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Effect of *Vitexdoniana* ethanol leaf extract on renal function in diabetic rats

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ABSTRACT

Diabetes was induced intraperitoneally using 50mg/kg streptozotocin, while diabetic rats were treated with 100mg/kg ethanol extract and glibenclamide (2.5mg/kg) respectively for 28 days. Normal rats received distilled water. Changes in fasting blood sugar (FBS) serum creatinine and urea levels thiobarbituric acid reactive substances (TBARS) in the kidney as well as kidney catalase (CAT) and superoxide dismutase (SOD) activities were assayed. The results revealed that the FBS, TBARS, creatinine and urea levels were increased while SOD and CAT activities decreased significantly ($P<0.05$) in control rats. Treatment with extract was able to revert these parameters to normal by increasing SOD and CAT and decreasing FBS, TBARS, urea and creatinine. Extract treatment demonstrated more effect compared to glibenclamide treatment. However, the extract was able to manage hyperglycaemia and diabetes -induced oxidative changes in the kidney, thus suggesting its use for the management of diabetes and its complications affecting the kidney.

Key word: hypoglycaemic, streptozotocin-induced diabetic, *Vitexdoniana*.

INTRODUCTION

Streptozotocin (STZ) is a naturally occurring nitrosourea with molecular weight of 265 and empirical formula of C14 H27 N5 O12 [1]. It is widely used to induce insulin-dependent diabetes mellitus in experimental animals because of its toxic effects on islet beta cells[2, 3]. The diabetogenic action of STZ is the direct result of irreversible damage to the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin [4]. The effects of STZ on different organs have been extensively studied. STZ has various biological actions, including the production of acute and chronic cellular injury, carcinogenesis, teratogenesis and mutagenesis [5]. STZ is a nitrosourea compound which generally shares similar fate of disposition with other nitrosoureas and is a drug of choice in islet cell carcinoma and malignant carcinoid tumors. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastriculceration [6, 7]. STZ given intravenously or intraperitoneally to laboratory mice in multiple sub-diabetogenic doses, induces pronounced pancreatic insulitis with eventual destruction of insulin secreting beta cells and diabetes mellitus. In an experimental study in rats, streptozotocin given intraperitoneally in a dose of 45 mg/kg body weight of animals, effectively produced hyperglycaemia [2, 3].

In another study in rats, STZ injected in a dose of 65 mg/kg body weight effectively produced hyperglycemia and gastricmucosal ulcerations [6, 7]. The incidence and severity of lesions produced by STZ in pancreas, liver, kidney and GIT, progressively increased with time from one to six weeks post treatment [6]. Studies have shown an association between specific diabetic complications and liver enzyme alterations [8, 9] but only limited data is

available on the use of ethanol extract of Vitexdoniana in the management of diabetic nephropathy and kidney enzyme alterations.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials:
Fresh leaves of *V. doniana* were collected from its natural habitat in Ankpa, Kogi State, and it was identified and authenticated by the Ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/6415 was deposited at the herbarium of the department. The plant material was dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle.

Extraction
The pulverized sample mixture was defatted with n-Hexane and extracted with ethanol using soxhlet extractor.

Animal management
Male albino rats (7-8weeks old) were purchased from the animal house of the Department of Biosciences, Salem University, Lokoja, Nigeria. They were acclimatized for two weeks prior to commencement of experiment. They were kept at room temperature and maintained *ad libitum* on growers mash (feed) and weighed prior to experiment.

Induction of diabetes
Rats were fasted overnight and experimental diabetes was induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 50mg/kg body weight. STZ was dissolved in a freshly prepared 0.1M cold citrate buffer pH4.5 [9]. Control rats were similarly injected with citrate buffer. Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ treated rats were provided with 10% glucose solution after 6 hr for the next 24 hr to prevent severe hypoglycemia. After 3 days for development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration 250mg/dl) that exhibited hyperglycemia were selected for experiment [10].

Experimental design
In the experiment, the rats were divided into 5 groups of 5 rats each. Treatment was carried out orally for four weeks.

Normal Control (N. control) Distilled water (5ml/kg)
Diabetic Control (D.Control) Distilled water (5ml/kg)
Diabetic Glibenclamide (D.STD) (2.5mg/kg)
Diabetic Extract (D. Ethanol) ethanol extract (100mg/kg)
Non diabetic Extract (N. Ethanol) ethanol extract (100mg/kg)

On the 28th day of post-treatment, the animals were fasted overnight, anesthetized with chloroform and sacrificed by humane decapitation. The blood was collected in test tubes and serum collected and stored in deep-freezer prior to analysis. Fasting blood glucose was and packed cell volume was monitored weekly. Liver and kidneys were surgically removed, immediately washed with ice-cold normal saline and stored in deep freezer.

Tissue Preparation
Weighed kidney samples were homogenised separately in 10 parts (w/v) of ice-cold 50mM Tris-HCl, (pH 7.4) using a homogeniser (Janke and Kunkel, Germany). The homogenates were centrifuged at 3,000 rpm for 15 minutes and the supernatant collected. The supernatants were used for measurement of scavenging enzyme activities and lipid peroxides (TBARS).

Collection of Blood Sample and Estimations
Blood was collected from retro-orbital plexus under mild chloroform anesthesia from overnight fasted rats. Serum was separated and analyzed for serum creatinine[11], serum urea [12], alkaline phosphatase [13], Superoxide Dismutase [14] and catalase [15], were estimated.

Thiobarbituric Acid Reactive Substances (TBARS)
Kidney Lipid peroxidation was determined as thiobarbituric acid reactive substances as described by [16]. Lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde, a product which react with thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 535nm. The

extinction coefficient, 1.56x10-5 M-1 Cm-1 was used in the calculation of TBARS and values were expressed as nmol/mg protein.

Fasting Blood Sugar

Fasting blood sugar (FBS) was measured using Accu-Check Advantage glucometer.

Statistical analysis

All the values estimations were expressed as mean ± standard deviation and analyzed for Duncan's post-hoc ANOVA and student's t-test using statistical package for social sciences (SPSS). Differences between groups were considered significant at P <0.05 levels.

RESULTS AND DISCUSSION

Table 1.Showsfasting blood sugar (FBS) from day 0 (before induction of diabetes) to week 4 of treatment. There was no significant difference (P<0.05) in FBS across the group at week zero. At week one, there was significant increase in FBS in d diabetic groups, treatment of diabetic rats with the extract significantly reduced the FBS levels across the week. However, there was no statistical difference in FBS in the control groups across the week

FASTING BLOOD GLUCOSE (FBS) (mg/dl)					
TREATMENT	**DAY 0**	**WEEK 1**	**WEEK 2**	**WEEK 3**	**WEEK 4**
N. CONTROL	104.2±8.6[a]	108.8±08.6[a]	110.5±11.5[a]	104.8±06.7[a]	108.2±07.3[a]
D. CONTROL	97.4±5.2[a]	318.7±25.0[b]	236.5±95.4[b]	236.3±26.8[b]	248.5±20.9[c]
D. STD	96.2±5.2[a]	365.2±23.7[c]	268.4±29.1[c]	170.7±25.3[d]	123.5±28.0[b]
D.ETH 100mg	106.6±7.6[a]	399.0±47.0[d]	330.6±37.3[d]	150.6±27.1[d]	113.4±12.1[ab]
N.ETH 100mg	99.2±6.6[a]	102.8±08.5[a]	108.1±06.0[a]	103.4±13.9[a]	101.6±12.9[a]

N. CONTROL= Normal Control; D. CONTROL = diabetic Control; D. STD = Diabetic Standard Drug (Glibenclamide); D.ETH = Diabetic Ethanol extract; N. ETH = Non Diabetic Ethanol extract.

Table 2.Presents alkaline phosphatase (ALP), urea and creatinine. Significant (P<0.05) increase in ALP, urea and creatinine was observed in the control groups owing to diabetic condition. Extract treatment was able to revert these parameters to nomal but caused no statistical significance in the non-Diabetic treated rats compared to the normal

	KIDNEY FUNCTION		
TREATMENT	**ALP (U/L)**	**UREA (mg/dl)**	**CREAT (mg/dl)**
N. CONTROL	22.81±04.00[a]	29.98±10.24[a]	0.78±0.45[a]
D. CONTROL	79.20±24.09[c]	52.07±1.89[b]	1.86±0.11[b]
D. STD	39.67±08.53[b]	35.80±12.18[c]	0.74±0.32[a]
D.ETH 100mg	30.06±08.85[ab]	37.48±13.97[c]	0.77±0.29[a]
N.ETH 100mg	23.12±07.79[ab]	29.62±11.34[a]	0.75±0.04[c]

Table 3.presents superoxide dismutase (SOD) catalase (CAT) and thiobarbituric acid reactive substance (TBARS) all in the kidneys. There was significant (P<0.05) decrease in SOD and catalase activities in the kidneys as well as increase in TBARS level in the control groups. Similarly treatment with extract was able to revert the condition to normal in catalase and TBARS but to near-normal in SOD activity

	KIDNEY PARAMETERS		
TREATMENT	**SOD (U/L)**	**CAT (U/L)**	**TBARS (nmol/mg prtn)**
N. CONTROL	32.16±06.08[a]	14.26±2.51[a]	0.56±0.13[a]
D. CONTROL	25.58±05.16[b]	08.13±1.21[b]	1.23±0.00[b]
D. STD	34.11±07.16[a]	11.08±2.11[ab]	0.52±0.10[a]
D.ETH 100mg	47.22±05.43[c]	13.08±1.07[a]	0.54±0.08[a]
N.ETH 100mg	44.49±04.87[c]	16.04±0.99[c]	0.44±0.09[a]

Elevated activities of serum alkaline phosphates (ALP)are a common sign of kidney damage and are observed more frequently among people with diabetes than in general population [2, 17-20].

[21, 22] proposed that the glomerular damage in diabetic kidney was due to the increased production of Kallikrein and prostaglandin E2 which caused hyperfiltration and vasodilatation in diabetes. Diabetes mellitus (hyperglycaemia) caused increase in cellular production of eicosanoids from kidney tissues as investigated by[23]. These eicosanoids included vasodilatory prostaglandins (PGE 2 and PGI 2). There also occurred smaller concurrent increase in thromboxane (TX)A2 within one week after induction of diabetes. This increase in eicosanoids has been linked to the glucose-induced activation of the glomerular protein kinase-C signalling system that enhances phospholipase A2 activity and therefore release of membrane bound arachidonic acid for oxygenation. [24] proposed that STZ in hyperglycemic animals causes a time dependent rise in AST, ALT, and ALP levels.

In our study, the levels of ALP was significantly increased in the kidney of control animals as shown in table2, but treatment with the extract restored the anomaly compared with the control. Alkaline phosphatase is a membrane

bound glycoprotein enzyme.[25] observed increased levels of serum ALP in pathological conditions involving the kidneys. The increase in serum ALP might be derived from injury to the brush border membrane of the renal tubular cells. Renal function impairment might also be responsible for the increased serum ALP. [26] reported that human intestinal alkaline phosphatase (hALP), a specific marker of proximal tubular S3 segment, was elevated in the urine of microalbuminuric diabetic patient. This suggested that tubular alterations were present at an early stage of diabetic nephropathy especially at S3 segment. Increase in the levels of ALP in diabetic rats was also reported by [17, 18, 19].

The current investigation revealed that induction of diabetes resulted in elevation of serum urea, and creatinine concentrations. These parameters are considered as significant markers of renal dysfunction [27, 28, 29]. *Vitexdoniana* extract administration resulted in decrement of these parameters, a finding that was in agreement with that of [15, 19] who reported ameliorated renal dysfunction of diabetic rats by the ginseng extract or 20 (S)-ginsenoside Rg3 administrations.

Concerning the renal antioxidant status, the current study revealed increased oxidative stress due to diabetes which was evidenced by increased tissue concentration of malondialdehyde and depletion of antioxidant enzymes concentration (catalase and SOD). *V. doniana* ethanol extract was able to manage the condition by reverting the altered parameters to normal. The recorded rise in tissue concentration of malondaldehyde, an index of endogenous lipid peroxidation, has been also reported by [27] in diabetic patients and [19] in diabetic rats reflecting increased state of oxidative stress. Moreover, ginsenoside fractions have been shown to induce the cytosolic antioxidant enzyme superoxide dismutase via enhanced nuclear protein binding to its gene regulatory sequences [30, 31].The reported results concerning the decrease in superoxide dismutase during diabetes by *V. doniana* ethanol extract agreed with those of [28]who showed a decrease in Cu-Zn SOD activity in renal tissues during diabetes, however they contradicted those of [31] who demonstrated either a no change or an increase in SOD activity in renal tissues of diabetic rats.

In conclusion, *V. doniana* ethanol extract was able to manage hyperglycaemia and its complications in the kidney, an effect that seems to be rather dependent on antioxidant property of *Vitexdoniana.*

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