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Kidney Function Status of Diabetic Male Wistar Albino Rat Treated with *Senna tora* Extract

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A B S T R A C T

The kidney plays a central role in detoxification and excretion of harmful metabolites and therefore is susceptible to toxicity by xenobiotics. The main goal of this study is to analyze the kidney function status of diabetic Male Wistar albino rat treated with *Senna tora* extract. Samples of matured and healthy-looking leaves of *Senna tora* were collected from the school environment, Federal University Wukari, Taraba State, Nigeria. Extraction of the plant was done using absolute ethanol and fractionation with different solvents of varied polarity via separating funnel, starting from ethanol, n-hexane, acetone and lastly, distilled water where four (4) fractions were collected in 100mL beakers each. The animals were treated with the extract *Senna tora* after they were induced with diabetes using alloxan monohydrate. The animals were kept for about twenty-one (21) days under treatment. After the experimental period, animals were sacrificed under chloroform anesthesia and blood was collected by cardiac puncture and liver was harvested. Determination of serum kidney biochemical parameters were analyzed using various kit of each parameter, such as sodium ions (Na^+), potassium ions (K^+), chloride ions (Cl^-), bicarbonate (HCO_3^-), urea and creatinine. This study shows that *Senna tora* have little negative effect on kidney function when used at moderate amounts though the little damage to the kidney have been observed within the limits of experiment, at the concentration dosage of the *Senna tora* used. There was no level of significance ($p \leq 0.05$) in the serum Sodium and Chloride concentration of the results obtained. But the K^+ and HCO_3^- has level of significance ($p \leq 0.05$), there was little evidence of kidney damage at high dosages and longer duration of consumption. Conclusion: The finding from this study has suggested the use of *S. tora leaf extract* in the treatment and management of kidney diseases, though dosage dependent.

Introduction

A medicinal plant is one whose one or more of its organs contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Ahmad *et al.,* 2008).

Herbal medicine, which is the use of medicinal plants in the treatment and cure of sicknesses and diseased conditions, has been with man since the beginning of time. In terms of recorded history (Ahmad *et al.,* 2008), medicinal plants have been in use for the past fifty centuries, which until the last two and a half centuries was the main source of treatment to man and his domestic animals. Early 20th century witnessed the arrival of hormones, chemotherapy, vitamins, antibiotics and more recently, the biotechnological products, which marked a sharp decline in the contribution of herbal medicine to health care delivery. Fortunately, there is a revival of herbal medicine at the close of the 20th century.

This is especially so with the rising cost of imported medication to the extent that governments cannot meet the demands of the people. Moreover, the scarcity and cost of the commodities used to manufacture drugs locally have made modern medicine too expensive for the common man to afford (Ahmad *et al.,* 2008). It is therefore important that we continually evaluate and develop our indigenous plant genetic resources for the improvement and sustenance of our health care delivery system.

International Diabetes Federation (IDF) defined diabetes as a chronic metabolic disease characterized by elevated levels of blood glucose (or blood sugar) which over time leads to serious damage to the heart, blood vessels, eyes, kidneys and nerves (World Health Organization, 2006). It is characterized by abnormally elevated levels of blood glucose due to complete or relative insufficiency in insulin secretion and /or insulin action together with chronic hyperglycemia (high fasting sugar level above 126 mg/dl) as well as disturbances in carbohydrate, fat and protein metabolism (Parsons and Cuthbertson, 1998). International Diabetes Federation (IDF) and the World Health Organization (WHO) have reported that about 1.5 million deaths are directly attributed to diabetes each year predicting that it will be the seventh leading cause of death in 2030, the world over, and that about 9% of adults in the world have diabetes of which 90% have type 2 diabetes (Parsons and Cuthbertson, 1998). Furthermore, Zarah *et al.,* (2000) reported an estimated increase in diabetes cases of about 38.20% in 2007 when compared to 2002 incidence in Katsina state, Nigeria.

Senna tora is a prominent plant in Africa and Asia and can also be referred to as *Cassia tora* (Pawar and Lalitha, 2015). It has long pinnate leaves resembling a feather, with each leaf having three pairs of leaflets that are opposite, ovate, oblong and oblique at the base. The yellow-colored flowers are bearded in the axil of the leaves. The flowers of *Senna tora* is made up of half inch diameter five petals. The seeds have three equal axes and oblique angles (rhombohedrl) and are brown in colour.

The flowers usually blossoms in the rainy season and fruits in the winter. *Senna tora* leaves, roots and seeds are used as food ingredients and additives (Ingle *et al.,* 2012; Shukla *et al.,* 2013).

Senna tora has indicated a wide range of pharmacological activities. It is well-known for its laxative, antimutagenic, antiperiodic and antihepatotoxic properties. It is also used for the treatment of cardiac disorders, bronchitis, ophthalmic diseases, ringworm, leprosy, haemorrhoids cough, hepatic disorder, skin diseases and liver tonic (Meena *et al.,* 2010; Bhandirge *et al.,* 2016). Certitude has been given to this plant *Senna tora* because of its many medicinal properties (Bhandirge *et al.,* 2016).

Medicinal plants are now being proposed as convincing alternatives for the treatment of various infections (Alao and Chukwujioke, 2013). This work was designed to evaluate the effects of *Senna tora* leaf supplement on blood glucose levels, kidney enzymes and total protein in alloxan induced diabetic wistar rats.

Materials and Methods

Animal Management and Care

Twenty-five (25) Wistar albino rats were used for the study. The rats weighed between 130 g - 204 g. The rats were purchased from the Animal House, Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria. All the Wistar albino rats were allowed free access to feed and water *ad libitum* throughout the experimental period. Standard laboratory protocols for animal studies were maintained as approved by the Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, Nigeria.

Sample collection and preparation

Matured and healthy-looking leaves of *Senna tora* leaves were collected from the school environment, Federal University, Wukari, Taraba State, Nigeria. The plant leaves were identified and authenticated at the Herbarium Unit, Department of Biological Sciences, Federal University Wukari. The leaves were thoroughly washed with tap water in order to remove the dust and soil particles. The leaves were air-dried under the shade to prevent ultra-violet rays from inactivating the chemical constituents (Ncube *et al.,* 2008; Das *et al.,* 2010).

The dried leaves were pounded into fine powder using mortar and pestle, and then stored and labelled in dry containers until needed. Only healthy plants were used as the leaves were examined to be free from diseases.

Ethanolic Extraction of *Senna tora* leaves

The dried *Senna tora* leaves weighing 500 g was macerated in 2,000 ml of ethanol (1:4 w/v) for 48hrs at room temperature. It was continually stirred after every 5 hours during the maceration period. The extracted juice was first sieved with clean white mesh before the use of Whatman No 1 filter paper. The filtrate was poured into a beaker and allowed to air dry. The resultant extract (18 g) was placed into the incubator for further studies.

Experimental Design

The rats were randomly divided into four groups (n = 5)

Five groups of animals, three in each, received the following treatment schedule:

Group I: Non-alloxan monohydrate induced rats (Normal control).

Group II: Alloxan monohydrate-induced untreated diabetic rats (140 mg/kg).

Group III: Alloxan monohydrate-induced diabetic rats treated with standard drug (Glibenclamide) (100 mg/kg).

Group IV: Alloxan monohydrate-induced diabetic rats treated with 100 mg/kg body weight of the extract.

Group V: Alloxan monohydrate-induced diabetic rats treated with 200 mg/kg body weight of the extract.

The rats in the treatment groups (III, IV & V) received standard drug (Glibenclamide), 100 mg/kg and 200 mg/kg body weight respectively of ethanolic extracts of *Senna tora* orally through an orogastric tube on a daily basis.

The control group received an equal volume of distilled water without the extract of *Senna tora* added for 2 weeks**.** After the experimental period, animals were sacrificed under chloroform anesthesia and venous blood was collected by cardiac puncture and liver and kidney was harvested. Blood samples were collected and serum was obtained by centrifuging at 3000 rpm for 5 mins.

Sacrifice and sample collection

At the end of the experimental period, the rats were withdrawn from the cages in each group and placed in a desiccator containing cotton wool soaked in light chloroform to anesthetize the rats partially. Blood samples were collected from the heart via cardiac puncture using a sterile syringe and needle.

The blood sample was divided into two fractions: One fraction was put into plain sample tubes while whole blood samples were put in Ethylene diamine tetraacetate (EDTA) treated sample bottles (Palmer, 2014). The serum was collected from the clotted sample in the sample container by letting it stand for 2 hours at room temperature to clot before centrifugation at 3,000 rpm for 20 minutes using an MSE England benchtop centrifuge.

Sera obtained from each sample were gently separated using Pasteur pipettes and dispensed into respective dry specimen bottles labeled accordingly. These were kept frozen in a refrigerator until when needed for various biochemical assays. The blood samples collected into the EDTA bottles were corked immediately, shaken gently to allow the blood to mix with the anticoagulant and prevent clotting and cell hemolysis. The hematological analyses were carried out as soon as the blood sample was collected.

Statistical Analysis

The mean ± SD of all values was calculated and changes observed between the treatment group and the control were subjected to analysis of variance (ANOVA) using SPSS version 23. Differences between groups were considered significant at $P<0.05$.

Results and Discussion

The kidney has an excretory organ, is central to the normal function of the body. It plays a major role in the maintenance of the body homeostasis, excretion of waste materials or products of metabolism, drugs and chemical which are vital to maintenance of health (Yakubu *et al.,* 2021).

The results of kidney function parameters such as Sodium (Na^+), Chloride (Cl^-), Potassium(K^+), Urea, Bicarbonate and Creatinine obtained are presented in SD ± Mean error in Tables 3 and 4. Results showed that the extract could significantly ($p<0.05$) mitigate the harmful effect of the toxicity caused by the extract-treated group (Group 3) compared with Group 2, which was induced but not treated.

In the present study, 140 mg/kg of alloxan was administered to all animals except animals in group 1 (control), the administration of alloxan significantly increased the blood glucose levels of the animals. The blood glucose levels of all the animals were all below 130 mg/dl but the induction of alloxan led to an elevation in their blood glucose levels to over 350 mg/dl making them diabetic or hyperglycemic.

The kidney helps maintain the body's homeostasis by reabsorbing important material and excreting waste products (David *et al.,* 2014). Creatinine is a breakdown waste product formed in the muscle by creatinine phosphate metabolism.

Creatinine is synthesized in the liver, passes into the circulation, and takes up almost entirely by skeletal muscle for energy production (Showkat *et al.,* 2011). Creatinine retention in the blood is evidence of kidney impairment. Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of the urea cycle, where ammonia is converted into urea and excreted through urine (David *et al.,* 2012). It represents 90% of the total urinary nitrogen excretion.

Urea varies directly with protein intake and inversely with the rate of excretion. Renal diseases that diminish urea's glomerular filtration rate will lead to its retention in the blood (Vasudevan *et al.,* 2011). The kidney also maintains a marginal concentration of electrolytes in the body. Electrolytes are small inorganic ions prevalent in body fluid that are important in normal physiological functions (Parsons and Cuthbertson, 1998). They are mainly sodium ion Na+, chloride ion Cl-, potassium ion K^+, bicarbonate ion HCO3, and hydrogen ion H+.

The volume of extracellular fluid (ECF) depends on the body's sodium content because Na+ and its salt are the major osmotic solute in ECF (Showkat *et al.,* 2011). Renal regulation of these ions is controlled by renal sympathetic, atrial natriuretic peptide, and aldosterone actions which may result in reabsorption or excretion of these ions at the distal tubule of the nephrons. However, nephrology defects caused by xenobiotics such as CCl4 and DEN toxicity may truncate these functions and result in irregular distribution of these ions in the ECF (World Health Organization, 2002).

Electrolytes (sodium, potassium, chloride, and bicarbonate) balance in the blood is a good indicator of kidneys and heart functions. Therefore, adequate information on serum electrolytes can assist in the determination of organ functions. For example, liver function assessment can be done by evaluating liver enzyme (AST, ALT, and ALP) parameters, total protein, albumin, and total bilirubin (David *et al.,* 2014).

Diabetes mellitus is a metabolic disease associated with impaired glucose metabolism which adversely alters the intermediary metabolism of lipids and carbohydrate (World Health Organization, 2002). Diabetes is now recognized as one of the major killer diseases and a leading cause of death, claiming many lives all over the world (Garba *et al.,* 2015).

Oral hypoglycemic agents, especially the sulphonylureas and biguanides, have been commonly used in the management of the disease, especially type II diabetes, as although they have serious side effects. Consequently, attention has been focused on the use of plants and herbal remedies believed to be safer and devoid of serious side effects as alternatives in the treatment of diabetes (Showkat *et al.,* 2011).

This result is found to be in agreement with that of Garba *et al.,* (2015), who reported that various dose levels of the methanolic extracts of *Senna tora* was able to reduce the blood glucose significantly ($p<0.05$) but however, not dose-dependently. Furthermore, this is in accordance with work of Emmanuel *et al.,* (2011) and Singh *et al.,* (2011) who reported that the aqueous extracts of the leaves of *Senna tora* in male albino Wistar rats has shown a significant hypoglycemic activity.

Table.1 Effect of *Senna tora* leaves extract on serum electrolyte parameters on Wistar rat induced with diabetes

Groups/Treatments	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^-(mmol/L)
1 Normal Control	5.30±0.92[a]	142.64±0.66[a]	102.03±0.55[a]	7.09±0.67[a]
2 Positive Control	9.27±1.33[b]	139.27±3.22[a]	107.62±3.22[a]	28.71±2.33[b]
3 Negative Control	10.11±1.33[b]	144.19±1.64[a]	106.00±4.56[a]	34.63±2.55[b]
4 100 mg of Extract	10.31±2.53[b]	148.37±3.66[a]	109.11±3.45[a]	36.04±2.67[b]
5 100 mg of Extract	14.15±3.57[c]	136.25±2.43[a]	98.43±21.55[a]	35.23±5.67[b]

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05).

Table.2 Effect of *Senna tora* leaves extract on serum Creatinine and serum Urea parameters on Wistar rat induced with diabetes

Groups/Treatments (mmol/L)	Creatinine	UREA (mmol/L)
1 Normal Control	2.13±0.55[b]	113.14±5.34[a]
2 Positive Control	2.37±0.33[b]	115.43±4.66[a]
3 Negative Control	1.20±0.56[a]	121.90±7.55[a]
4 100 mg of Extract	3.19±0.74[c]	231.55±6.56[c]
5 200 mg of Extract	4.17±0.57[d]	189.52±4.79[b]

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05).

Table.3 Effect of *Senna tora* leaves extract on Lipid Profile

Group/Treatment	TAGs (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TCHOL (mg/dL)
1 Normal Control	110.05±2.55[c]	1.24±0.31[a]	221.05±5.76[a]	331.35±3.56[b]
2 Positive Control	63.76±3.65[a]	1.20±0.25[a]	392.75±5.97[b]	456.76±7.25[c]
3 Negative Control	67.45±3.75[ab]	1.14±0.12[a]	445.84±7.33[c]	513.51±6.52[d]
4 100 mg of Extract	72.40±4.35[b]	1.63±0.21[a]	372.14±6.64[b]	444.87±5.78[c]
5 100 mg of Extract	61.92±2.33[a]	1.37±0.22[a]	224.97±2.45[a]	287.16±3.66[a]

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05)

Table.4 Effect of Induction on body weight of Wistar Albino Rats

Parameters	Before Induction (g)	First Week (g)	Second Week (g)	Third Week (g)
Group 1	172.80±4.67[ab]	182.60±3.98[ab]	147.00±4.53[a]	133.80±2.45[a]
Group 2	182.00±3.56[b]	184.20±5.44[b]	170.60±7.43[ab]	152.80±4.34[ab]
Group 3	158.40±3.43[a]	170.40±3.67[ab]	164.80±4.33[ab]	158.20±6.32[ab]
Group 4	184.40±5.78[b]	183.60±6.32[ab]	218.80±6.34[c]	194.20±4.55[c]
Group 5	155.40±3.33[a]	159.80±4.55[a]	184.20±3.65[b]	174.80±5.66b[c]

Result presented as mean ± Standard deviation. Result within a column with the same superscript indicate no levels of significance while result within the same column with different superscript indicate level of significances (p≤0.05)

This study shows that *Senna tora* have no negative effect on kidney function when used at moderate amounts. Within the limits of experimental error, at the dosage of the *Senna tora* used, there was little evidence of kidney damage at high dosages and longer duration of consumption.

The presence of active ingredients and their biological activities in the ethanolic extract of *Senna tora* leaves makes it a prospective alternative to synthetic drugs in preventing kidney toxicity in both man and animals.

Significance Statement

This study evaluated the kidney function parameters of *Senna tora* leaves extract against alloxan monohydrate induced diabetes. Significantly, the results have shown that *S. tora* has a potential to ameliorate liver and kidney diseases.

However, further research should be carried out on other parts of the plants with an aim to acquire comprehensive information on phytochemical and biological potential of this medicinally useful plant species.

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Author Contributions

Ojochenemi Ejeh Yakubu: Investigation, formal analysis, writing—original draft. Mubarak Sanusi Tani: Validation, methodology, writing—reviewing. Onuh Gabriel Emmanuel:—Formal analysis, writing—review and editing. Caleb Enejoh Omede: Investigation, writing—reviewing. Janya Danjuma: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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