, AST and ALP is presented in Table 1. Animals in extracts treated groups show significantly ($p<0.05$) lower ALT compared with untreated diabetic group. There was no significant ($p>0.05$) difference in ALT of animals in extract treated group and insulin treated group.

The level of ALT for animals treated diabetic groups were significantly ($p<0.05$) lower compared with diabetic non treated groups, there was no significant ($p>0.05$) difference in AST between the treated group and normal control groups.

The strong positive significant ($p<0.05$) correlation study between fasting blood glucose and marker enzymes is shown in Fig. 2, of all the marker enzyme ALT has the highest correlation study. Significantly ($p<0.05$) lower ALP value was observe for all treated group compared with non treated diabetic groups while significantly ($p<0.05$) higher value was observed in animals treated with *Vitex doniana* bark extracts and insulin when compared with animals in control group. There was positive significant ($p<0.05$) correlation between the hyperglycaemia level and the marker enzyme with ALT value higher than AST and ALP values (Fig. 2).

Table 1: Effect of combined aqueous extracts of *phyllanthus amarus* plants and *vitex doniana* stem bark on hepatic marker enzymes of *streptozotocin* induced diabetic rats

Animals group	(ALT) (U/I)	AST (U/I)	ALP (U/I)
Group A	2.65±0.50a	9.83±0.75ab	103.15±17.60a
Group B	16.18±2.10c	23.55±4.47c	287.98±1.58d
Group C	6.84±3.39b	11.08±0.55b	155.63±6.29abc
Group D	7.22±3.08b	9.30±1.19ab	208.03±20.33c
Group E	7.20±2.87b	9.68±1.36ab	203.03±11.85bc
Group F	4.05±1.63ab	6.43±1.77a	146.88±65.80ab

Values are mean of four determination ± SD
Values with different letter in a row differ significantly ($p<0.05$)

Result of total bilirubin and total protein is presented in Table 2 non treated diabetic group is significantly ($p<0.05$) higher in total bilirubin compared with non-diabetic control and animals in treated groups. Animals in non-diabetic control groups show no significantly ($p<0.05$) difference when compared with the animals in treated group. Significantly ($p<0.05$) lowered total protein was observed with animals in non-treated diabetic group compared with normal and treated groups, the treated groups are significantly ($p<0.05$) higher in total protein compared with the normal control group.

Results of the present study establish a scientific basis for the utility of the two plants. The *Vitex doniana* and the *Phyllanthus amarus* in the treatment of diabetes. The possible mechanism by which the drug brings about its hypoglycemic action may be either by potentiating the insulin effect or by increasing the pancreatic secretions of insulin from the cells of islets of langerhan's. This may be

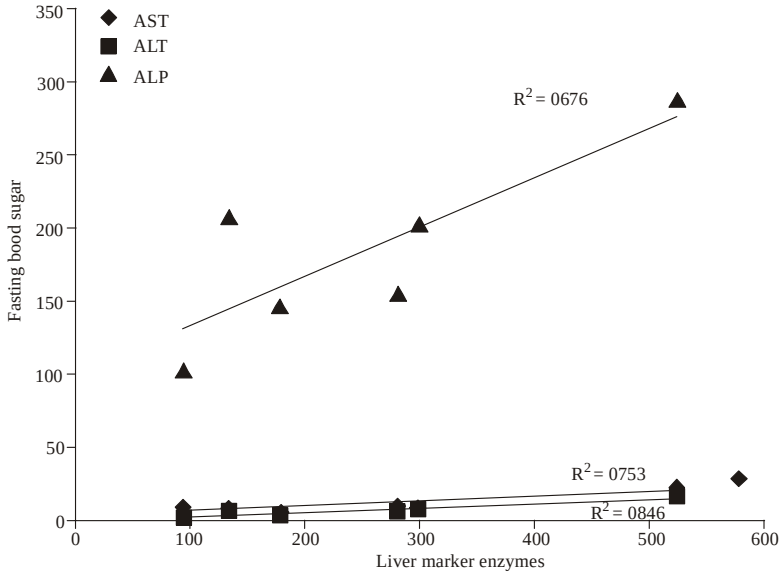

Fig. 2: Correlation between fasting blood sugar and liver marker enzymes in rats treated with *Phyllanthus amaru* and *Vitex doniana* stem bark extracts

Table 2: Effect of combined aqueous extracts of *phyllanthus amarus* plant and *vitex doniana* stem bark on some biochemical parameters of *streptozotocin* induced diabetic rats

Animal groups	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)	Unconjugated bilirubin (mg/dL)	Total protein (g/100 mL)
Group A	0.26±0.14a	0.14±0.03a	0.17±0.13a	5.09±0.220b
Group B	1.03±0.29b	0.46±0.04c	0.56±0.31b	3.49±0.52a
Group C	0.31±0.15a	0.25±0.03b	0.11±0.12a	7.84±1.14c
Group D	0.29±0.11a	0.28±0.02b	0.08±0.05a	6.86±0.55c
Group E	0.28±0.05a	0.28±0.02b	0.05±0.02a	6.94±0.60c
Group F	0.34±0.12a	0.24±0.01b	0.18±0.05a	7.03±0.19c

Values are mean of four determination ± SD; Values with different letter in a row differ significantly ($p<0.05$)

due to the presence of various constituents like saponins, flavonoids and tannins. In many studies the hyperglecemic effects of these agents is already well documented. They have been reported to potentiate plasma insulin effect by increasing the pancreatic secrection of insulin from cells of islets of langerhan's (Achrekar *et al*., 1991; Yanardag and Colak, 1998; Pari *et al*., 2001) and/or may increase peripheral uptake of glucose (Bajaj and Srinivasan, 1999). Significant ($p<0.05$) decrease in blood glucose level of serum show by the polyherbal formulations extracts compared with the *Vitex doniana* effect of the two extracts shows that the Vitex doniana exert more hypoglycaemic than polyherbal formulation.

The present result also shows that injection of steptozotocin induces hepatocellular damage, which is one of the characteristic changes in diabetes as evidenced by high serum levels of AST, ALT and ALP in untreated diabetic group. These increases may be due to the leakage of these enzymes from the liver cytosol into the blood stream and change in the permeability of liver membranes. The liver releases of aminotransferase; ALT and AST together with ALP is an indication of liver damage caused by exposure to toxic substances (Ranjna, 1999).

AST and ALP are not specific to the liver only but are also located in organs like heart, brain, kidney and skeletal muscle. ALT is more liver specific enzyme for diagnostic use, when the intergrity 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 extracts treated groups suggest that the extracts are hepatoprotective against streptozotocin induced hepatocellular damaged.

The strong positive significant ($p<0.05$) correlation study between fasting blood glucose and marker enzyme shows that Hyperglycaemia is responsible for liver damage during diabetes especially as evidence by significant ($p<0.05$) higher correlation in ALT.

The result also shows that the extracts has the ability to enhance the bilirubin conjugating and protein synthesizing function of the liver as observed by the decrease in blood total bilirubin as well as an increased in blood protein concentration of the treated animals.

In it can be concluded that both plants and its combination exerts a significant ($p<0.05$) antidiabetic and hepatoprotective effect, however it cannot be concluded that combination of the two plants may have synergistic or additive effect, although further studies remain to be concluded to investigate this hypothesis , the herbal formulation can be considered as safe supplementary therapy for a long time management of diabetics patients.

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REFERENCES

Achrekar, S., G.S. Kaklij, M.S. Pote and S.S. Kelkar, 1991. Hypoglycemic activity of Eugenia jambolana and Ficus benalensis: Mechanism of action. *In vivo*, 5: 143-147.

Ahmad, I., F. Agil and M. Owais, 2006. Modern Phytomedicine: Turning Medicinal Plants into Drugs. West-Sussex England: John Wiley and Sons, pp: 2-24.

Babalola, E.O., 1993. The persistence of African Traditional medicine in contemporary Nigeria Society. African Marburgensia, 26: 4.

Bajaj, S. and B.P. Srinivasan, 1999. Investigations in to the anti-diabetic activity of Azadirachts indica. India J. Pharmacol., 31: 138-141

Chidi, U.I., A.N. Linus and O.U. Cosmas, 2007. Assesment of the hepatic effect, phytochemical and proximate composition of Phyllanthus amarus. Afr. J. Biotechnol., 6(6): 728-731.

Doumas. B.T., B.W. Perry, E.A. Sasse and J.V. Straumfjord, 1973. Standardization in bilirubin assays: Evaluation of selected methods and stability of bilirubin solutions. Clin. Chem., 19: 984-993.

Gornall, A.G., C.S. Bardwill and M.M. David, 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem., 177: 751-766.

Haussament, T.U., 1977. Qualitative determination of serum alkaline phosphate Clin. Chem. Acta, 35: 271-273.

Hostettmann, K., A. Marston, K. Ndjoko and J. Wolfender, 2000. The potential of african plants as a source of drug. Curr. Org. Chem., 4: 973-1010.

James, D.B., O.A. Owolabi, M. Bisalla and H. Jassium, 2010. Effects of aqueous extracts (leaves and stem) of vitex doniana on carbon tetrachloride induced liver injury in rats. Br. J. Pharmacol. Toxicol., 1(1): 1-5.

Kim, J.S., J.B. Ju., C.W. Choi and S.C. Kim, 2006. Hypoglycemic and antihyperglycemic effect of four korean medicinal plants in alloxan induced diabetic rats. Am. J. Biochem. Biotech., 2(4): 154-160.

Moss, D.W and A.R. Henderson, 1996. Enzymes, In: Tietz, N.W., (Ed.), Tietz Fundamental of Clinical Chemisty. 4th Edn, W.B Sounders Company Philadalphia, pp: 283-335.

Pari, L., R. Ramakrishnan and S. Venkateshwaran, 2001. Antihyperglycaemic effect of Diamed, a herbal formulation, in experimrntal diabetes in rats. J. Pharm. Pharmacol., 53: 1139-1143.

Ranjna, C., 1999. Practical Clinical Biochemistry Methods and Interpretation. 2nd Edn.,

Jaypee Brother Medical Publishers (P) Ltd., Delhi, India, November, pp: 117.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Path., 28: 56-63.

Ruffo, C.K., A. Birnie and B. Tengas, 2002. Edible Wild Plants of Tanzania. Relma Technical Handbook Series 27. Nairobi, Kenya, pp: 766.

Tiwari, A.K and J.M. Rao, 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr. Sci., 83(1): 30-37.

World Health Organization (WHO), 1998. Second report of the WHO Expert Committee on DiabetesM ellitus. Technical Report Series 646, Geneva, pp: 66.

Yanardag, R and H. Colak, 1998. Effect of chard (*Beta vulgaris* L. *var. cicla*) on blood glucose levels in normal and alloxan –induced diabetic rabbits. Pharm. Pharmacol. Commum., 4: 309-311.