

*Full Length Research Paper*

# **Ethanol extracts of *Terminalia catappa* leaves and *Persea americana* seed attenuate renal damage associated with Streptozotocin-induced diabetic rats**

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**Kidney is an essential organ responsible for excretion and detoxification. The use of streptozotocin in rat model experiments has provided rooms for researchers to test the effectiveness of medicinal plants that might possess nephroprotective and antidiabetic properties; so as to help in the treatment of diabetes complications. The study was aimed to evaluate the attenuating effects of ethanol extracts of *Terminalia catappa* leaves and *Persea americana* seed on renal damage associated with streptozotocin-induced diabetic rats. Thirty male wistar rats were randomly distributed into six groups (n = 5). Group 1: (Distilled water only); Group 2: 80 mg/kgbw streptozotocin; Group 3: (80 mg/kgbw streptozotocin + 200 mg/kgbw T. catappa leaf extract); Group 4: (80 mg/kgbw streptozotocin + 200 mg/kgbw *Persea americana* seed extract); Group 5: (80 mg/kgbw streptozotocin + 200 mg/kgbw extracts-mixture); Group 6: (80 mg/kgbw streptozotocin + 5 mg/kgbw glibenclamide (standard drug)). Streptozotocin was administered intraperitoneally, and glibenclamide orally. Blood sample was collected for biochemical analyses, and kidney for histopathology. Extracts of the T. catappa leaves and P. americana seed contributed significantly (p < 0.05) in bringing the levels of serum creatinine and urea; activities of alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) to normalcy. Both plant extracts equally produced significant (p < 0.05) regeneration of kidney cells.**

**Key words:** Nephroprotective, antidiabetic, avocado pear, diabetes mellitus, indian almond, persea americana, streptozotocin, terminalia catappa.

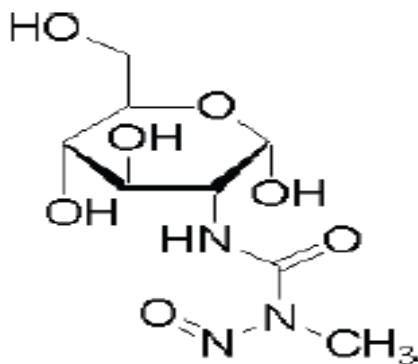
## **INTRODUCTION**

Diabetes mellitus (DM) is a known metabolic disorder connected with kidney impairment, high blood sugar

levels (hyperglycemia) over a prolonged period, frequent urination (polyuria), thirst (polydipsia), and increased

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**Scheme 1.** Streptozotocin.  
Source: Sithole (2009).

hunger (polyphagia), inadvertent weight loss if left untreated, delay in healing of wounds, itchy skin and vision impairment (El-Wakf et al. 2011; Adeyi et al., 2015). DM could cause complications such as ketoacidosis, stroke, foot ulcers, or even death (Dodda and Ciddi, 2014). Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced (Rolo and Palmeira, 2006; Akinsanya et al., 2021).

The kidney plays a key role in detoxifying and excreting harmful chemical toxicants ingested as drugs or food. This has greatly been helpful in maintenance of homeostasis and normal cellular function.

Streptozotocin (Scheme 1) is an organic substance used as therapeutic agent in the treatment of some cancers of the islets of Langerhans. It that has been reported to be toxic to the insulin-producing  $\beta$ -cells and nucleic acids of pancreas and some other vital organs like kidney and liver in mammals, at overdose (Brentjens and Saltz, 2001). The use of streptozotocin in experiments has given more rooms for researchers to test the effectiveness of new drugs that could exert hypoglycemic and tissue repair in the general treatment of diabetes and its associated complications (Sithole, 2009).

However, streptozotocin ( $C_8H_{15}N_3O_7$ ) is a glucosamine-nitrosourea compound that has a similar structure with glucose molecule and forms an N-N bond in the N-nitrosourea (a part of the structure that is responsible for its biological and pharmacological interaction). It can be transported into glucose needing cells by a glucose transport "GLUT2", though not recognized by other glucose transport proteins (Sithole, 2009).

Over the past centuries, phytomedicine has become a thing of worldwide significance with medicinal and economic importance for treating different diseases. The use of medicinal plants, from leaves, fruit, stem to roots have contributed impressively to well-being (Palombo, 2006; Jachak and Saklani, 2007); subsequently, the use of medicinal plants, for decades now has been accepted

as complementary medical option to improve the health conditions of the folk through decreasing the adverse effects and costs of synthetic medicines (Rouhi-Boroujeni et al., 2015; Hassani et al., 2016).

Moreover, over 80% of the populations in developing countries use traditional medicinal plants to treat a variety of metabolic disorders and diseases like diabetes, dyslipidemia, obesity and hypertension amongst others (Rouhi-Boroujeni et al., 2015). Herbs are regarded to be safe as they exist naturally, and there is limited evidence regarding the use of herbal medicines during pregnancy and breastfeeding among mothers (Izzo et al., 2016).

*T. catappa* and *P. americana* plants are widely grown in tropical regions of the world as ornamentals, grown for the deep shade their leaves provide. Fruits from both plants are edible. However, *T. catappa* fruit tastes slightly acidic, and its leaves extract has shown activity against *Plasmodium falciparum* chloroquine (CQ)-resistant (FcB1) and CQ-sensitive (HB3) strains (Adeyemi and Oluwasegun, 2019). Also, several studies have revealed that *P. americana* seed may improve dyslipidemia, and be useful in the treatment of diabetes and other associated conditions (Dabas et al., 2013).

Many available synthetic drugs administered for patients with diabetes and impaired kidney function are costly, and some may not be recommended for pregnant or nursing mothers. Hence, this study aimed to investigate the attenuating effects of ethanol extracts of *T. catappa* leaves and *P. americana* seed on some kidney function biomarkers (serum creatinine and urea levels; activities of alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST) and histology of kidney) in streptozotocin-induced rat model.

## MATERIALS AND METHODS

### Collection of plant materials and preparation of extracts

Tender leaves of *T. catappa* (Indian almond plant) and matured fruit of *P. americana* (avocado) were collected from Bells University of Technology, Sango-ota, Ogun State, Nigeria. Leaves of *T. catappa* and seed obtained from the fruit of the *P. americana* were shade dried until they became crispy, after which they were pulverized in a clean and dry grinding machine. Approximately 200 g of the powdered leaves and seed obtained, each was soaked into 2000 ml of 78 % ethanol for 48 h (Tabeshpour et al., 2017; Akinsanya et al., 2021). The extracts were filtered through Whatmann No. 1 filter paper. Filtrates were dried in a hot air oven at 45°C till a semi solid mass was produced.

### Experimental animals

Thirty (30) male wistar rats, weighing approximately (150 -180 g) were purchased from a local rearer at Abeokuta, Ogun state. They were kept in cages covered with net in a standard environment (12:12 hr light: dark cycle) and acclimatized for two weeks prior the commencement of the experiment. The rats were fed *ad libitum* with pellet feed; their beddings were changed every 24hrs in order to maintain cleanliness and prevent accumulation of ammonia gas.

**Table 1.** Experimental design.

Groups (n = 5)	Treatments
1	Distilled water only (negative control)
2	Streptozotocin (80 mg/kgbw) and untreated
3	Streptozotocin (80 mg/kgbw) + <i>T. catappa</i> (200 mg/kgbw)
4	Streptozotocin (80 mg/kgbw) + <i>P. americana</i> (200 mg/kgbw)
5	Streptozotocin (80 mg/kgbw) + <i>T. catappa</i> and <i>P. americana</i> (200 mg/kgbw)
6	Streptozotocin (80 mg/kgbw) + glibenclamide (5 mg/kgbw)

The rats were considered diabetic with blood glucose > 200 mg/dL.

### Grouping and exposure of the rats

Thirty wistar rats were randomly divided into six groups (n = 5). Excluding the negative control, all other groups were exposed to a single dose (80 mg/kgbw) of streptozotocin according to the method described by Dino et al. (2017), intraperitoneally. The blood glucose and body weight of the experimental rats were monitored for 1 week. Thereafter, the rats were treated orally with 200 mg/kgbw ethanol extracts of the leaves of *Terminalia catappa* (Ravalya et al., 2013) and *Persea americana* seed (Okon et al., 2018), and 5 mg/kgbw of glibenclamide orally (Daye et al., 2013) for 21 days as shown in Table 1.

### Sacrifice of animals

On the 22nd day of the experiment, the rats were subjected to a 12 h food fast, after which they were sacrificed by cervical dislocation.

### Collection of blood and organs

Blood sample was collected into plain tubes, allowed to stand for few min and centrifuged at 3000 rpm for 15 min to obtain serum. Serum obtained was used for selected biochemical analyses. Kidney was excised and preserved in 10% formalin solution in readiness for histopathological study.

### Biochemical analysis

The serum obtained was analyzed to determine the creatinine and urea levels according to the method described by Bartels and Bohmer (1972); activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), using the methods described by Arhoghro et al. (2009) for ALP activity determination; Frankel and Reitman (1984) for AST and ALT activities determination, following Randox kits manufacturer's instructions.

### Histopathology

Histopathology reveals the microscopic study of tissue(s). It is performed by examining a thin section of a tissue under a light microscope. The tissue is fixed in 10% formalin, embedded in paraffin, and then manually sectioned with a microtome to obtain 4-5 µm thick paraffin sections. Dewaxed sections are stained with hematoxylin and Eosin blue (H and E). After staining, a very thin glass is placed over the tissue section to protect it and to enhance optical evaluation of the tissue.

### Statistical analysis

All values were expressed as the mean ± standard error of mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range tests by statistical software package (SPSS, version 17.00). Significant means were separated at  $p < 0.05$ . Alphabetic superscripts indicate statistical differences.

## RESULTS

Table 2 showed effects of streptozotocin, *T. catappa* leaves and *P. americana* seed ethanol extracts on serum creatinine and urea levels (mg/dL) of the experimental rats. The creatinine level in serum of the positive control (2) showed a significant increase ( $p < 0.05$ ) when compared to the negative control (1). No significant difference ( $p > 0.05$ ) was observed in the creatinine levels in serum of groups 4, 5 and 6, when compared to the negative control (1). Group 3 showed a significant decrease ( $p < 0.05$ ) towards the negative control when compared to positive control (2).

A significant increase ( $p < 0.05$ ) was observed in the urea level of positive control (2) when compared with the negative control (1). No significant difference ( $p > 0.05$ ) was observed in the urea levels in serum of groups 3, 4 and 6, when compared to the negative control (1). Group 5 showed a significant decrease ( $p < 0.05$ ) towards the negative control when compared to the positive control (2).

In Table 3, effects of streptozotocin, *T. catappa* leaves and *P. americana* seed ethanol extracts on serum ATP, ALT and AST were shown. The positive control (2) showed a significant increase ( $p < 0.05$ ) in ALP activity, when compared to the negative control (1). Group 3 showed no significant difference ( $p > 0.05$ ) in ALP activity when compared with the negative control (1). Groups 4, 5 and 6 showed a significant decrease ( $p < 0.05$ ) towards the negative control level when compared to the positive control (2).

There was a significant increase ( $p < 0.05$ ) in the ALT activity of the positive control (2) when compared to the negative control (1). Groups 3, 4, 5 and 6 showed significant decrease ( $p < 0.05$ ) towards the negative control when compared to the positive control (2).

**Table 2.** Effects of streptozotocin, *T. catappa* leaves and *P. americana* seed ethanol extracts on serum creatinine and urea levels (mg/dL).

Group	Creatinine	Urea
1	40.33±2.73 <sup>a</sup>	5.10±0.50 <sup>a</sup>
2	53.00±2.00 <sup>c</sup>	6.96±0.23 <sup>c</sup>
3	45.77±1.14 <sup>b</sup>	5.20±0.00 <sup>a</sup>
4	42.67±0.88 <sup>a</sup>	5.20±0.40 <sup>a</sup>
5	37.67±3.18 <sup>a</sup>	5.70±0.10 <sup>b</sup>
6	36.00±4.16 <sup>a</sup>	5.20±0.10 <sup>a</sup>

Data were expressed as mean ± SEM ( $n = 5$ ). Values having different alphabetic superscripts within the same column are significantly different ( $p < 0.05$ ). Group 1: Distilled water only (negative control); Group 2: Streptozotocin (80 mg/kgbw) and untreated; Group 3: Streptozotocin (80 mg/kgbw) + *T. catappa* (200 mg/kgbw); Group 4: Streptozotocin (80 mg/kgbw) + *P. americana* (200 mg/kgbw); Group 5: Streptozotocin (80 mg/kgbw) + *T. catappa* and *P. americana* (200 mg/kgbw); Group 6: Streptozotocin (80 mg/kgbw) + glibenclamide (5 mg/kgbw)

**Table 3.** Effects of streptozotocin, *T. catappa* leaves and *P. americana* seed ethanol extracts on serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities of the experimental rats (g/L).

Group	ALP	ALT	AST
1	25.67±3.48 <sup>a</sup>	34.67±9.33 <sup>a</sup>	65.33±3.53 <sup>a</sup>
2	46.67±4.41 <sup>c</sup>	67.67±9.06 <sup>c</sup>	89.00±17.35 <sup>b</sup>
3	32.00±0.00 <sup>a</sup>	34.67±2.67 <sup>b</sup>	69.00±7.00 <sup>a</sup>
4	35.00±0.00 <sup>b</sup>	35.00±2.00 <sup>b</sup>	65.00±8.66 <sup>a</sup>
5	34.33±3.67 <sup>b</sup>	34.33±3.67 <sup>b</sup>	51.00±13.05 <sup>a</sup>
6	35.22±1.45 <sup>b</sup>	38.33±1.67 <sup>b</sup>	86.67±3.33 <sup>b</sup>

Data were expressed as mean ± SEM ( $n = 5$ ). Values having different alphabetic superscripts within the same column are significantly different ( $p < 0.05$ ). Group 1: Distilled water only (negative control); Group 2: Streptozotocin (80 mg/kgbw) and untreated; Group 3: Streptozotocin (80 mg/kgbw) + *T. catappa* (200 mg/kgbw); Group 4: Streptozotocin (80 mg/kgbw) + *P. americana* (200 mg/kgbw); Group 5: Streptozotocin (80 mg/kgbw) + *T. catappa* and *P. americana* (200 mg/kgbw); Group 6: Streptozotocin (80 mg/kgbw) + glibenclamide (5 mg/kgbw).

The positive control (2) and Group 6 showed significant increase ( $p < 0.05$ ) in AST activities when compared to the negative control (1). Groups 3, 4 and 5 showed no

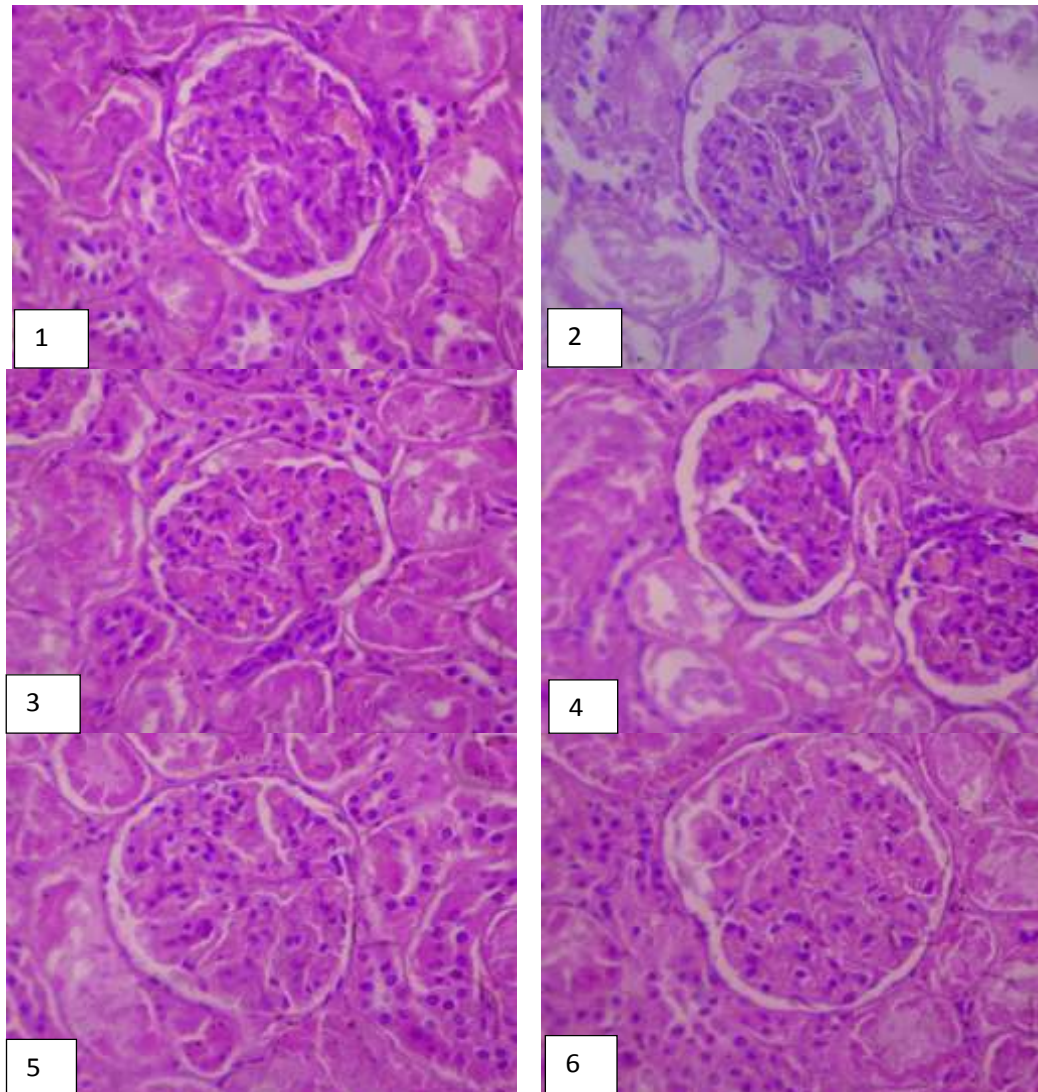
## DISCUSSION

For decades now, several medicinal plants have been used as an accepted complementary medical option to improve health conditions and decrease the cost effects of the synthetic drugs (Rouhi-Boroujeni et al., 2015; Hassani et al., 2016). In developing countries, more than 80 % of the population now practices herbal medicine to treat several disease conditions (Rouhi-Boroujeni et al., 2015). Generally, herbs are regarded to be natural and safe (Izzo et al., 2016); hence, the attenuating effects of ethanol extracts of *T. catappa* leaves and *P. americana* seed were investigated on a renal damage associated

significant difference ( $p > 0.05$ ) in AST activities when compared with the negative control (1) (Figure 1).

with streptozotocin-induced diabetic rats. Creatinine and urea levels, including alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important biomarkers which activities are important in the diagnosis of kidney and other tissue damage caused during toxicity, injury or infection.

An increase in creatinine and urea concentrations; ALP, ALT and AST activities in serum of streptozotocin exposed group compared with the negative control was as a result of streptozotocin toxicity, which must have caused renal damage and renal dysfunction. Treatment with *T. catappa* leaves extract and *P. americana* seed extract contributed to the decrease in the concentrations and activities of these parameters in the experimental rats. This is in line with the the work of Ezejiofor et al.



**Figure 1.** Effects of streptozotocin, *T. catappa* leaves and *P. americana* seed ethanol extracts on the kidney of the experimental rats: Negative control (1): kidney showing normocellular glomerular tufts disposed on a background containing renal tubules, and compact tissues. No abnormality was observed in group 1; Positive control (2): kidney showing profuse infiltration by inflammatory cells, areas with dissolved cells and an absence of glomerulus; Group 3 (administered 80 mg/kgbw streptozotocin + 200 mg/kgbw *T. catappa* leave extract): kidney showing Bowman's space, glomeruli and no inflammatory cells; Group 4 (administered 80 mg/kgbw streptozotocin + 200 mg/kgbw *P. americana* seed extract): kidney showing Bowman's space, glomeruli and no inflammatory cells; Group 5 (administered 80 mg/kgbw streptozotocin + extracts-mixture): kidney showing glomeruli and a near compact kidney architecture; Group 6 (administered 80 mg/kgbw streptozotocin + 5 mg/kgbw glibenclamide): kidney showing glomeruli and a near compact kidney architecture (Magnification:  $\times 400$ ).

(2013); Mahadeva et al. (2011) which concentrated on the mechanism of the antidiabetic activities of *T. catappa* leaked into the blood circulation and serve as biomarkers of kidney injury. The histopathological study of the kidney in the group exposed to streptozotocin showed cellular infiltration, tubular damage, hardening of tissues and vascular congestion compared with negative control (where no abnormality was observed). On treatment with *T. catappa* leaves and *P. americana* seed extracts, a positive association with the negative control was

and *P. americana* respectively. ALT and AST measure the activities of intracellular kidney enzymes that have been observed in the kidney of treated groups. This study confirmed that leaves of *T. catappa* and *P. americana* seed extracts attenuate kidney function impairment, damage to tissue and renal dysfunction. This is in line with the study of Edem et al. (2009), that extracts of both *T. catappa* and *P. americana* equally had significant healing effects and reversed the histopathological damage that occurred to rat kidney on exposure to

streptozotocin, comparable with the effects of glibenclamide (Ezejiofor et al., 2013; Akinsanya et al., 2021).

## Conclusion

Conclusively, leaves of *T. catappa* and *P. americana* seed could attenuate renal damage caused by exposure to streptozotocin.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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