HISTOPATHOLOGICAL EXAMINATION AND CHANGES IN SERUM ELECTROLYTES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS OWING TO TREATMENT WITH ETHANOL EXTRACT OF VITEX DONIANA LEAVES

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ABSTRACT
In this study, the antidiabetic effects, histopathological examination and changes in serum electrolytes of Vitex doniana leaves treatment on Wistar albino rats was studied. V. doniana leaves were extracted using ethanol and water as solvents. Diabetes mellitus was induced with a single intraperitoneal dose of 50 mg/kg b.w of streptozotocin and treatment of diabetic and normal rats was carried out using the doses of 25 and 50 mg/kg b.w for each extract. Fasting blood sugar (FBS), Na⁺, K⁺, Cl⁻, HCO₃⁻ and Ca²⁺ were analysed as well as the histopathological examination of the liver and kidney. At 25 and 50 mg/kg, the extracts significantly (P<0.05) reduced the FBS (groups 3, 4 and 5) compared to control (group 2). Treatment of diabetic rats with 25 and 50 mg/kg (groups 3, 4 and 5) revealed non-significant (p<0.05) increase in the electrolyte concentrations compared to control (group 2), while histopathological examination of liver and kidney showed mild toxicity on the tissues. However, these results suggest that ethanol and aqueous extracts of the leaves of Vitex doniana have remarkable anti-diabetic effects with promising clinical relevance of reducing diabetic complications.

Key word: histopathology, streptozotocin-induced diabetic, Vitex doniana.

INTRODUCTION
Medicinal plants are continued to be a powerful source for new drugs, now contributing about 90% of the newly discovered pharmaceuticals [1]. Traditional medicine provides better health coverage for 80% of the world population, especially in the developing countries [2].

Several authors have evaluated the medicinal application of the V. doniana. Sofowora, [3] reported that various parts of the plant are used for treatment of several disorders which include rheumatism, hypertension, cancer and inflammatory diseases. It has been reported to have significant analgesic and anti-inflammatory activities mediated through sequential inhibition of the enzymes responsible for prostaglandin synthesis from arachidonic acid [4]. The essential oil extracted from the plant has been shown in vitro to have antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumonia, Candida albicans, Streptococcus pyogens, Shigelladyenseriae, Listeria monocytogenes and Bacillus cereus [5]. Research reported by Shukla et al., [6] on the antihapatotoxic effects of aqueous leaves and stem extracts of V. doniana showed that it was effective against carbon tetrachloride induced liver injury in rats. The anti-hypertensive effect of extract of stem bark of V. doniana has been reported by Olusola et al., [7], and shows that the extract exhibited a marked hypotensive effect in both normotensive and hypertensive rats. Extracts of stem bark of V. doniana have also demonstrated some level of in vitro trypanocidal activity against Trypanosoma brucei [8]. The aqueous and methanolic extracts have been reported to exhibited anti-diarrhea activity [9]. Phenolics are found in V. doniana and have been reported to have multiple biological effects, including antioxidant activity [10,11].

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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 was defatted with n-Hexane and extracted with ethanol using soxhlet extractor as modified by [11].

Animal management
Male albino rats (Wistar strain, weighing 150-200g) were purchased from Department of Biochemistry, Salem University, Lokoja, Nigeria. They were housed under standard husbandry conditions (30°C ± 2°C, 60 –70 % relative humidity and 12h : 12h day-night cycle) and allowed standard pelleted rat feed and water *ad libitum*.

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. 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 [12].

Experimental design
In the experiment, the rats were divided into 7 groups of 5 rats each. Treatment was carried out using 25mg and 50mg/kg body weight/day respectively by oral intubation method for 28 days.

Group 1. Normal Control (N. control) Distilled water (5ml/kg)
Group 2. Diabetic Control (D.Control) Distilled water (5ml/kg)
Group 3. Diabetic + Glibenclamide (D.STD) (2.5mg/kg)
Group 4. Diabetic + Extract (D. Ethanol) Ethanol extract (50mg/kg)
Group 5. Diabetic + Extract (D. Ethanol) Ethanol extract (25mg/kg)
Group 6. Non Diabetic + Extract (N. Ethanol) Ethanol extract (50mg/kg)
Group 7. Non Diabetic + Extract (N. Ethanol) Ethanol extract (25mg/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 sample tubes and serum collected and stored in deep-freezer prior to analysis. Meanwhile, fasting blood glucose was monitored weekly.

Assay of serum electrolytes
The concentrations of Na⁺ and K⁺ in the serum were determined by the flame photometry method Mac Donald, [13]. Serum chloride and calcium ion concentrations were determined using the method described by Schales and Schales, [14], while bicarbonate ion concentration was estimated by a modified direct titration method as described by Sigaard-Anderson, [15].

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

Histopathological Examinations
Liver and kidneys were surgically removed, immediately washed with ice-cold normal saline and stored in 10% formalin and subsequent analysis.Histopathological examination of liver and kidney of the rats was carried out according to the method of Sarkar et al.,[16].

Data analysis
Statistical analysis was performed using SPSS software package, version 20.0. Experimental results were analysed by one way analysis of variance (ANOVA) followed by Duncan’ multiple range test (DMRT). All the results were expressed as mean ±SD for five rats in each group *p*-Values <0.05 were considered as significant.
Results

STZ treatment increased blood glucose levels significantly (p<0.05) in experimental rats when compared to control rats. Administration of extract decreased the blood glucose levels significantly (p<0.05) in STZ – induced diabetic fasted rats. The hypoglycaemic activity was equivalent to that of glibenclamide (2.5 mg / kg body weight / day) treated positive control rats.

Table 1. Fasting blood sugar concentration in streptozotocin-induced diabetes in rats treated with 25mg and 50mg/kg of aqueous and ethanol extracts of *Vitex doniana* leaves

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>WEEK 0</th>
<th>WEEK 1</th>
<th>WEEK 2</th>
<th>WEEK 3</th>
<th>WEEK 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NORMAL</td>
<td>103.8±6.1a</td>
<td>107.2±8.0a</td>
<td>110.6±7.8a</td>
<td>87.2±43.4a</td>
<td>104.6±10.5a</td>
</tr>
<tr>
<td>2. D. CONTROL</td>
<td>107.4±7.4a</td>
<td>300.8±32.3b</td>
<td>272.7±47.3b</td>
<td>252.7±59.2c</td>
<td>226.6±37.0f</td>
</tr>
<tr>
<td>3. D. ETH 50mg</td>
<td>102.4±9.2a</td>
<td>404.7±50.0b</td>
<td>283.6±43.9b</td>
<td>169.3±38.3b</td>
<td>120.2±14.1b</td>
</tr>
<tr>
<td>4. D. ETH 25mg</td>
<td>108.2±9.4a</td>
<td>374.2±41.1b</td>
<td>223.2±61.7b</td>
<td>138.3±27.4ab</td>
<td>122.3±09.2b</td>
</tr>
<tr>
<td>5. D. STD (2.5mg)</td>
<td>102.2±14.5a</td>
<td>391.8±90.9b</td>
<td>223.6±59.0b</td>
<td>143.3±25.6ab</td>
<td>118.3±15.2b</td>
</tr>
<tr>
<td>6. N. ETH 50mg</td>
<td>111.4±7.3a</td>
<td>102.2±13.8a</td>
<td>88.2±08.9a</td>
<td>109.4±06.7a</td>
<td>103.2±11.4a</td>
</tr>
<tr>
<td>7. N. ETH 25mg</td>
<td>103.2±11.4a</td>
<td>98.8±11.5a</td>
<td>104.6±10.5a</td>
<td>101.6±13.1a</td>
<td>101.2±13.5a</td>
</tr>
</tbody>
</table>

Table 2 shows significant increase (p<0.05) in Na⁺, K⁺, Cl⁻ and Ca²⁺ in diabetic control rats except in HCO₃⁻ which was not statistically significant compared to normal control group. There was decrease in the concentration of Na⁺ (142.92±2.52), K⁺ (5.16±0.57), Cl⁻ (98.36±1.64) and Ca²⁺ (8.55±0.21) owing to treatment with aqueous extract at 50 mg/kg b.w. and Na⁺ (141.12±1.79), K⁺ (4.95±0.90), Cl⁻ (99.22±2.71), Ca²⁺ (8.62±0.56) owing to treatment with ethanol extract at the same dosage compared to the diabetic control groups; Na⁺ (148.45±2.06), K⁺ (6.10±0.88), Cl⁻ (104.02±2.30) and Ca²⁺ (9.72±0.560). Treatment of normal rats with the extracts at the different doses caused non significant difference (p>0.05) in the electrolytes concentration compared with the control and normal rats and there was no significant difference (p<0.05) in HCO₃⁻ concentration along the groups.

Table 2. Serum electrolytes concentration in streptozotocin-induced diabetes in rats treated with 25mg and 50mg/kg of aqueous and ethanol extracts of *Vitex doniana* leaves

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Na⁺ (mmol/L)</th>
<th>K⁺ (mmol/L)</th>
<th>Cl⁻ (mmol/L)</th>
<th>HCO₃⁻ (mmol/L)</th>
<th>Ca²⁺ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>142.42±2.18a</td>
<td>4.54±0.61a</td>
<td>101.96±0.49a</td>
<td>23.38±4.59a</td>
<td>8.82±0.40d</td>
</tr>
<tr>
<td>D. CONTROL</td>
<td>138.45±2.06a</td>
<td>6.10±0.88b</td>
<td>96.02±2.30a</td>
<td>21.49±4.70a</td>
<td>7.72±0.56e</td>
</tr>
<tr>
<td>D. STD (2.5mg)</td>
<td>140.16±2.63ab</td>
<td>5.31±1.18ab</td>
<td>98.72±3.34ab</td>
<td>22.86±2.23a</td>
<td>8.52±0.54ab</td>
</tr>
<tr>
<td>D. ETH 50mg</td>
<td>141.12±1.79ab</td>
<td>4.95±0.90a</td>
<td>99.22±2.71bc</td>
<td>23.88±2.47a</td>
<td>8.62±0.56ab</td>
</tr>
<tr>
<td>D. ETH 25mg</td>
<td>140.64±1.55ab</td>
<td>5.10±1.28ab</td>
<td>98.36±0.88ab</td>
<td>22.32±1.72a</td>
<td>8.44±0.64ab</td>
</tr>
<tr>
<td>N. ETH 50mg</td>
<td>142.64±1.34ab</td>
<td>5.33±1.20ab</td>
<td>102.22±2.59ab</td>
<td>26.14±1.71b</td>
<td>9.07±0.63ab</td>
</tr>
<tr>
<td>N. ETH 25mg</td>
<td>140.40±3.79ab</td>
<td>5.35±1.53ab</td>
<td>101.5±1.83ab</td>
<td>24.38±3.80a</td>
<td>8.74±0.83b</td>
</tr>
</tbody>
</table>

The histopathological findings are summarized as follow;

Figures below show the structural features of hepatocytes in the experimental animals. Fatty liver was shown by H&E staining as an unstained area in liver parenchymal cells (Fig. 4B). In the untreated diabetic rats, microvascular vacuolization, focal necrosis and inflammation in the portal area were significantly apparent in figure 2 (diabetic control) compared with the normal group (Figure 1) and extract treated diabetic rats (Figure 3 and 4) improved these findings. Interstitial matrix deposition was studied in Azan-Mallory-stained liver sections.
as an index of interstitial fibrosis. Diabetic rats showed higher interstitial fibrosis than normal rats, which was improved in the extract treated diabetic rats.

Histological examination of kidney of the STZ-induced diabetic rats showed significant changes in the morphology of kidney cells including mild swelling and inflammation. Oral administration of extracts reduced the inflammation and swelling in kidney tissues.

Discussion

Our observations in this study are in well agreement with the reports by several workers that STZ-induced diabetes mellitus and insulin deficiency leads to increased blood glucose [17]. It has been reported that STZ at lower doses produces partial destruction of pancreatic β-cells with permanent diabetes condition [18] and there may be more possibility of many surviving β-cells [19]. Since a much low dose of STZ was chosen for this study, there may be many surviving β-cells, capable of undergoing regeneration. Administration of extracts decreased the elevated blood glucose level across the days. Prolonged administration may stimulate the β-cells
of islets of Langerhans to produce insulin. The antihyperglycemic effect of extract was compared with glibenclamide, a standard hypoglycemic drug. Glibenclamide has long been used to treat diabetes, to stimulate insulin secretion from the pancreatic β-cells. From the results, it appears that still insulin producing β-cells are functioning in STZ treated diabetic rats and stimulation of insulin release could be responsible for the most of the observed metabolic activities. Further the observed blood glucose-lowering effect in fasted normal and STZ induced diabetic rats could possibly be due to the increased peripheral glucose utilization. A number of other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release [20, 21]. It has been reported that chemically (STZ) induced diabetes produces partial or total deficiency of insulin that results in decrease in the concentration of glycolytic enzymes [22].

In the present investigation, diabetes was associated with electrolyte imbalance, where a significant increase (P<0.05%) in Na+, K+, CI and Ca2+ in diabetic control rats except in HCO3− which was not statistically significant (Table 2). There was slight decrease in the concentration of Na+, K+, CI and Ca2+ in diabetic rats treated with the extracts (25 and 50mg/kg respectively), but not statistically significant (p>0.05) compared with the control and normal rats. There was no significant difference in HCO3− concentration across the group. This may be attributed to the state of hyperglycemia that produces an osmotic diuresis that causes marked urinary loss of water and electrolytes, a condition that may be aggravated by urinary excretion of ketones which obligates additional electrolyte loss. Concerning sodium, additionally, there is translocation of Na,K-ATPase pumps from the basolateral membrane of proximal convoluted tubules to the cytosol which leads to a decrease in sodium pumping from renal tubules to the blood [23]. Also, expression of sodium channel proteins in the collecting ducts and distal convoluted tubules was altered leading to increased fractional excretion of sodium in urine. As for serum calcium previous researches showed lower concentration in diabetes which was associated with a decrease in bone mineral content and increased urinary excretion of calcium [24]. The increase was a result of several factors and in particular to hyperglycaemia, but a wide range of metabolic changes including chronic acidosis, insulin deficiency, impaired parathormone action, and changed vitamin D metabolism could be implicated [25]. On the contrary, the rise in serum potassium, is evidenced because of the extracellular migration in response to acidosis, however, potassium is also lost in large quantities in urine. V. doniana extracts at the doses of 25mg and 50mg/kg, in the present investigation, resulted in slight modification of such electrolyte imbalance as well as those implicated in the tissues.

REFERENCES


