



RESEARCH ARTICLE

IN-VIVO ANTIDIABETIC POTENTIALS AND TOXICOLOGICAL EFFECT OF DENNETTIA TRIPETALA SEEDS ON ALLOXAN-INDUCED DIABETIC MALE ALBINO RATS

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Abstract



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Background: Hyperglycemia and dyslipidemia are hallmarks of diabetes mellitus. Diabetes mellitus is a complex metabolic disorder characterized by disturbances in carbohydrates, protein, and lipid metabolism due to insufficient insulin production. **Aim:** Present study was aimed to estimate the effects of *Dennettia tripetala* seed methanol extract (DTSE) on blood sugar and toxicological effect in alloxan-induced diabetes models using male wistar rats.

Methods: A total of thirty (30) male albino rats were divided into six distinct groups. Group A served as normal control group, where rats were neither induced nor treated. Group B acted as the negative control group, where rats were induced but not treated. Group C served as the positive control group, where rats were induced and treated with glibenclamide. Group D consisted of rats that were induced and treated with 100 mg of DTSE. Group E included rats that were induced and treated with 200 mg of DTSE. Lastly, group F comprised rats that were induced and treated with 400 mg of DTSE. Liver and kidney functions were determined using established analytical procedures. Blood samples were collected through ocular puncture for the evaluation of biochemical parameters.

Results: Blood glucose level in all the alloxan-induced diabetic rats treated with DTSE showed a relative significant ($p < 0.05$) reduction when compared with the controls. Histological investigation of diabetic rat's liver and kidney indicated degradation of normal tissue architecture, however after the treatment with DTSE minor reparative alteration were seen.

Conclusions: The study suggests that DTSE possesses a hypoglycemic effect, hepatoprotective and anti-atherogenic effect but has toxicological effect on the kidney of the rats treated with high dose of DTSE as pathological changes were elicited in the organ of the rats.

Keywords: Diabetes, lipid, *Dennettia tripetala*, Glibenclamide, rats.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by disturbances in carbohydrates, protein, and lipid metabolism due to insufficient insulin production. Unlike a single disease, it manifests as a syndrome with elevated blood glucose levels resulting from disruptions in insulin production, secretion, or functionality¹. According to estimates from the International Diabetes Federation, the prevalence of this condition among adults is projected to reach approximately 387 million individuals². The absence of adequate insulin triggers a cascade of intricate

physiological responses. The liver responds by enhancing its uptake of glucose from the bloodstream, aiming to counteract the escalating glucose levels. Interestingly, the kidney, despite its independence from insulin for normal function, works to manage the heightened glucose levels associated with diabetes³. However, existing synthetic anti-diabetic agents, although effective, often come with considerable financial burdens and the potential for severe side effects, highlighting the urgent need for alternative therapeutic approaches.

The natural world has long been a source of potential remedies, and herbal medicines derived from medicinal

plants have emerged as promising alternatives for addressing the challenges posed by diabetes mellitus. One notable advantage of medicinal plants is their relatively lower likelihood of inducing adverse effects, providing a safer avenue for treatment⁴. This movement towards harnessing the potential of natural remedies has gained momentum as researchers and healthcare providers strive to overcome the limitations of synthetic medications. Recent medical investigations have shed light on the intricate relationship between type 2 diabetes mellitus and liver diseases. Notably, type 2 diabetes has been associated with a heightened susceptibility to liver-related disorders, which can lead to severe consequences, including mortality⁵.

A particularly intriguing candidate for exploration in this context is *Dennettia tripetala*, commonly known as pepper fruit. Indigenous to the rainforests of Nigeria and other regions of West Africa, this plant produces fruits with a distinctive pungent flavor. Referred to as Ako, Mmimi, and Ata-Igbere in the Edo, Igbo, and Yoruba tribes of Nigeria respectively, *D. tripetala* has a deep-rooted history in traditional medicine⁶. As a member of the *Annonaceae* family, *D. tripetala* is prevalent in the tropical rainforests of Nigeria⁷. Its fruits, which transition from green to pink as they ripen, boast an alluring spiciness. Traditional medicine practices have documented the use of *D. tripetala* seeds in the treatment of various ailments, including cough, fever, diarrhea, and rheumatism⁸. Exploring the potential therapeutic properties of this plant holds the promise of advancing our understanding of natural remedies for diabetes while also uncovering new avenues for managing related conditions, particularly those impacting the liver.

In essence, diabetes mellitus represents a multifaceted challenge in modern healthcare, and the pursuit of novel therapeutic strategies draws attention to the potential of medicinal plants like *D. tripetala*. With its rich history in traditional medicine and the emerging insights into its biological properties, this natural resource could herald a new era of holistic and potentially more benign interventions for diabetes and its complex array of ramifications.

MATERIALS AND METHODS

Sampling and authentication process

Fresh seeds of *D. tripetala* were procured from a farm situated in Lodu-Ndume, Umuahia North LGA, Abia State, Nigeria. These seeds underwent verification and validation by the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture, Umudike.

Sample preparation and extraction

The plant specimens were subjected to an air-drying procedure spanning three weeks. Subsequently, the samples were meticulously ground into a fine powder using a blender. The methanol extract was acquired by immersing 700 g of powdered leaves in an 80% methanol solution (to state the grade). This mixture was then subjected to a 72 hours of fermentation process, during which the container underwent periodic agitation (to include the speed) to ensure thorough

mixing. To prevent air leakage, the container employed for this process was hermetically sealed. The mixture then underwent filtration and subsequent concentration utilizing a rotary evaporator, culminating in the acquisition of *D. tripetala* seed. The method used was modified in a previous study⁶.

Animal subjects for experimentation and ethical issues and approval

A total of thirty (30) adult male albino rats, weighing between 110 and 130 g were sourced from the animal facility within the Department of Veterinary Medicine at MOUAU, Nigeria. They were granted full access to water in an aerated environment. The rats were accommodated in stainless steel cages and given a two-week acclimatization period prior to the commencement of the study. Adherence to the principles and protocols of laboratory animal care as outlined by the NRC (2011) was maintained throughout the study. All the experimental procedures undertaken in this study were subjected to scrutiny and approval by the Research and Ethics Committee of MOUAU.

Induction of experimental diabetic state

For the induction of the experimental diabetic condition, a freshly prepared dose of alloxan monohydrate (Sigma Ltd., USA), equivalent to 120 mg/kg was administered. Blood samples were collected after a lapse of 72 hours to ascertain the establishment of diabetic conditions.

Design of experimental groups

A: (Control group): Administered only distilled water

B: (Negative control): Induced diabetic condition without treatment

C: (Positive control): Induced diabetic condition, treated with glibenclamide

D: Treated with 100 mg/Kg of DTSE for induced diabetic condition

E: Treated with 200 mg/Kg of DTSE for induced diabetic condition

F: Treated with 400 mg/Kg of DTSE for induced diabetic condition

Blood glucose level measurement

The animals' blood glucose concentrations were assessed through employment of a Glucometer Accu-check (Tyson Bio Evolve glucometer, Tyson Bioresearch Inc., Hangzhou, China). Subsequently, these measurements were conducted on a weekly basis at days 0, 7, 14, and 21 during the entire treatment period involving the standard drug and the extract⁹.

Analysis of biochemical parameters

The total cholesterol content was quantified using the enzymatic colorimetric chod-pap test. Concurrently, the determination of triglyceride levels was carried out spectrophotometrically, utilizing the Tietz method¹⁰. Assessment of high-density lipoproteins (HDL) was executed according to the technique devised by Grove (10) LDL levels were determined by subtracting the cholesterol concentration present in the supernatant from the overall cholesterol levels¹¹.

Statistical interpretation

The data acquired were presented as the Mean \pm Standard Error of the Mean (SEM). To compare the means, a one-way analysis of variance (ANOVA) was

implemented. Statistically significant distinctions between means were observed at a significant level of $p < 0.05$.

RESULTS

The treatment group exhibited significantly lower glucose levels after one week. A noteworthy reduction in glucose levels was observed in group C compared to group B in week 1. By the second week, groups D, E, and F exhibited a further significant decline compared to group B. Group F showed the highest reduction

(100.0±42.16) in blood glucose level after week 3, followed by group E (107.0±20.12) which is pretty the same with glibenclamide. This suggests DTSE's potential for beta cell regeneration leading to increased insulin secretion or enhanced glucose uptake through improved insulin sensitivity. Also, the medicinal plant could act as this condition by improving peripheral glucose uptake. Our finding showed some potential for controlling diabetes through significant antihyperglycemic activity at a concentration of 100, 200, and 400 mg/kg body weight when compared to the Glibenclamide (Standard drug).

Table 1: Mean of the blood glucose levels (mg/dl) of all the experimental groups treated with *D. tripetala* seed extract.

Group	Week 0	Week 1	Week 2	Week 3
A (Normal control)	110.3±1.34	100.3±1.43	109.1±1.14	111.2±1.23
B (Negative Control)	456.0±12.36*	546.0±23.31*	416.0±32.36*	356.0±42.65*
C (Positive Control)	410.0±22.62* ^d	199.0±22.30* ^d	126.0±12.01* ^d	107.0±11.04 ^d
D 100 mg/Kg B. WT of DTSE	356.0±32.12* ^d	206.0±12.36* ^d	197.0±52.11* ^d	122.0±13.06 ^d
E 200 mg/Kg B. WT of DTSE	377.0±12.35* ^d	216.0±21.13* ^d	196.0±32.43* ^d	107.0±20.12 ^d
F 400 mg/Kg B. WT of DTSE	387.0±31.35* ^d	205.0±12.15* ^d	134.0±31.12* ^d	100.0±42.16 ^d

Values were expressed as mean±SEM. *Significant difference at $p < 0.05$ compared to group A and (d) Indicates a significant difference at $p < 0.01$, compared to group B. n= 5 animals.

The values were reported as the mean ± standard error of the mean (SEM). * Significant difference observed at a p -value < 0.05 when comparing to group A, while (d) significant difference was observed at a p -value < 0.01 when comparing to group B. The sample size consisted of five animals. Diabetic rats treated with DTSE (100, 200, and 400 mg/kg b.w.) experienced reduced blood glucose levels (Table 1).

Table 2 presents the activities of liver function markers in rats administered *D. tripetala* methanol seed extract and is indicative that comparative to negative control group, there was a significant decrease in liver ALP in rats administered 100 mg/kg, 200 mg/kg and 400 mg/Kg body weight respectively. Similar trends were observed in significant decrease in serum AST for 100 mg/Kg, 200 mg/Kg and 400 mg/Kg respectively

when compared to the negative control group. ALT activities also showed significant decrease in serum for 100 mg/Kg, 200 mg/kg and 400 mg/Kg when compared to the negative control.

Table 3 presents the activities of kidney function markers in rats administered *D. tripetala* methanol seed extract and is indicative that comparative to negative control group, there was a significant decrease ($p < 0.05$) in serum creatinine of all the groups treated with DTSE (100 mg/kg, 200 mg/Kg and 400 mg/kg) respectively. Group E treated with 400 mg/kg body weight showed the highest reduction level (0.79±0.08) which is similar with the group treated with standard drug (Glibenclamide) when compared to the negative control.

Table 2: Effect of *D. tripetala* methanol seed extract effect on liver markers (ALP, AST and ALT).

Treatment	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Group A (Normal Control)	11.28±0.65*	51.87±2.63*	5.12±0.37*
Group B (Diabetic control)	53.61±1.37	84.20±1.11	17.07±0.38
Control C (Glibenclamide)	19.28±1.01*	56.13±2.54	12.15±0.85*
Group D (DTSE, 100 mg/kg)	25.56±1.19*	62.73±3.17*	9.17±0.38*
Group E (DTSE, 200 mg/kg)	21.78±0.40*	66.60±0.46*	11.93±0.46*
Group F (DTSE, 400 mg/kg)	19.67±2.61*	56.93±1.75*	12.68±1.07*

Values were expressed as mean±SEM. *Significant difference at $p < 0.05$ when compared with the negative control group, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase

Table 3: Effect of *D. tripetala* methanol seed extract on serum kidneyfunction marker (creatinine, urea and urea-creatinine ratio).

Treatment	Creatinine (mg/dL)	Urea (mg/dL)	Urea-Creatinine ratio $\mu\text{mol/L}$
Group A(Normal Control)	1.12±0.08*	27.25±2.39*	64.26±0.49*
Group B(Diabetic control)	2.42±0.03	98.53±1.07	101.78±3.15
Control C(Glibenclamide)	0.79±0.08*	26.79±2.28*	68.17±2.19
Group D (DTSE, 100 mg/kg)	0.96±0.03*	32.67±0.40*	64.57±0.62*
Group E (DTSE, 200 mg/kg)	1.01±0.05*	23.14±1.49*	62.65±6.56*
Group F (DTSE, 400 mg/kg)	0.79±0.08*	27.07±1.34*	67.87±0.81*

Values were expressed as mean±SEM. *Significant difference at $p < 0.05$ when compared with the negative control group.

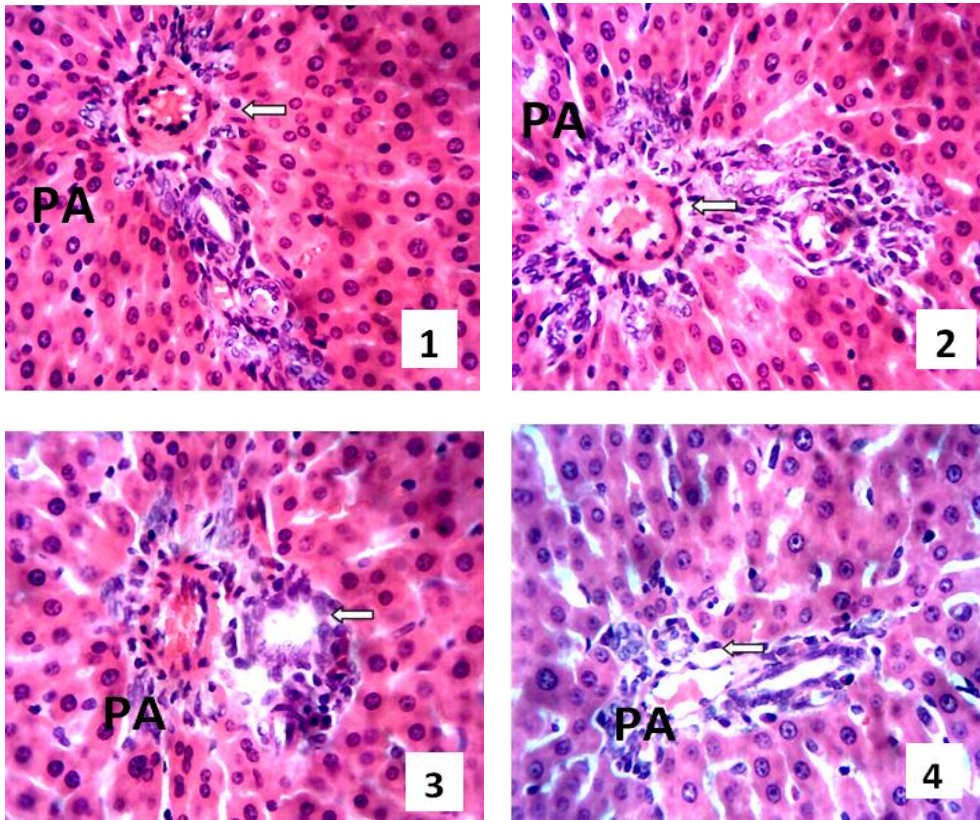


Plate 1: Photomicrograph of liver section of rat groups.

1 (normal control), 2 (100 mg/kg body weight of DTSE) 3 (200 mg/kg body weight of DTSE) and 4 (400 mg/kg body weight of DTSE) showing the portal area (PA) with normal histological features of the hepatic vein, hepatic portal vein and bile ductules. The hepatocytes are apparently normal (arrows). H and E stain, ×400

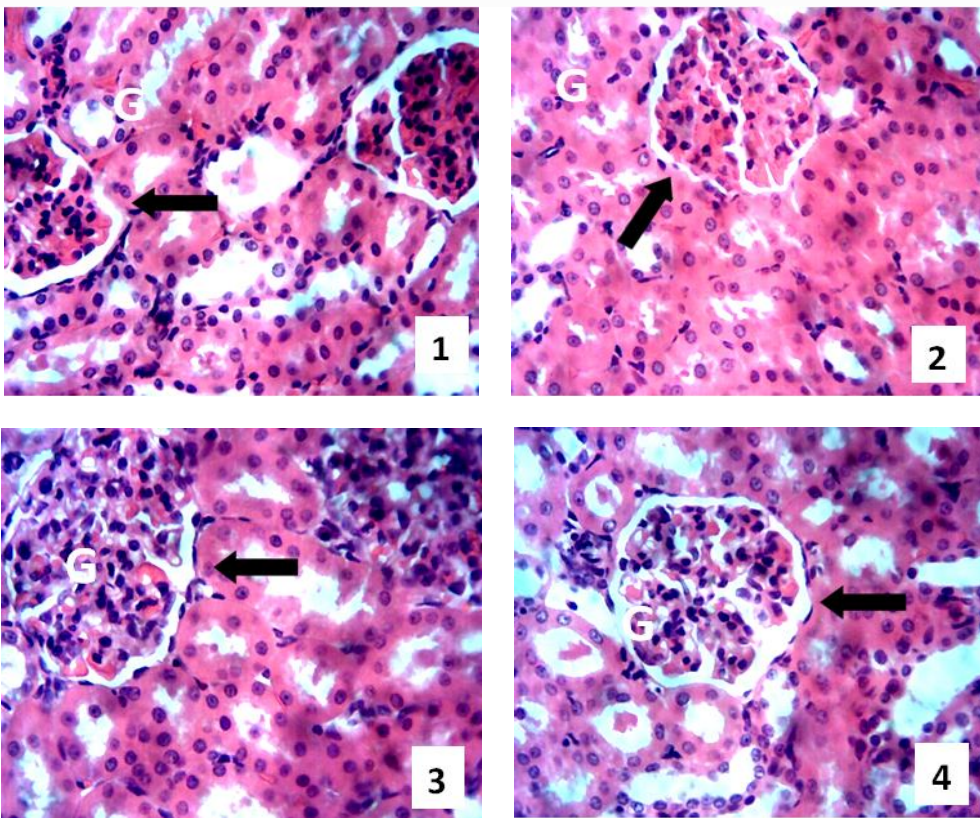


Plate 2: Photomicrograph of kidney section of rat groups.

1 (normal control), 2 (100 mg/kg body weight of DTSE) 3 (200 mg/kg body weight of DTSE) and 4 (400 mg/kg body weight of DTSE) showing apparently normal glomerulus (G) and renal tubular epithelium (arrows). H and E stain, ×400.

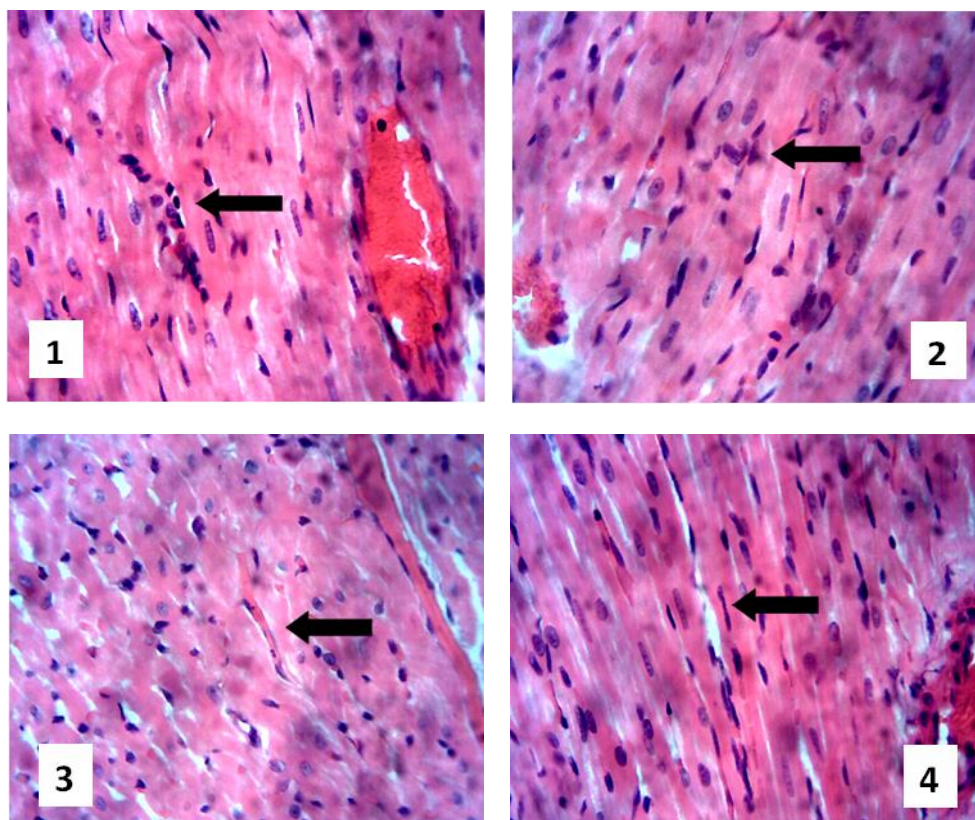


Plate 3: Photomicrograph of cardiac tissue section of rat groups.

1 (normal control), 2 (100 mg/kg body weight of DTSE) 3 (200 mg/kg body weight of DTSE) and 4 (400 mg/kg body weight of DTSE) showing apparently normal striated muscle fibres (arrows). H and E stain, $\times 400$

There was significant decrease in serum urea for 100 mg/kg, 200 mg/kg and 400 mg/kg respectively when compared to the negative control group. Group E treated with 400 mg/kg body weight showed the highest reduction level (27.07 ± 1.34) which is similar with the group treated with standard drug (Glibenclamide). Urea-creatinine activities also showed significant decrease in serum kidney for 100 mg/kg, 200 mg/kg and 400 mg/kg when compared to the negative control. The DTSE has great potential in the management of kidney disease since decrease in the creatinine levels of the experimental rats. Also, the extract reduced the level of the urea in the experimental rats which was able to protect the kidney from kidney dysfunction.

An examination of liver, kidney and cardiac tissue through histopathological analysis, following exposure to varying DTSE doses, reveals encouraging safety outcomes. Liver tissue from animals receiving 100, 200, and 400 mg/kg DTSE doses display no negative histological changes, signifying the absence of detrimental impacts on hepatic structures. Similarly, kidney tissues within these groups present sustained glomerular integrity but the group treated with (DTSE, 400 mg/kg) showed mild degeneration of the renal tubular epithelium (black arrows). Cardiac tissues showcase typical striated muscle fibers, indicating DTSE's non-harmful influence on heart tissue. These favorable outcomes could potentially be attributed to the diverse active constituents within DTSE. These findings augment the accumulating evidence that bolsters DTSE's safety profile when used in moderated quantity. Although comparable investigations may

have explored DTSE's effects on different organs or employed varying dosages, this current inquiry bolsters the comprehension of DTSE's organ-specific repercussions. Future research may extend to encompass more exhaustive toxicity evaluations, metabolic profiling, or investigations on human cell lines, delving further into the extract's safety attributes and mechanisms of action. Overall, the demonstrated lack of adverse effects on liver, and cardiac tissues but not on the kidney tissues.

DISCUSSION

The study presented findings indicating the potential antihyperglycemic, hypo-lipidemic and anti-atherogenic effects of DTSE in alloxan-induced diabetic rats. Alloxan-induced diabetes involves the alteration of pancreatic beta cells responsible for insulin production¹². *In vitro* studies have highlighted alloxan's toxicity to these cells, causing necrosis due to reactive oxygen species and elevated calcium levels^{13,14}. Lower alloxan doses (120 mg/kg b.w.) partly damaged beta cells leading to permanent diabetes but with the potential for cell regeneration¹⁵. Glibenclamide, a second-generation sulfonylurea achieves its anti-hyperglycemic effect through multiple mechanisms. It stimulates insulin release from pancreatic beta cells, enhances insulin availability by reducing hepatic clearance, and suppresses glucagon secretion, lowering glucose release¹⁶. The pattern of blood sugar reduction observed with DTSE compared to the positive control suggests potential shared pharmacological activity.

The lipid profile serves as a diagnostic indicator for cardiovascular disorders and, to a lesser extent, diabetes and cancer^{17,18}. Plant components like fruits and leaves encompass a diverse array of antioxidants with free radical scavenging attributes¹⁹. These antioxidants aid in converting reactive oxygen species (ROS) into less reactive forms, defending against the free radicals generated due to metabolic processes²⁰. An examination of liver, kidney, and cardiac tissue through histopathological analysis, following exposure to varying DTSE doses, reveals encouraging safety outcomes. Liver tissue from animals receiving 100, 200, and 400 mg/kg DTSE doses display no negative histological changes, signifying the absence of detrimental impacts on hepatic structures. Similarly, cardiac tissues showcase typical striated muscle fibers, indicating DTSE's non-harmful influence on heart tissue²¹. Kidney tissues, the group treated with (400 mg/kg body weight of DTSE) showed mild degeneration of the renal tubular epithelium (black arrows) and renal tubular epithelium which could indicate tissue damage causing kidney failure or acute renal damage. Cardiac tissues showcase typical striated muscle fibers, indicating DTSE's non-harmful influence on heart tissue. These favorable outcomes could potentially be attributed to the diverse active constituents within DTSE²². These findings augment the accumulating evidence that bolsters DTSE's safety profile. Although comparable investigations may have explored DTSE's effects on different organs or employed varying dosages, this current inquiry bolsters the comprehension of DTSE's organ-specific repercussions²³. Future research may extend to encompass more exhaustive toxicity evaluations, metabolic profiling, or investigations on human cell lines, delving further into the extract's safety attributes and mechanisms of action²⁴. Overall, the demonstrated lack of adverse effects on liver and cardiac tissues underscores the latent safety and therapeutic applicability of DTSE but could cause kidney damage when used for long term treatment²⁵.

CONCLUSIONS

In conclusion, our data show that DTSE possesses hypoglycemic and hepatoprotective effects and may suggest the idea that this medicinal plant could be a potential therapy the management of diabetes and associated disorders but could impose threat to the kidney organ of the body when used in high doses or prolonged treatment, so caution should be applied when using the extract for the treatment of diabetes.

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AUTHOR'S CONTRIBUTIONS

Alaabo PO: designed and supervised the study. **Egbuonu ACC:** reviewed and co-supervised the study. **Achi NK:** writing original draft. **Obike CA:** literature search. **Ukpabi-Ugo JC:** statistical analysis. **Ezeigwe OC:** interpreted the results. **Chukwu UD:** conceptualization. **Wisdom CE:** data collection. All the authors approved the finished version of the finished version of the manuscript.

DATA AVAILABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICTS OF INTERESTS

None to declare

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