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Ameliorative Effect of Aqueous Extracts of Seeds of *Delonix regia* (Hook) Raf on the Liver, Kidney and Spleen of High-fat Diet Streptozotocin-induced Diabetes in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Plants have been relied upon by people for treatment, control and management of diabetes. The local residents around University of Port Harcourt, Nigeria, use cooked *Delonix regia* (Hook.) Raf. Seeds as food supplement for the management of diabetes.
Aim: Based on the available evidence on this plant species, this study was carried out to evaluate the ameliorative potentials of aqueous cooked and uncooked seed extracts of *Delonix regia* (Hook.) Raf. On the liver, kidney and spleen of high-fat diet streptozotocin-induced diabetes in female Wistar rats.
Methods: Forty-eight rats were grouped into eight. Group 1 served as normal control and was fed with normal diet. The diabetic state was achieved by feeding the rats with high-fat diet which contained 20% sucrose, 20% lard and 60% grower mash for six weeks, followed by 40 mg/kg body weight of a single dose intraperitoneal injection of streptozotocin. Seven days after induction of

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diabetes, rats in group 2 did not receive any treatment and were designated as the negative control. Rats in groups 3 (Positive control) and 4 (Second positive control) received metformin 100mg/kg and metformin/vildagliptin 50/25 mg/kg body weight respectively as standard drugs while groups 5 to 8 designated (A1), (A2) (B1) and (B2) respectively, were induced and treated daily with 150 and 300 mg/kg body weight orally cooked seeds (A1), (A2) and uncooked seeds (B1), (B2) extracts for six weeks. Blood was obtained through cardiac puncture after the rats were anaesthetised and sacrificed. Histopathology of the liver, kidney and spleen were studied.

Results: Both extracts significantly ($p<0.05$) decreased the bilirubin, potassium, bicarbonate concentrations, alkaline phosphatase (ALP) and alanine transaminase (ALT) activities as well as granulocyte count in dose and time-dependent manner when compared to group 2 respectively. Photomicrographs of the spleen of diabetic untreated rats showed significant hemosiderin pigment deposition compared to the splenic architecture of the normal rats. Treatment with *Delonix regia* (Hook.) Raf extracts prevented hemosiderin pigment deposition in groups 5 to 8.

Conclusion: This study, therefore, provides useful resources about the ameliorative potentials of seed extracts of *Delonix regia* (Hook.) Raf. on streptozotocin-induced diabetes in female Wistar rats.

Keywords: Delonix regia (Hook.) Raf; streptozotocin; diabetes; liver; kidney; spleen.

ABBREVIATIONS

Met : Metformin
VDG : Vildagliptin
$LD_{(50)}$: Lethal dose

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia, glucose intolerance and caused by an imbalance between insulin demands or relative deficiency of insulin secretion with/without varying degree of insulin resistance. It is also a chronic diseases associated with development over time of micro-vascular and macro-vascular complications and neuropathies. It is reported to have an adverse effect on carbohydrate, lipid and protein metabolism resulting in chronic hyperglycaemia and abnormality of lipid profile leading to severe diabetic complications such as polyuria, polyphagia, ketosis, retinopathy as well as cardiovascular disorder [1,2,3]. Over the years, due to the high incidence of diabetes and other related diseases, search for drugs and dietary supplements gotten from plants have accelerated. The use of plant medicine has continued till today in China, India and many other countries in Africa and South America [4]. Medicinal plants are currently investigated worldwide by scientists such as biochemists, botanists, pharmacologists, microbiologists and natural product chemists for phytochemicals and lead compounds that could be developed for the treatment of diseases [5]. India, China and the USA are the top three countries in terms of number of hyperglycaemic patients [6]. Plants have been relied upon by people for nourishment and medicine for treatment control and management of varieties of diseases that have threatened their existence and survival dated back since the existence of human kind [7]. One of such plants used by the locals in management of diseases is the *Delonix regia* plant.

Delonix regia (Hook.) Raf. is a species in the genus Delonix (family Leguminosae). It is an accepted name derived from the record of the International Legume Database and Information Service (ILDIS), reported by Roskov et al. [8]. *Delonix regia* (Hook.) Raf also referred to as the royal *Poinciana flamboyant* or flame tree is reportedly used traditionally for management of various diseases such as diabetes [9], wound healing, ulcer, obesity and heart problems was used for this study. It is a well-known ethanomedical plant extract with its various parts performing several functions. It has been used for the treatment of various diseases; the flower is traditionally used as an antihelmintic [10], insecticides [11], gynaecological disorders or dysmenorrhoea, inflammation and diarrhoea [12]. The aqueous extract of the flower was used as an additive in herbal sunscreen formulation [13], while the back was reported to be traditionally used as antiperiodic febrifuge [10], biomonitor and bioaccumulators of atmospheric trace metals [14] and also for removal of inorganic nitrogen from aqueous solutions through adsorption [15]. The leaves are used for the treatment of bronchitis and pneumonia in infants [16], for anti-diabetic treatment [17], gastric problems, body and rheumatic pains treatment [18]. The seed of *Delonix regia* (Hook.) Raf contain flavonoids which are used as wound healing agent in

households [19]. Plant extract was reported to possess hepatoprotective ability [20]. The seed extract of *Delonix regia* (Hook.) Raf. at lethal dose $LD_{(50)}$, index for acute toxicity, is not toxic [21]. It has high Ca, Na, Mg, K, Fe and bioactive substances such as saponin, tannins, alkaloids, flavonoids, and phytate [22]. A study by Omoedu et al. [23], suggests that seed extract of *Delonix regia* (Hook.) Raf possess hypoglycaemic activity and may have the potential to reduced elevated serum enzyme activities caused by alloxan-induced diabetes. Out of every hundred people suffering from diabetes eighty percent are type 2 diabetic. It is estimated that by the year 2030, 438 million people (7.8%) of adult population will be diabetic as over 150 million people currently are living with diabetes mellitus [6]. Due to these shortcomings, researchers globally have continued to search for anti-diabetic remedies with the expectation of finding new natural products that could be used or developed into harmless, cheap and efficient anti-diabetic remedies. Furthermore, the local residents around University of Port Harcourt, Nigeria, also use cooked *Delonix regia* (Hook.) Raf. seeds as food supplement for the management of diabetes. Based on the available evidence on this plant species, this study was carried out to evaluate the ameliorative potentials of aqueous extracts of cooked and uncooked *Delonix regia* (Hook.) Raf. seeds on the liver, kidney and spleen of high-fat diet streptozotocin-induced diabetes in female Wistar rats.

2. MATERIALS AND METHODS

2.1 Reagent and Chemicals

Streptozotocin (Sigma-Aldrich, Germany), Randox assay kits (Randox Laboratories, Crumlin, County Antrim, England) Formaldehyde (90%), Chloroform (90%). All other reagents and apparatus were of analytical grades.

2.2 Procurement of Animal

Forty-eight (48) female Wistar rats weighing 100-130g were bought from animal house of Department of Biochemistry, University of Port Harcourt Choba, Rivers State. All animals used for this study were maintained according to the rules and regulations outlined in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures. The rats were weighed and divided into eight (8) groups of six (6) rats each and housed differently in a plastic cage covered with wire gauze. They were left to acclimatise for one week and fed with grower mash and access to clean water, *ad libitum*.

2.3 Collection of the *Delonix regia* (Hook.) Raf. Seeds and Sample Preparation

Seeds of *Delonix regia* (Hook.) Raf, were collected at the premises of the University of Port Harcourt Teaching Hospital (UPTH) Rivers State, Nigeria and were authenticated by a biotechnologist, Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria. Plant with seed samples were deposited at the University of Port Harcourt Herbarium. Its voucher specimen Number is UPH /V/1269. The pods from *Delonix regia* (Hook.) Raf. tree were collected and split open in order to abstract its seeds; the hard seeds were collected and ground into a fine powder. The ground fine powder for the uncooked sample was weighed and soaked in distilled water for 24 hours after which it was filtered. The powder for the cooked sample was also weighed, soaked in distilled water, cooked for 15mins and then filtered after cooling. The extracts were quantified by drying 1ml of the homogeneous filtrate in an oven at 40^0 C in a pre-weighed watch glass. This was based on the fact that most preparations used in traditional medicines are formulated in cold or hot water [24].

2.4 Experimental Design

Forty-eight (48) female Wistar rats weighing 100-130g were used for the study. The rats were weighed and divided into eight (8) groups of six (6) rats each and were housed differently in a plastic cage covered with wire gauze. They were left to acclimatise for one week and fed with grower mash and access to clean water, *ad libitum*. Rats in group 2 to 8 were fed with high-fat diet (20% sucrose+ 20% Lard + 60% grower mash) for six weeks and afterward injected with 40 mg/kg body weight (BW) streptozotocin in distilled water to induce diabetes [25]. After seven days of induction of diabetes treatment which lasted for 6 weeks started. Diabetes was not induced in rats in group 1 designated as normal control. They received normal feed and water. Group 2 received high fat diet + streptozotocin (40 mg/kg), group 3 received high fat diet HFD + streptozotocin (40 mg/kg) + Met (100 mg/kg) and group 4 received high fat diet HFD + streptozotocin (40 mg/kg) + Met +VDG

(50mg+25 mg)/kg. Groups 5 and 6 designated (A1) and (A2) were treated respectively with 150 and 300mg/kg BW cooked *Delonix regia* (Hook.) Raf. seeds while groups 7 and 8 designate (B1) and (B2) were treated respectively with 150 and 300 mg/kg BW of uncooked seeds extracts. Three rats from each group were sacrificed at the end of every 3 weeks. Histological evaluations of the liver, kidney and spleen were done after six (6) weeks. Blood was collected through a cardiac puncture into heparin bottles and the organs preserved with 10% formalin for histological analysis. The blood samples in heparin bottles were centrifuged, after which the supernatants were collected and designated plasma stored in the refrigerator (-4^{0}C) for further analyses.

2.5 Determination of Haematology Indices, Liver and Kidney/Electrolyte Biomarkers

Haematology test was done using the method described by Cheesbrough [26]. Total Bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Creatinine, Urea, Potassium, Sodium and Bicarbonate concentrations were analysed using methods as outlined in Randox assay kits (Randox Laboratories, Crumlin, County Antrim, England) and absorbance of parameters were measured using Commodity Visible Spectrophotometer (G. Bosch Germany).

Histopathological slides were prepared at Anatomical Pathology Laboratory, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State. Small tissues of liver, kidney and spleen were collected in 10% formalin for proper fixation. They were processed and embedded in paraffin wax. Section of 5µm in thickness was cut, mounted on a slide and stained with haematoxylin and eosin.

2.6 Slide Examination

The photomicrographs were taken and analysed at Anatomy Department Laboratory, Faculty of Basic Medical Science, Madonna University, Nigeria, Elele, Rivers State. The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 9.0 megapixels microscope camera at X200 and X400 magnifications.

2.7 Statistical Analysis

Data were expressed as mean ± S.E.M. (Standard error of the mean) and were analysed for statistical differences from test control groups of animals by One-Way Analysis of Variance (ANOVA). At $p<0.05$, differences between groups were considered statistically significant.

Data represent mean ± S.E.M., n = 3 per group. Superscripts “a, b” indicate significant differences ($p<0.05$) when the normal and negative control groups are compared to the induced and treated groups respectively. Superscripts “c, d” indicate significant differences ($p<0.05$) when the positive and second positive control groups are compared to the groups treated with extracts respectively.

3. RESULTS

3.1 Results of Haematological as Well as Biochemical Indices of the Liver, Kidney and Spleen are Presented in Tables 1 to 6

Diabetic-induced rats presented a significantly reduced packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb) concentration and platelet count. Reductions in PCV, RBC and Hb concentrations in extracts treated rats, were observed when compared to diabetic untreated rats and decreased significantly ($p<0.05$) when compared to normal control. Administration of extracts (cooked and uncooked) led to decrease in platelet counts in rats. WBC count and its’ differential lymphocyte were increased in diabetic untreated rats, however, administration of the extracts resulted in a reduction in the white blood counts and its related indices.

An elevated level of urea and decreased creatinine concentrations were observed in diabetic untreated rats when compared to normal control group. Administration of extracts effectively decreased and increased these markers respectively. The levels of Potassium and bicarbonate ions significantly increased in the untreated diabetic rats when compared to the normal control. The administration of the extracts of cooked and uncooked *Delonix regia* (Hook.) Raf. seeds significantly decreased the levels of these parameters in the diabetic treated rats when compared to the rats in the normal control group. There was a reduction in Sodium (Na^+) concentration in diabetic rats and this was significantly increased ($p<0.05$) following the oral administration of extracts when compared to normal control group.

Results obtained after administration of high-fat diet streptozotocin-induced diabetes showed that all the rats became diabetic after 7 days. The bilirubin concentration, ALT, and ALP activities in the serum were significantly ($p<0.05$) increased in the untreated diabetic rats while AST activities were significantly ($p<0.05$) reduced in the rats of the negative control group when compared to the normal control group.

Table 1. Effect of aqueous extracts of *Delonix regia* (Hook.) Raf. seeds, metformin/vildagliptin on red and white blood cell concentration of high fat diet streptozotocin-induced diabetes in female Wistar rats at weeks 3 and 6

Treatment group	Red blood cell ($\times10^6$/ml)		White blood cell ($\times10^9$/L)	
	Week 3	Week 6	Week 3	Week 6
Group 1	7.83 ± 0.16	7.83 ± 0.16	13.57 ± 2.51	13.57 ± 2.51
Group 2	7.23 ± 0.14	6.30 ± 0.05[a]	14.00 ± 2.80	10.10 ± 0.87
Group 3	6.73 ± 0.44	6.82 ± 0.05	11.63 ± 2.18	7.70 ± 0.29[ab]
Group 4	6.85 ± 0.44	6.60 ± 0.13	15.53 ± 1.74	9.60 ± 1.79 [ab]
Group 5 (A1)	7.13 ± 0.16	4.70 ± 0.03[abcd]	10.30 ± 0.51	3.87 ± 0.99 [abcd]
Group 6 (A2)	6.27 ± 0.72[a]	5.87 ± 0.02[a]	13.00 ± 2.83	10.27 ± 0.09[ac]
Group 7 (B1)	7.50 ± 0.28	5.70 ± 0.29[a]	11.83 ± 1.51	9.67 ± 0.90 [ab]
Group 8 (B2)	6.26 ± 0.30[a]	5.57 ± 0.13[a]	10.70 ± 3.87	6.27 ± 0.03 [abcd]

Table 2. Effect of aqueous extracts of *Delonix regia* (Hook.) Raf. seeds, metformin/vildagliptin on haemoglobin concentration and packed cell volume of high fat diet streptozotocin-induced diabetes in female Wistar rats at weeks 3 and 6

Treatment group	Hb (g/dl)		PCV (%)	
	Week 3	Week 6	Week 3	Week 6
Group 1	14.60 ± 0.00	14.60 ± 0.00	40.10 ± 0.29	40.10 ± 0.29
Group 2	14.20 ± 0.10	12.10 ± 0.17	39.40 ± 0.84	33.97 ± 0.15
Group 3	13.50 ± 0.38	17.33 ± 4.33	36.43 ± 1.48	36.10 ± 0.06
Group 4	13.63 ± 0.96	12.47 ± 0.03	37.37 ± 2.67	36.67 ± 0.38
Group 5 (A1)	13.67 ± 0.58	8.97 ± 0.03[abcd]	36.97 ± 1.87	24.47 ± .15[abcd]
Group 6 (A2)	12.07 ± 1.07	11.40 ± 0.40[ac]	32.90 ± 2.83	31.87 ± 1.47[ac]
Group 7 (B1)	14.53 ± 0.60	10.90 ± 0.64[ac]	41.23 ± 2.60	31.77 ± 1.99[ac]
Group 8 (B2)	12.90 ± 0.52	10.53 ± 0.20[abcd]	35.20 ± 2.02	30.10 ± 0.52[abcd]

Table 3. Effect of aqueous extracts of *Delonix regia* (Hook.) Raf. seeds, metformin/vildagliptin on platelets count of high fat diet streptozotocin-induced diabetes in female wistar rats at weeks 3 and 6

Treatment group	Platelets count (10^3/L)	
	Week 3	Week 6
Group 1	661.00 ± 3.46	661.00 ± 3.46
Group 2	700.33 ± 68.86	486.33 ± 23.96[a]
Group 3	593.00 ± 19.08	452.67 ± 11.84[a]
Group 4	435.67 ± 112.48[ab]	307.67 ± 5.49[a]
Group 5 (A1)	685.33 ± 88.85	178.50 ± 11.26[ab]
Group 6 (A2)	522.00 ± 21.36	466.00 ± 42.72[a]
Group 7 (B1)	676.00 ± 95.77	420.00 ± 43.30[a]
Group 8 (B2)	625.67 ± 86.89	372.00 ± 36.37[ab]

Table 4. Effect of aqueous extracts of *Delonix regia* (Hook.) Raf. seeds, metformin/vildagliptin on lymphocyte and granulocyte count of high fat diet streptozotocin-induced diabetes in female Wistar rats at weeks 3 and 6

Treatment group	Lymphocyte (%)		Granulocyte (%)	
	Week 3	Week 6	Week 3	Week 6
Group 1	82.87 ± 0.03	82.87 ± 0.03	4.17 ± 0.32	4.17 ± 0.32
Group 2	80.23 ± 8.01	67.47 ± 2.40	7.67 ± 4.02	27.00 ± 2.08[a]
Group 3	55.03 ± 27.52	74.50 ± 1.73	3.13 ± 1.65	13.40 ± 1.85
Group 4	77.73 ± 2.71	70.97 ± 2.17	7.87 ± 2.60	11.47 ± 0.61[b]
Group 5 (A1)	77.67 ± 0.33	66.87 ± 3.67	5.70 ± 0.50	15.17 ± 3.15
Group 6 (A2)	50.33 ± 25.17	70.50 ± 0.81	4.50 ± 2.26	14.00 ± 0.75
Group 7 (B1)	80.97 ± 4.08	74.37 ± 1.76	7.07 ± 3.48	11.45 ± 1.41[b]
Group 8 (B2)	81.90 ± 1.27	60.80 ± 5.31	5.30 ± 0.87	27.10 ± 13.90[a]

3.2 Plate 1, 2 and 3, Shows Respectively, Photomicrographs of Thin Sections (5 µm) of the Liver, Kidney and Spleen of Experimental Rats Harvested at the End of 6 Weeks of Treatment and Stained with H&E (X40)

Plate 1: Photomicrographs of sections of the liver

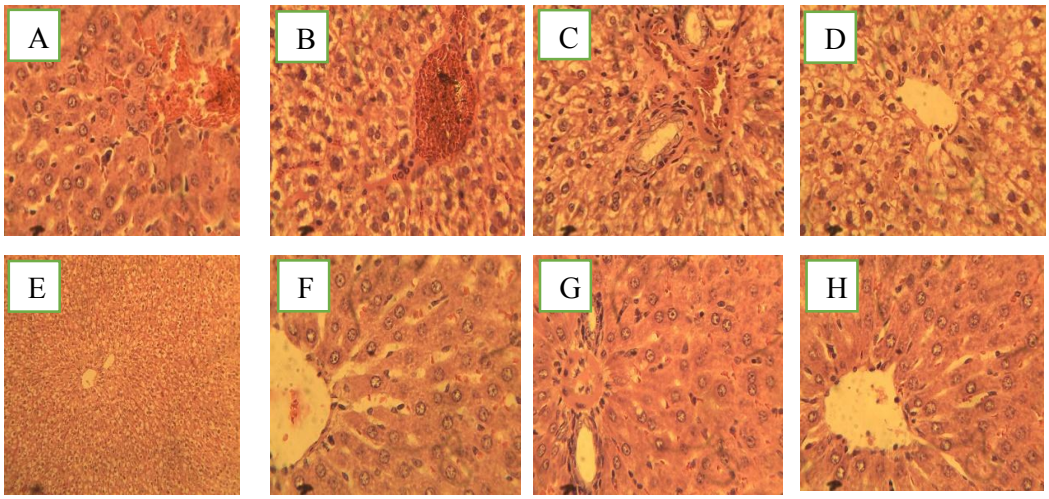

Plate 1. Photomicrographs of sections of the liver collected from group 1 - the normal control showed a normal architecture of the hepatocytes (A). Normal hepatic lobules consisting of normal hepatocytes arranged in radiating chords around central veins were observed. The hepatic chords are separated by hepatic sinusoids and diverge towards the periphery of hepatic lobule. Normal components of hepatic triads (hepatic artery, hepatic vein and bile duct) were also observed around central vein. Group 2 – the negative control (B), showed a severe diffused vascular degeneration of hepatocytes. The hepatocytes in all subzones of the hepatic lobule are affected. All the affected hepatocytes appear swollen, with multiple coalescent clear cytoplasmic vacuoles and normal nuclei around central vein. Groups 3, [positive control], 4 [second positive control] and 5 [A1 – 150 mg/kg body weight], (C, D & E [X10]), showed severe diffused vacuolar degeneration of the hepatocytes while groups 6 [A2 – 300mg/kg body weight], 7 [B1 – 150 mg/kg body weight], and 8 [B2 – 300mg/kg body weight], (F, G & H), showed normal architecture of the hepatocytes

Table 5. Effect of aqueous extracts of *Delonix regia* (Hook.) Raf. seeds, metformin/vildagliptin on kidney/electrolyte biomarkers of high fat diet streptozotocin-induced diabetes in female Wistar rats at weeks 3 and 6

Treatment group	Urea (mmol/l)	Creatinine (mmol/l)	Sodium (Na) (mmol/l)	Potassium (K) (mmol/l)	Bicarbonate (HCO_3) (mmol/l)
			WEEK 3		
Group 1	9.44 ± 1.10	34.64 ± 6.93	136.30 ± 6.95	4.51 ± 0.03	27.17 ±3.15
Group 2	7.64 ± 0.13	11.55 ± 4.62[a]	139.17 ± 7.99	8.27 ± 0.65[a]	28.46 ±3.42
Group 3	7.38 ± 0.60	16.17 ± 4.62	139.03 ±19.16	6.15 ± 0.12[a]	33.06 ±1.84[ab]
Group 4	8.09 ± 0.17	20.79 ± 8.00	131.73 ±11.76	6.47 ± 0.22[a]	40.71 ±1.32[ab]
Group 5 (A1)	7.72 ± 0.34	27.72 ± 13.86	132.37 ± 3.81	6.60 ± 0.10[a]	45.65 ±2.86[abc]
Group 6 (A2)	7.99 ± 0.29	20.71 ± 6.96	123.31 ± 7.32	6.83 ± 0.11[a]	26.19 ±1.19[cd]
Group 7 (B1)	7.21 ± 0.36	28.26 ± 6.58	159.10 ± 1.90[ab]	6.76 ± 0.33[a]	35.76 ±2.17[ab]
Group 8 (B2)	7.37 ± 0.15	17.47 ± 0.64[a]	119.08 ± 0.57	7.60 ± 0.59[a]	25.71 ±2.16[cd]
			WEEK 6		
Group 1	9.44 ± 1.10	34.64 ± 6.93	136.30 ± 6.95	4.49 ± .04	27.17 ± 3.15
Group 2	12.09 ± 0.07[a]	9.24 ± 2.31[a]	78.38 ± 1.55[a]	11.7567 ± 0.96[a]	41.88 ±1.65[a]
Group 3	11.91 ± 0.37	16.69 ± 2.10[a]	76.92 ± 1.66[a]	6.6833 ± 1.03[b]	24.36 ±0.54[b]
Group 4	11.41 ± 0.17	25.31 ± 9.99	74.02 ± 0.38[a]	3.3467 ± 0.39[b]	27.19 ±1.51[b]
Group 5 (A1)	11.31 ± 0.12	20.79 ± 8.00[a]	72.13 ± 1.84[a]	5.0867 ± 0.89[b]	44.92 ±2.26[acd]
Group 6 (A2)	12.19 ± 0.20[a]	20.69 ± 10.49[a]	81.39 ± 0.55[a]	6.7767 ± 1.33[b]	29.35 ±1.48[b]
Group 7 (B1)	10.42 ± 1.18	9.24 ± 2.31[a]	84.25 ± 1.47[a]	6.5833 ± 1.90[b]	26.56 ±0.18[b]
Group 8 (B2)	10.88 ± 0.19	20.79 ± 8.00[a]	110.43 ± 11.42	5.1900 ± 1.58[b]	25.74 ± 0.49[b]

Table 6. Effect of aqueous extracts of *Delonix regia* (Hook.) Raf. seeds, metformin/vildagliptin on liver biomakers of high fat diet streptozotocin-induced diabetes in female Wistar rats at weeks 3 and 6

Treatment group	Bilirubin (mmol/l)	Aspartate transaminase (U/L)	Alkaline phosphatase (U/L)	Alanine transaminase (U/L)
		WEEK 3		
Group 1	12.40 ± 0.32	81.67 ± 7.33	25.77 ± 1.94	4.00 ± 0.00
Group 2	7.90 ± 0.61	35.67 ± 16.18[a]	33.16 ± 4.49[b]	12.00 ± 0.00[a]
Group 3	11.60 ± 0.59	46.67 ±3.18	36.78 ± 4.47	6.67 ± 1.33
Group 4	10.61 ± 1.14	47.33±12.91	34.96 ± 3.88	6.67 ± 1.33
Group 5 (A1)	10.66 ± 0.27	31.00 ±8.00[a]	21.82 ± 6.42	8.00 ± 0.00
Group 6 (A2)	10.92 ± 0.64	43.67 ± 6.57	30.24 ± 2.02	5.33 ± 1.33
Group 7 (B1)	9.44 ± 1.68	43.33 ± 3.67	27.27 ± 0.50	6.67 ± 2.67
Group 8 (B2)	8.98 ± 0.37	24.33 ±3.53[a]	32.57 ± 1.53	9.33 ± 1.33[a]
		WEEK 6		
Group 1	12.40 ± 0.32	81.67 ± 7.33	26.55 ± 1.77	4.00 ± 0.00
Group 2	63.00 ± 0.05[a]	58.00 ± 13.75[a]	64.78 ± 0.82[a]	84.67 ± 1.67[a]
Group 3	61.68 ± 0.68[a]	70.00 ± 3.00	78.84 ± 0.07[a]	73.67 ± 1.67[a]
Group 4	59.85 ± 4.65[a]	69.33 ± 10.74	89.22 ± 3.01[a]	77.33 ± 5.33[a]
Group 5 (A1)	56.52 ± 4.32[a]	69.33 ± 10.74	64.56 ± 2.08[ad]	75.33 ± 1.67[a]
Group 6 (A2)	52.45 ± 1.88[ab]	89.00 ± 0.00	51.47 ± 10.13[acd]	75.33 ± 1.67[a]
Group 7 (B1)	50.88 ± 1.17[abc]	74.33 ± 7.33	40.38 ± 5.25[acd]	51.00 ± 23.54[a]
Group 8 (B2)	55.32 ± 1.71[a]	81.00 ± 7.02	44.96 ± 7.76[abcd]	77.33 ± 3.18[a]

Plate 2: Photomicrographs of sections of the kidney

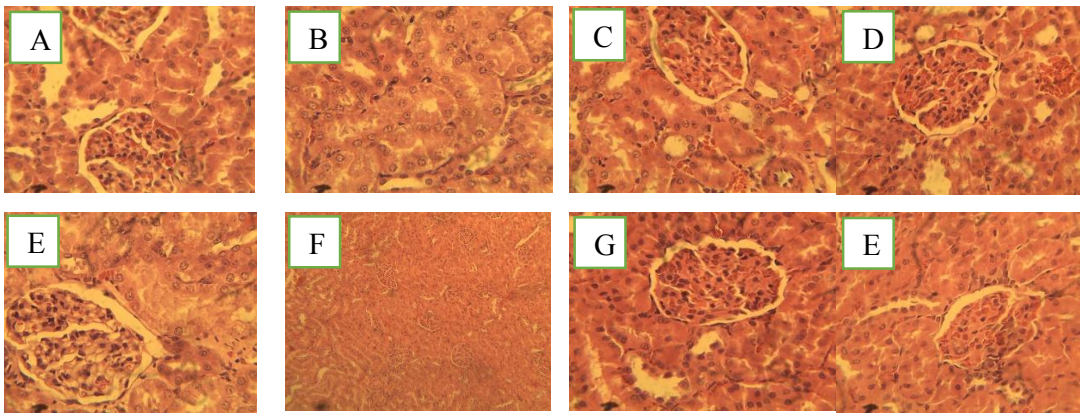

Plate 2. Photomicrographs of sections of the kidney showed: Group 1 - normal control (A), showed a normal renal architecture of the group. Normal glomeruli in their Bowman's capsules were observed, surrounded by a sea of normal renal tubules (proximal convoluted tubules, pars recta, distal convoluted tubules and collecting ducts). The renal tubules in outer and inner medulla were also normal as well as renal interstitium. Group 2 - - the Negative control (B), showed a mild multifocal vacuolar degeneration of the renal tubular epithelial cells. The affected tubules were randomly present in the cortex and outer medulla, showing epithelial cells with swollen vacuolated cytoplasm compare with the normal renal tubules. All the treated groups (C, D, G & H), showed normal renal architecture and normal bowman's capsules (F) [X10] except group 5 – A1 [150 mg/kg body weight] (E) which showed a mild multifocal vacuolar degeneration of the renal tubular epithelial cells

Plate 3: Photomicrographs of sections of the spleen

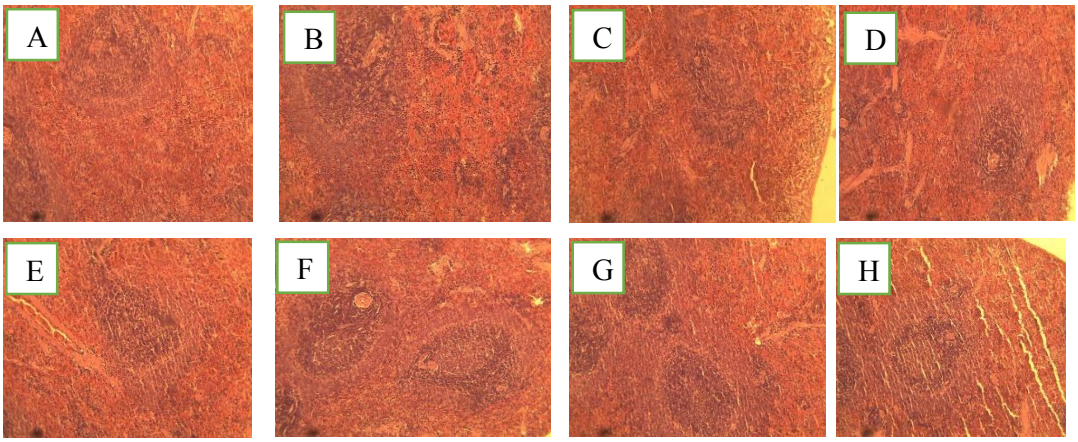

Plate 3. Photomicrographs of sections of the spleen collected from group 1 - the normal control group (A), showed a normal splenic architecture of experimental animals. The slides showed normal red pulp and white pulp consisting of normal lymphoid follicles and periarteriolar lymphoid sheaths. The untreated group 2 - Negative control - (B), showed significant hemosiderin pigment deposition. All the treated groups (C, D, E, F, G & H), showed a normal splenic architecture

4. DISCUSSION

Diabetes mellitus is often simply considered as a syndrome of disordered metabolism with abnormally high blood glucose levels (hyperglycaemia) both in clinical and experimental settings [27]. In this study results obtained after administration of high fat diet streptozotocin-induced diabetes showed that all the rats became diabetic after 7 days [28].

Damage to hepatic tissues mainly releases the enzymes AST, ALT and ALP from the hepatic cells into the plasma, and, hence, elevation of plasma activities of these enzymes is considered a reliable indicator of liver damage, since these enzymes are intracellular [29]. The activities of AST, ALT and ALP have been reported to increase in diabetic rats [23,30]. In this study, a significant ($p<0.05$) increase in the activities of plasma serum ALT and ALP in the diabetic untreated rats was observed when compared with the normal control. The 150mg/kg (BW) uncooked sample extract significantly ($p<0.05$) decreased the activities of these enzymes. This result is consistent with earlier report by Omoedu et al. [23] on the effect of *Delonix regia* (Hook.) Raf. seed extract on some liver enzyme markers in alloxan-induced diabetic rats. The AST activities significantly ($p<0.05$) decreased compared to the normal control. Elevated levels of ALT and GGT (positive correlation) and the lowest levels of AST/ALT (negative correlation) are associated with a higher prevalence for type 2 diabetes and pre-diabetes [31].

Significant elevation in the level of plasma bilirubin was observed in all the groups at the end of the sixth week when compared to the normal control. Similar observation was also reported using *Artemisia afra* extract to reverse bilirubin level in diabetic rats [32]. Elevation of plasma bilirubin could be as a result of the decrease of liver uptake, conjugation or increase in bilirubin production from haemolysis [33]. The effect of cooked and uncooked extracts in liver impairment, especially 300mg/kg and 150mg/kg BW respectively, are more efficacious compared to other doses and those of the standard drugs.

A significant elevated ($p<0.05$) level of urea and decreased creatinine concentrations were observed in diabetic untreated rats (group 2) with respect to normal rats. Oral administration of extracts effectively decreased and increased these markers respectively, which indicates protection against renal impairment due to diabetes. An increase in urea and creatinine concentration indicates an impaired renal function of diabetic animals [34]. Harita et al. [35], hypothesised that, lower serum creatinine is associated with an increased risk of type 2

diabetes, which might reflect a lower volume of skeletal muscle. Skeletal muscle is a major target tissue of insulin and a lower volume of skeletal muscle would mean fewer target sites for insulin which causes increase in insulin resistance which leads to the development of type 2 diabetes [36].

The levels of Potassium (K^+) and bicarbonate (HCO_3^-) ions significantly increased in the untreated diabetic rats when compared with the normal control. The administration of the extracts of cooked and uncooked *Delonix regia* (Hook.) Raf. seeds significantly decreased the levels of these parameters in the diabetic treated rats and the efficacy is comparably same to metformin and metformin/vildagliptin standard hypoglycaemic drugs treated groups. A similar observation was also reported using *Moringa oleifera Lam* seeds powder to ameliorate the concentrations of the renal function analytes and potassium [37]. Sodium (Na^+) concentration was observed to decrease significantly ($p<0.05$) across the experimental groups when compared to the normal control group at week six of the experiment except for group 8 (B2) treated with 300mg/kg body weight of uncooked seeds extract. Decrease in sodium concentration may be due to osmotic diuresis caused by diabetes. According to Loeb [38], Eteng et al. [39], the electrolyte panel is frequently used to screen for an electrolyte or acid-base imbalance and to monitor the effect of treatment on a known imbalance that is affecting bodily organ function. Also, glucose excretion in urine by diabetes, imposes an osmotic diuresis (dehydration), with the consequence of electrolyte lost with dehydration. The test for electrolytes includes the measurement of sodium, potassium and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions. An attempt by the kidney to buffer the urine decreases the alkaline metals including potassium and sodium in serum [40]. Alteration in bicarbonate concentration in the blood could lead to disturbance in acid-base balance. Hyperkalaemia or excess potassium in the blood occurs in cases of renal failure as the kidney loses the ability to excrete the mineral [41,42]. The efficacy of the extracts may be compared to the standard drugs used for the management of diabetes.

Haematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems [43]. The inducement of streptozotocin into rats significantly reduced the PCV, RBC, Hb concentration and platelet count. This is assumed to be associated with retarded haemopoeisis, destruction and shrinkage of RBC. Platelets count in experimental animals has been reported to indicate adverse effect on the oxygen carrying capacity of the blood as well as thrombopoietin [44]. Since RBC and Hb are very important in transferring respiratory gases. From the results of this study, administration of extracts (cooked and uncooked) led to decrease in platelet counts in rats. Administration of 150 mg/kg cooked sample extract led to statistically significant ($p<0.05$) reduction in platelets count in rats. In this study, reduction in platelets counts obtained suggests that the administration of the extracts may cause disruption in the oxygen-carrying capacity of the blood. Reductions in PCV, RBC and Hb concentrations in extracts treated rats, were observed when compared to diabetic untreated rats and decreased significantly ($p<0.05$) compared to normal control. Administration of 150 mg/kg cooked sample extract led to statistically significant ($p<0.05$) reduction in PCV level in rats when compared to negative control. This implies that the hard seeds from *Delonix regia* (Hook.) Raf. could cause disturbances in the osmoregulatory system of the blood cells and/or oxidative injury to the cell membrane, suppress the haemopoietic system and disrupt haemoglobin production in dose and time-dependent manner. The failure to produce haemoglobin occurs in many diseases, including iron deficiency anaemia, thalassemia and anaemia associated with chronic infection or disease [45,46]. The reduction may have occurred due to lysis of blood cells. Sule et al. [47] also observed a decrease in RBC, PCV, haemoglobin and lymphocytes in rats fed with extracts of *Acalypha wilkesiana*. The inducement of diabetes with high fat diet and streptozotocin into rats reduced WBC count and its' differential lymphocyte while granulocyte was elevated. WBC defends the body against infection and tissue damage. The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection [48]. The white blood counts and its related indices were favourably restored to near normal after extracts administration. 150mg/kg uncooked sample extract significantly ($p<0.05$) decreased granulocyte count and the efficacy is comparably same to metformin/ vildagliptin standard hypoglycaemic drugs treated groups. The presence of some phytochemicals in the extract could be responsible for the

observed decrease in white cell count of the treated rats [49]. This suggests that the extracts may have immune boosting effect on the animals.

High fat diet and streptozotocin caused significant changes in the histology of the liver of the diabetic untreated rats showing a severe diffused vacuolar degeneration of the hepatocytes, as well as the positive control, second positive control and A1, treated groups compared to normal rats, whereas A2, B1 and B2 treated diabetic rats showed normal architecture of the liver. These reports correlate the results of the biochemical analysis which indicates that diabetes induces liver damage while the damage was ameliorated by the administration of the extracts. Despite changes in the body, the kidney keeps its biomarkers in the blood constant. The plasma serum biomarkers values are usually indicative of the renal functions or dysfunctions [50]. Correlating the biochemical changes with the histopathology investigation revealed that high-fat diet and streptozotocin caused significant damage in renal structure showing a mild multifocal vacuolar degeneration of the renal tubular epithelial cells as well as that of A1 treated diabetic rats. Treatments with both cooked (A) and uncooked (B) seed extracts of *Delonix regia* (Hook.) Raf. Prevented the alterations and protected the histological aspects of the kidney. There were alterations in the histology of the spleen of diabetic untreated rats which showed significant hemosiderin pigment deposition compared to the splenic architecture of the normal rats. Treatment with *Delonix regia* (Hook.) Raf. seed extracts prevented hemosiderin pigment deposition in the experimental groups.

5. CONCLUSION

In conclusion, this present study showed that *Delonix regia* (Hook.) Raf. seeds have shown to be protective against disturbance of acid-base balance, nephroprotective and hepatoprotective in treated diabetic rats, and also exhibited a greater effectiveness in resolving adverse effects in the liver, kidney and spleen of high-fat diet streptozotocin-induced diabetic rats. It is therefore advisable that the use of this extract in herbal medicine should be with some cautious measures to avoid the risk of anaemia due to an observed decrease in erythropoietic parameters. Increase in the WBC count observed in rats suggests that extracts contain agents that could effectively attenuate the altered lymphocytes and granulocytes and boost the immune system production, therefore the plant extracts could serve as an immune booster. This further suggests that the crude extract of the plant exhibits very impressive potency and promise in the management of diabetes and its complications and hence a potential source for the discovery of new orally active anti-diabetic drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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