

## Change in Organs Weight and Antioxidant Potential of Combined Effects of Aqueous Extracts of *Phyllanthus amarus* and *Vitex doniana* Stem Bark on Streptozotocin-Induced Diabetic Rats

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**Abstract:** *Phyllanthus amarus* and *Vitex doniana* are used folklore plants in several disorders due to their excellent properties and potent phyto-constituents. In this study, change in organs weight and antioxidant potential of combined effects of aqueous extracts of *Phyllanthus amarus* and *Vitex doniana* stem bark on streptozotocin - induced diabetic rats was investigated. In a 21 days study, animals were divided into six groups (A- F) of six rats each. Group A served as normal control, group B as diabetic control, Groups C, D and E were administered with three different plant extracts (100 mg/kg of *Phyllanthus amarus*, *Vitex doniana* and combination of the two plants respectively, group F was treated with standard drug insulin (5 units/kg). Administration of *Vitex doniana* extracts and *Phyllanthus amarus* and their combination significantly ( $p < 0.05$ ) decrease fasting blood glucose. Significant ( $p < 0.05$ ) increase in weight gain for animals in treated groups when compared with diabetic group was observed. Treated groups significantly ( $p < 0.05$ ) decrease levels of thiobarbituric acid (TBARS), while catalase and Superoxide Dismutase (SOD) in liver and kidney were significantly ( $p < 0.05$ ) increase when compared with diabetic control group. *Phyllanthus amarus* treated groups shows significantly ( $p < 0.05$ ) higher SOD in the liver and kidney when compared with standard drug. While kidney catalase was significantly ( $p < 0.05$ ) higher in the groups treated by *Vitex doniana* and *Phyllanthus amarus* compared with other treated group. These results suggest that the use of aqueous extract of these plants and their combination possess antidiabetic and antioxidant activity, which could exert a beneficial action against the disease associated with free-radicals complications.

**Key words:** Antioxidant, aqueous extracts, albino rats, catalase, diabetes, polytherapy, *Phyllanthus amarus*, superoxide dismutase, *Vitex doniana*

### INTRODUCTION

Diabetes Mellitus (DM) is a complex and chronic disease associated with a myriad of debilitating complications, causes of which are usually multi-factorial. The lesions in the patho-physiology of diabetes are multiple and therefore would require more than a single drug agent to reverse all or majority of the aspects of the disease. The effective therapeutic approach should be multimodal and in this light, several traditional medicinal herbs have been preferred given the plethora of active ingredients present in a single herb (Tiwari and Rao, 2002; Atangwho *et al.*, 2009).

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. The use of plant derived natural compounds as part of herbal preparations for alternate source of medicament continues to play major roles in chemotherapy especially in third world countries

(Joy *et al.*, 1998). Several studies carried out have shown that traditional medicines could provide better glycaemic control than currently used conventional drugs (Rates, 2001; Roja and Rao, 2000). Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate a disease condition by acting on a variety of targets (various modes and mechanisms) in the host organism. On the basis of the above, polyherbal therapy is considered the preferred therapeutic approach to management of diabetes mellitus given its multi-factorial pathogenicity (Tiwari and Rao, 2002; Ebong *et al.*, 2008).

The maximum therapeutic efficacy with minimum side effects (Ebong *et al.*, 2008) of polyherbal therapy were thought to be derive from phytochemicals, Such phytochemicals include tea polyphenols which suppress Post-Prandial Hyperglycaemia and glucose transport across the small intestine (Yoshikawa *et al.*, 1999) and saponins which delay glucose transfer from the stomach to the small intestine (Yuan *et al.*, 1998; Chattopadhyay, 1998). Epicatechin has a restorative effect on pancreatic

$\beta$ -cells against alloxan damage (Chakravathy *et al.*, 1982), and plant flavonoids which exert their antidiabetic activity via antioxidant properties (Bnouham *et al.*, 2006). These reports have accelerated the global efforts to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes related complications (Tiwari and Rao, 2002).

The maximum number of drugs so far combined as trial in conventional therapy is three: glyburide, metformin and thiazolidinedione, or glyburide, metformin and alpha-glucosidase inhibitor (Luna and Fienglos, 2001). But more than three agents may be present in a medicinal herb with a variety of intervention targets via various mechanisms of action. Besides, polyherbal formulations (Singh, 2005) have proved more useful and beneficial in the management of various ailments including those that seem to defile conventional medication (Ebong *et al.*, 2008). This concept originated from Ayurvedic medicine (Singh, 2005) but is today, beginning to gain acceptance as an effective therapeutic approach in sourcing medicament for most degenerative ailments

The herbs for this study are selected based on the disease and have several advantages over monotherapies, and according to Tiwari and Rao (2002) seems to have reduced toxicity and side effects and synergistic therapeutic efficacy. Some investigators have equally demonstrated and reported these relative advantages (Singh, 2005; Arjuman *et al.*, 2007; Shah *et al.*, 2006).

In our laboratory we have demonstrated a hepatoprotective effect with the aqueous extracts from *Phyllanthus amarus* and *Vitex doniana*. Under the prevailing circumstance investigation into the concept of polyherbal therapy is warranted. Polyherbal therapy allows for combination of secondary metabolites and potentiation of biological effects. Therefore, an attempt to establish their hypoglycaemic and antioxidant effect for possible development into anti-diabetic drugs. It is in the light of these that we evaluated antioxidant potential of combined effects of aqueous extracts of *Phyllanthus amarus* and *Vitex doniana* stem bark on streptozotocin-induced diabetic rats

## 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 45°C and the weight of sample taken. The concentrated extracts were then stored in a refrigerator until required for analysis

**Lethal dose at 50 (LD<sub>50</sub>):** 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<sub>50</sub> 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 used 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 A:** Control animals given water and feed only  
**Group B:** Diabetic animals given water and feed only  
**Group C:** Diabetic animals given water, feed and 100 mg/Kg body weight of *Phyllanthus amarus* extract

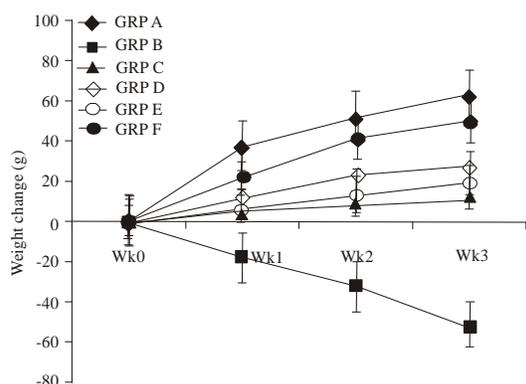


Fig. 1: Effect of combined aqueous extracts of *Phyllanthus amarus* plant and *Vitex doniana* stem bark on change in rats body weights of *Streptozotocin* induced diabetic rats

Table 1: Effect of combined aqueous extracts of *Phyllanthus amarus* plant and *Vitex doniana* stem bark on Blood glucose levels in streptozotocin-induced diabetic rats

Group	Blood glucose level (mg/dL)	
	Initial FBG	Final FBG
A. Normal control	90.40±10.85 <sup>a</sup>	93.30±3.51 <sup>a</sup>
B. Diabetic control	262.40±35.50 <sup>a</sup>	525.50±77.93 <sup>b</sup>
C. <i>Phyllanthus amarus</i>	344.60±08.23 <sup>b</sup>	281.00±25.46 <sup>a</sup>
D. <i>Vitex doniana</i>	317.00±68.86 <sup>b</sup>	133.00±12.73 <sup>a</sup>
E. Combined extract	329.40±15.74 <sup>b</sup>	299.00±1.41 <sup>a</sup>
F. Insulin	481.40±82.41 <sup>b</sup>	178.00±1.00 <sup>a</sup>

Values are mean ± SD of four rats per groups; Values with different superscripts across the row differ significantly (p<0.05).

**Group D:** Diabetic animals given water, feed and 100 mg/Kg body weight of *Vitex doniana* extract

**Group E:** Diabetic animals given water, feed and 100mg/Kg body weight of both polyherbal (1:1 *Phyllanthus amarus* and *Vitex doniana* extract).

**Group F:** 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 21 days, 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 while Liver and kidney were removed and placed in ice-cold containers and used for various biochemical estimations. Thiobarbituric Acid Reactive Substance (TBARS) in tissues was estimated by the method of Fraga *et al.* (1988). Superoxide dismutase activity assay was carried out according to the method described by Martin *et al.* (1987) and catalase by method.

**Statistical analysis:** The results were analysed for statistical significance by one-way ANOVA using the SPSS statistical program and Post Hoc Test (LSD) between groups using MS excel program. All data were expressed as Mean±SEM. p-values <0.01 and 0.05 were considered significant.

## RESULTS AND DISCUSSION

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

Percentage change in organs weight is presented in Table 1, significant (p<0.05) increase in percentage change in liver and kidney with non significant (p>0.05) increase in spleen was observed for animals in diabetic untreated group compared with the animals in normal control. Diabetic treated groups shows significantly (p<0.05) lower percentage change in organs weight compared with diabetic untreated groups. Figure 1 shows significant (p<0.05) decrease in weight change for animals in untreated diabetic group compared with that normal and treated diabetic group.

The antioxidant potential is presented in Table 3 and 4. There was a significant (p<0.05) decrease in catalase (CAT) and superoxide dismutase (SOD) with significant (p<0.05) increase in thiobarbituric acids level (TBARS) in the liver and kidney of untreated diabetic rats when compared with animals in normal control group and diabetic treated groups.

Animals in *Phyllanthus amarus* and *Vitex doniana* treated groups shows significantly (p<0.05) higher catalase in kidney when compared with other treated diabetic groups. Superoxide dismutase in the liver was observed to be significantly (p<0.05) higher in animal treated with *Phyllanthus amarus*, while TBARS was significantly (p<0.05) reduced in the animals treated with *Vitex doniana* in both liver and kidney.

The treated groups increase glucose metabolism and thus enhance body weight in STZ-induced diabetic rats to certain extent. However, it did not normalize the body weight completely as it remained lesser than normal control rats. The decrease in body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source (Chen and Ianuzzo, 1982). The possible mechanism by which the extracts brings about decrease in blood glucose may be by potentiation of the insulin effect by increasing either the pancreatic secretion of insulin from  $\beta$  cells of islets of langerhans or its responsiveness (Padmini and Chakrabati, 1982).

Table 2: Effect of combined aqueous extracts of *Phyllanthus amarus* plant and *Vitex doniana* stem bark on the percentage change in organs weight of *Streptozotocin* induced diabetic rats

Parameters (% change)	Group A Normal	Group B diabetic control	Group C phyllanthus amarus	Group D vitex doniana	Group E vitex doniana +phyllanthus amarus	Group F Insulin
Body	62.20±10.28 <sup>c</sup>	-53.00±7.75 <sup>a</sup>	12.40±4.88 <sup>b</sup>	27.25±8.73 <sup>c</sup>	21.00±10.44 <sup>c</sup>	49.50±11.30 <sup>c</sup>
Liver	3.99±0.25 <sup>a</sup>	6.96±0.39 <sup>d</sup>	4.10±0.04 <sup>ab</sup>	3.96±0.09 <sup>a</sup>	5.26±1.10 <sup>c</sup>	4.75±0.07 <sup>bc</sup>
Kidney	1.27±0.06 <sup>a</sup>	1.99±0.15 <sup>b</sup>	1.18±0.08 <sup>a</sup>	1.23±0.03 <sup>a</sup>	1.20±0.38 <sup>a</sup>	1.16±0.04 <sup>a</sup>
Spleen	0.57±0.05 <sup>ab</sup>	0.69±0.21 <sup>b</sup>	0.34±0.05 <sup>a</sup>	0.48±0.05 <sup>a</sup>	0.40±0.10 <sup>a</sup>	0.47±0.06 <sup>a</sup>

Values are mean of four determination±SD.; Values with different superscript across the row differ significantly (p<0.05)

Table 3: Effect of combined aqueous extracts of *Phyllanthus amarus* plant and *Vitex doniana* stem bark on anti-oxidant enzyme in liver of *Streptozotocin* induced diabetic rats

Group	Catalase (μmol/min)	SOD (u/mL)	TBARS (nmole/mg) protein)
A. Normal control	66.25±1.77 <sup>d</sup>	87.05±3.154 <sup>e</sup>	0.700±0.150 <sup>a</sup>
B. Diabetic contro	123.75±1.77 <sup>a</sup>	34.86±5.09 <sup>a</sup>	3.10±0.150 <sup>a</sup>
C. <i>Phyllanthus amarus</i>	52.50±3.54 <sup>bc</sup>	76.61±0.740 <sup>d</sup>	1.67±0.010 <sup>c</sup>
D. <i>Vitex doniana</i>	58.75±8.84 <sup>cd</sup>	66.07±7.58 <sup>cd</sup>	1.37±0.050 <sup>b</sup>
E. Combined extract	43.75±1.77 <sup>b</sup>	59.66±4.02 <sup>bc</sup>	2.04±0.050 <sup>d</sup>
F. Insulin	50.00±3.53 <sup>bc</sup>	51.21±1.71 <sup>b</sup>	1.69±0.066 <sup>c</sup>

Values are mean±SD of four determinations; Values with different superscripts down column differ significantly (p<0.05)

Table 4: Effect of combined aqueous extracts of *Phyllanthus amarus* plant and *Vitex doniana* stem bark on anti-oxidant enzyme in kidney of *streptozotocin* induced diabetic rats

Group	Catalase (μmol/min)	SOD (u/mL)	TBARS (nmole/mg protein)
A. Normal control	72.50±3.54 <sup>c</sup>	79.93±2.55 <sup>c</sup>	0.664±0.079 <sup>a</sup>
B. Diabetic control	26.25±1.77 <sup>a</sup>	37.95±9.47 <sup>a</sup>	3.36±0.75 <sup>e</sup>
C. <i>Phyllanthus amarus</i>	66.25±1.77 <sup>c</sup>	74.38±2.42 <sup>c</sup>	1.69±0.070 <sup>bc</sup>
D. <i>Vitex doniana</i>	65.00±14.14 <sup>c</sup>	69.83±5.71 <sup>bc</sup>	1.45±0.033 <sup>b</sup>
E. Combined extract	43.75±5.30 <sup>b</sup>	59.77±1.34 <sup>b</sup>	2.04±0.160 <sup>d</sup>
F. Insulin	46.25±1.76 <sup>b</sup>	58.60±5.52 <sup>b</sup>	1.80±0.146 <sup>cd</sup>

Values are mean±SD of four determinations: Values with different superscripts down the column differ significantly (p<0.05)

Hyperglycemia results in free radical formation through various biochemical reactions. Free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radical produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn results in elevated production of free radicals (Lery *et al.*, 1999). Lipid peroxide mediated tissue damage has been observed in the development of diabetes. It has been observed that insulin secretion is closely associated with lipoxigenase-derived peroxides (Walsh and Pek, 1974). The increased lipid peroxidation in the diabetic animals may be due to the observed remarkable increase in the concentration of TBARS in the liver and kidney of diabetic rats (Stanely *et al.*, 2001).

In the present study, TBARS level in liver and kidney were significantly lower in the treated groups compared to the diabetic control group. The above result suggests that treatment with insulin and extracts may exert antioxidant activities and protect the tissues from lipid peroxidation while the significant (p>0.05) decrease observed with animals treated with extract of *Vitex doniana* in both liver and kidney suggests the significant (p<0.05) reduction of hyperglycaemic effect of that extract, the result in Table 3, 4 shows that the extract significantly (p<0.05) reduce blood glucose level.

Superoxide dismutase is one of the most important enzymes in the enzymatic antioxidant defense system

which catalyses the dismutation of superoxide radicals to produce H<sub>2</sub>O<sub>2</sub> and molecular oxygen (Mc Crod *et al.*, 1976), hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H<sub>2</sub>O<sub>2</sub> or by glycation of enzymes (Sozmen *et al.*, 2001). The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide (Chance *et al.*, 1952). Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase. Catalase (CAT) catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Searle and Wilson, 1980).

This decrease in CAT activity could result from inactivation by glycation of enzyme (Yan and Harding, 1997). Reduced activities SOD and CAT in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides (Wohaieb and Godin, 1987). The reductions of hepatic SOD and CAT activities in STZ - induced diabetic rats when compared with normal rats were reported (Saxena *et al.*, 1993) Whereas, the treated groups showed a significant increase in the liver and kidney SOD and CAT activities of the diabetic rats. This means that the treatments can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. In the

extract treated group *Vitex doniana* significantly increase the level of catalase while *Phyllanthus amarus* significantly increase superoxide dismutase compared with the combination of both extracts and that of insulin this suggest that the two extracts my reduce potential glycation of enzymes more than its combination and the insulin and thus improve the activities of antioxidant enzymes.

### CONCLUSION

It can be concluded that combination of the plant extracts significantly ( $p < 0.05$ ) possess potent antioxidant activity, which may be directly or indirectly responsible for its hypoglycaemic property. However the mode of action is yet to be known, although further studies remain to be conducted to investigate this hypothesis, the herbal formulation can be considered as supplementary therapy for a long time management disease associated with free-radicals complications

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