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Original Article

Residual aqueous fraction of Ethulia conyzoides L. extract regulates liver and kidney functions and prevents pancreatic β-cells damage in high fat diet-streptozotocin Type 2 diabetes in Rats

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ABSTRACT

Background: Complications of Type 2 Diabetes (T2D) include hypertension, oxidative stress, liver disease, and kidney disease. These complications are the main cause of death and disability in diabetic patients. **Objective:** The study investigated the effect of residual aqueous fraction of *Ethulia conyzoides L.* extract on liver and kidney function as well as the histology of liver, kidney, and pancreatic islets of type 2 diabetic rats. **Methodology:** Thirty-six rats were divided into six groups (n=6). Group I (non-diabetic rats) and II (diabetic control) were given feed and water only. Groups III-V were treated with 100mg/kg, 200mg/kg, and 400mg/kg *Ethulia conyzoides L*. while group VI received 100mg/kg metformin for 28 days. The liver and kidney function were evaluated while the liver, pancreas, and kidney were processed for light microscopy. **Results:** A significant reduction (P<0.05) in total protein and albumin levels were observed in diabetic control rats when compared with diabetic rats treated with 400mg/kg *Ethulia conyzoides L.* A significant decrease in high-density lipoprotein was observed in diabetic control rats when compared with diabetic rats treated with *Ethulia conyzoides L.* The liver and kidney of diabetic rats treated with *Ethulia conyzoides L*. showed normal hepatocytes/central vein and glomerulus respectively. The pancreatic islet of diabetic control rats treated with *Ethulia conyzoides L*. showed near-normal positive staining of insulin/β-cell by insulin antibody. **Conclusion:** *Ethulia conyzoides L.* prevented liver and kidney damage in Type 2 diabetic rats by improving liver and kidney functions and maintaining the normal pool of pancreatic β-cells.

Keywords: *Ethulia conyzoides L,* Type 2 diabetes, β-cells, liver enzymes.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, its prevalence is on the increase in developing and developed nations[1]. It is classified as type-1 (about 10%), and type-2 (about 90%) based on impaired insulin secretion (malfunction of beta cells) and insulin resistance respectively[2]. Type 2 diabetes (T2D) is largely due to insulin resistance and/or insensitivity of insulin receptors.[3] Overweight, low physical activity coupled with an unhealthy diet are the predisposing factors of T2D[3]. Complications of T2D include hypertension, oxidative stress, liver disease, hyperlipidemia, kidney disease, and retinopathy.[5-6] These complications are the main cause of death and disability in diabetic patients.[7] Previous studies have shown that diabetes is the leading cause of kidney-related diseases.[8] Diabetes was reported as the main cause of end-stage renal disease in some parts of Asia and South America.[9] The liver is an insulin-sensitive

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tissue that plays a role in regulating the interaction between gluconeogenesis and glucose utilization.[10] Liver injury induced by T2D leads to an increase in intracellular reactive oxygen species (ROS) levels, the accumulated ROS can activate the NF-κB pathway, causing inflammation, steatosis, and fibrosis.[7,11] The alteration in lipids and lipoproteins metabolism that occurs in a diabetic state can lead to an increased risk of chronic heart disease.[12]

Ethulia conyzoides L. (family: *Asteraceae*) is an herb native to Africa and Asia.[13] With Methyl coumarin as the most abundant constituents, *Ethulia conyzoides L*. was reported to have antihypertensive and antihyperglycemic properties.[14-16] The leaf and root of *Ethulia conyzoides L.* were reported to poses anti-lithic properties and were used in the treatment of wound[17-18].

To prevent the complications of T2D, early detection and treatment are very important as inadequate management can further worsen the complications.[19] Many synthetic drugs such as metformin, glibenclamide, troglitazone, albiglutide, taspoglutide, acarbose, miglitol, dapagliflozin, alogliptin, and canagliflozin are used in the management of diabetes.[20] While the anti-diabetic drugs provide some promising results, they also produce various adverse effects.[21] Hence, there is a need for the development of an effective drug with lesser side effects. The present study aimed to investigate the effect of *Ethulia conyzoides L.* on the liver and kidney function as well as the histology of liver, kidney, and pancreatic islets of type 2 diabetic rats.

Materials and Method

Plant material

The whole plant of *Ethulia conyzoides L.* was harvested from Okpokwu L.G.A Benue State, Nigeria. It was identified and authenticated at the Herbarium unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria (voucher number 7098). The whole plant was rinsed in clean water and dried at room temperature. It was grounded into powder using a mortar and pestle, and the powder was used to prepare the extract.

Experimental animals

Thirty-six (36) Wistar rats weighing 120-150g were purchased from the animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The rats were kept in metal cages. They were fed with grower mash (Vital Feed, Grand cereal, Jos, Nigeria) and water *ad libitum.* Ethical clearance was obtained from the Ahmadu Bello University Committee on Animal Use and Care (Approval number: ABUCAUC/2019/007) and was conducted according to the ARRIVE Guidelines.

Extraction procedure

The extraction was conducted according to the method described by Okoduwa *et al.*[22] Briefly, 1700g of the grounded sample was suspended in 70% methanol (1:10 w/v) for 48 hours at room temperature with frequent agitation (cold maceration). The mixture was passed through a mesh sieve (1 mm), filtered off using a filter paper and the filtrate was concentrated by drying in an oven at 45°C to obtain the dried extract.

Partitioning of the methanol extract

The methanol extract (100g) was suspended in 500ml of distilled water and then partitioned using n-hexane and ethyl acetate in increasing order of polarity.[23] The dissolved methanol extract was soaked in n-hexane in a separating funnel, shaken, and allowed to stand for phase separation into two fractions. The n-hexane fraction was carefully decanted after partitioning. More of the n-hexane solvent was added and the same process was repeated until when there was no further color change upon the addition of n-hexane. The n-hexane soluble portion was obtained and allowed to dry under room temperature to obtain the n-hexane fraction. The aqueous fraction was also partitioned with ethyl acetate following the procedure as in the n-hexane above. The resulting residue was dissolved in water and referred to as the residual aqueous fraction. Each fraction obtained was concentrated using a rotary evaporator and the remaining solvent in the extract was allowed to evaporate in the room.

Induction of Type 2 Diabetes

Type 2 Diabetes was induced in 30 rats according to the method described by De Magalhaes *et al.*[24] The feed was fortified with margarine in a ratio of 10:1. This was administered along with 20% fructose solution in drinking water *ad libitum* for six weeks. After six weeks, the rats were fasted overnight and injected intraperitoneally with 40mg/kg streptozotocin (STZ) (dissolved in a citrate buffer

pH 4.5). The rats were given 5% glucose solution in drinking water within the first 24 hours of STZ administration. An oral glucose tolerance test (OGTT) was performed on each rat, three days after diabetes induction. The rats were given 2.5 g/kg glucose and the blood glucose concentrations were measured at 30 minutes, 60 minutes, 90 minutes, and 120 minutes after induction using a glucometer (AccuChek, Roche, Switzerland). Rats that displayed a sustained increase in blood glucose level up to ≥200 mg/dL were confirmed to be diabetic and incorporated in the study.

Experimental Design

The rats were divided into six groups of six rats each: Group I consist of non-diabetic rats (normal control) and were given feed and water only. Group II consist of diabetic control rats and was given feed and water only. Groups III, IV, and V were treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg residual aqueous fraction of *Ethulia conyzoides L*. While group VI were treated with 100 mg/kg metformin (standard drug). They were also given normal feed and water throughout the administration. The administration was through an oral route and lasted for 28 days. On the 29th day, all the rats were given 2.5 g/kg glucose and the blood glucose concentrations were measured at 30 min, 60 min, 90 min, and 120 min. The rats were euthanized with Ketamine injection and the blood was collected in a plain bottle. The blood samples were centrifuged at 3000 rpm for 10 minutes and levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, creatinine, urea, electrolyte, and lipid profile were estimated on a UV-Vis spectrophotometer (Mettler Toledo, Switzerland) using enzyme-linked immunosorbent assay (ELISA) kit (Neoscientific, UK) according to manufacturer's instruction. The liver, pancreas, and kidneys were dissected fixed in neutral buffered formalin (NBF), processed for light microscopy, and sectioned at 5µm. Kidneys and liver sections were stained with hematoxylin and eosin (H&E). Antigen retrieval was performed on pancreas sections with a retrieval solution ($_{P}$H 8.0) and then neutralized with an endogenous peroxidase (Leica). The sections were then washed in tris-buffered saline solution. Initial incubation was performed with lyophilized rat monoclonal insulin antibody (Leica). A second incubation was performed with a secondary antibody (Leica) to form a complex (TAG) with the primary antibody. Peroxidase activity was developed with horseradish peroxidase (Leica). The labeled insulin target molecule was eventually visualized via reaction with diaminobenzyldehyde hydrochloride working solution. Sections were finally counter-stained with hematoxylin, dehydrated in graded changes of alcohol, cleared in xylene, and mounted with dibutylphthalate polystyrene xylene (DPX). A minimum of five slides per tissue were observed under the microscope.

Statistical analysis

Data were analyzed with Statistical Product and Service Solutions (SPSS) Version 21. One-way analysis of variance (ANOVA) followed by the Tukey test was used to determine the difference between groups and $P < 0.05$ was considered statistically significant. Values were presented as mean ± standard error of the mean (SEM).

Results

Blood Glucose Level

Significant increases ($p < 0.05$) in blood glucose levels were observed in diabetic control rats at 30 min, 60 min, 90 min, and 120 min when compared to the normal control rats and metformin-treated diabetic rats (Figure 1). The blood glucose level of *Ethulia conyzoides L.* treated diabetic rats were significantly decreased ($p < 0.05$) during 30 min, 60 min, 90 min, and 120 min intervals relative to diabetic control rats (Figure 1). The blood glucose level of metformin-treated diabetic rats were significantly lower ($p < 0.05$) up to 120 min intervals relative to diabetic rats treated with *Ethulia conyzoides L.* at 100 mg/kg and 200 mg/kg (Figure 1).

Liver Function

A significant increase ($p<0.05$) in ALT, AST, and ALP levels were observed in diabetic control rats relative to the normal control (Table 1). There was no significant difference in ALT, AST, and ALP of normal control rats compared to diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.* at $p> 0.05$ (Table 1). The ALT, AST, and ALP levels of diabetic rats treated with 200 mg/kg and 400 mg/kg *Ethulia conyzoides L.* were significantly lower ($p<0.05$) compared to the metformin-treated diabetic rats (Table 1). A significant reduction ($p< 0.05$) in total protein and albumin levels were observed in diabetic control rats compared to normal control and diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.*

(Table 1). No significant change ($p>0.05$) in albumin level was observed in normal control rats relative to the diabetic control rats treated with *Ethulia conyzoides L.* (Table 1).

Kidney Function

From Table 2, there was a significant reduction ($p< 0.05$) in the levels of potassium and sodium in diabetic control rats compared to the normal control rats. There was no significant change ($p > 0.05$) in the levels of potassium and sodium in normal control rats when compared with diabetic rats treated with *Ethulia conyzoides L.* A significant increase in chloride and bicarbonate levels was observed in diabetic control rats when compared to the normal control rats and *Ethulia conyzoides L.* treated diabetic rats at $p < 0.05$ (Table 2). A significant increase in urea and creatinine was observed in diabetic control rats when compared to the normal control rats ($p < 0.05$). Urea and creatinine levels of diabetic rats treated with 100 mg/kg and 200 mg/kg *Ethulia conyzoides L.* were significantly higher ($p < 0.05$) than those of normal control rats (Table 2). No significant change ($p > 0.05$) was observed in urea and creatinine levels of diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.* compared to the normal control rats (Table 2). No significant change ($p > 0.05$) was observed in potassium, sodium, and chloride levels of control rats relative to the diabetic rats treated with metformin. However, a significant increase ($p < 0.05$) in bicarbonate and urea levels was observed in metformin-treated diabetic rats relative to the control (Table 2).

Lipid Profile

Triglycerides, cholesterol, and low-density lipoprotein (LDL) levels were significantly higher ($p < 0.05$) in diabetic control rats compared to the normal control rats (Table 3). Also, the level of cholesterol, LDL, and triglycerides of normal control rats were significantly lower ($p < 0.05$) relative to *Ethulia conyzoides L.* treated diabetic rats. A significant decrease ($p < 0.05$) in high-density lipoprotein was observed in diabetic control rats when compared to the normal control and *Ethulia conyzoides L.* treated diabetic rats (Table 3). The triglycerides, cholesterol, and LDL levels of metformin-treated diabetic rats were significantly higher ($p < 0.05$) compared to the control. However, HDL levels of metformin-treated diabetic rats were significantly lower ($p < 0.05$) relative to the control (Table 3).

Histological and Immuno-Histochemical Studies

Photomicrograph of kidney of normal control rats showed normal glomerulus and normal renal tubules (Figure 2a). The kidney of diabetic control rats showed compacted glomerulus and renal tubules (Figure 2b). The kidney of diabetic rats treated with 100 mg/kg *Ethulia conyzoides L.* showed compacted glomerulus and distorted renal tubules (Figure 2c). The kidney of diabetic rats treated with 200 mg/kg *Ethulia conyzoides L.* showed normal glomerulus and distorted renal tubules (Figure 2d). The kidney of diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.* showed normal glomerulus and normal renal tubules (Figure 2e), while the kidney of diabetic rats treated with metformin showed compacted glomerulus and distorted renal tubules (Figure 2f).

Photomicrograph of liver of normal control rats showed normal central vein, normal hepatocytes, and normal sinusoids (Figure 3a). The liver of diabetic control rats showed an occluded central vein with numerous vacuoles around hepatocytes (Figure 3b). The liver of diabetic rats treated with *Ethulia conyzoides L.* at 100 mg/kg showed few vacuoles, normal central vein, and hepatocytes (Figure 3c). The liver of diabetic rats treated with *Ethulia conyzoides L.* at 200 mg/kg and 400 mg/kg showed normal hepatocytes central vein and sinusoids (Figure 3d & 3e), while the liver of diabetic rats treated with metformin showed enlarged sinusoids with distorted central vein (Figure 3f).

Photomicrograph of pancreatic islet of normal control rats showed a normal homogenous positive staining of insulin/β-cell by insulin antibody (Figure 4a). The pancreatic islet of diabetic control rats showed very few areas of positive staining of insulin/β-cell by insulin antibody (Figure 4b). Pancreatic islet of diabetic control rats treated with *Ethulia conyzoides L.* at 100 mg/kg, 200 mg/kg, and 400 mg/kg showed near-normal positive staining of insulin/β-cell by insulin antibody (Figures 4c, 4d & 4e), while the pancreatic islet of diabetic control rats treated with metformin showed normal positive staining of insulin/β-cell by insulin antibody (Figure 4f).

Table 1: Liver function of diabetic and non-diabetic rats treated with residual aqueous fraction of *Ethulia conyzoides L.* and Metformin

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (g/dL)	Albumin (g/dL)
Normal control	5.80 ± 0.49	10.60 ± 0.40	20.06 ± 0.49	11.76 ± 0.94	3.16 ± 0.21
Diabetic control	25.40 ± 1.56[a]	29.40 ± 3.41[a]	43.98 ± 1.78	7.90 ± 0.74[a]	2.26 ± 0.10[a]
100 mg/kg RAF of *Ethulia conyzoides L.*	14.80 ± 0.66[ab]	19.00 ± 1.04[ab]	35.28 ± 1.81[ab]	8.02 ± 0.39[a]	2.84 ± 0.08[b]
200 mg/kg RAF of *Ethulia conyzoides L.*	11.20 ± 0.48[ab]	17.00 ± 0.83[ab]	29.86 ± 1.67[ab]	9.06 ±0.19[a]	2.96 ± 0.02[b]
400 mg/kg RAF of *Ethulia conyzoides L.*	8.60 ± 0.40[ab]	14.40 ± 0.74[b]	24.04 ± 2.19[b]	10.44 ± 0.32[b]	2.86 ± 0.07[b]
100 mg/kg Metformin	14.60 ± 1.02[ab]	20.60 ± 0.74[ab]	34.84 ± 1.81[ab]	9.06 ± 0.19[a]	2.76 ± 0.11[b]

All values were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey post hoc test. [a] indicates a significant difference with normal control while [b] indicates a significant difference with diabetic control ($p < 0.05$), n=5. RAF= residual aqueous fraction.

Table 2: Kidney function of diabetic and non-diabetic rats treated with residual aqueous fraction of *Ethulia conyzoides L.* and Metformin

Groups	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mg/dL)	Bicarbonate (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Normal control	17.11 ± 1.69	156.12 ± 9.03	27.20 ± 1.74	79.60 ± 4.38	17.89 ± 2.89	0.7 ± 0.03
Diabetic control	10.15 ± 0.47[a]	89.80 ± 0.20[a]	36.20 ± 0.80[a]	111.40 ± 4.91[a]	53.44 ± 3.38[a]	1.26 ± 0.08[a]
100 mg/kg RAF of *Ethulia conyzoides L.*	17.55 ± 1.20[b]	145.20 ± 13.05[b]	29.40 ± 0.81[b]	91.40 ± 2.32[ab]	33.53 ± 1.58[ab]	1.02 ± 0.04[b]
200 mg/kg RAF of *Ethulia conyzoides L.*	16.64 ± 0.93[b]	137.80 ± 11.94[b]	28.20 ± 3.51[b]	84.80 ± 5.59[b]	30.97 ± 4.19[ab]	0.96 ± 0.05[a]
400 mg/kg RAF of *Ethulia conyzoides L.*	17.42 ± 1.46[b]	129.60 ± 10.82[ab]	29.80 ± 0.86[b]	88.20 ± 3.24[a]	23.98 ± 2.54[ab]	0.82 ± 0.03[a]
100 mg/kg Metformin	16.21 ± 1.45[b]	151.40 ± 14.29[b]	30.40 ± 1.33[b]	93.20 ± 7.85[a]	37.02 ± 3.38[ab]	1.00 ± 0.03[a]

All values were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey post hoc test. [a] indicates significant difference with normal control while [b] indicates significant difference with diabetic control ($p < 0.05$), n=5. RAF= residual aqueous fraction.

Table 3: Lipid profile of diabetic and non-diabetic rats treated with residual aqueous fraction of *Ethulia conyzoides L.* and Metformin

Groups	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Normal control	67.56 ± 1.11	78.56 ± 0.844	21.58 ± 0.87	47.38 ± 0.78
Diabetic control	198.50 ± 4.51[a]	181.86 ± 2.04[a]	72.76 ± 1.40[a]	17.80 ± 0.64[a]
100 mg/kg RAF of *Ethulia conyzoides L.*	147.18 ± 2.39[ab]	134.40 ± 1.67[ab]	56.92 ± 2.35[ab]	27.22 ± 0.95[ab]
200 mg/kg RAF of *Ethulia conyzoides L.*	124.62 ± 2.44[ab]	119.44 ± 1.37[ab]	37.10 ± 4.68[ab]	29.32 ± 1.55[ab]
400 mg/kg RAF of *Ethulia conyzoides L.*	109.52 ± 3.28[ab]	98.68 ± 3.06[ab]	33.31 ± 1.71[ab]	39.06 ± 2.01[ab]
100 mg/kg Metformin	136.48 ± 2.71[ab]	123.98 ± 1.85[ab]	60.72 ± 1.55[a]	28.20 ± 1.71[ab]

All values were presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey post hoc test. [a] indicates significant difference with normal control while [b] indicates significant difference with diabetic control ($p < 0.05$), n=5. RAF= residual aqueous fraction, LDL= low density lipoprotein, HDL= high density lipoprotein.

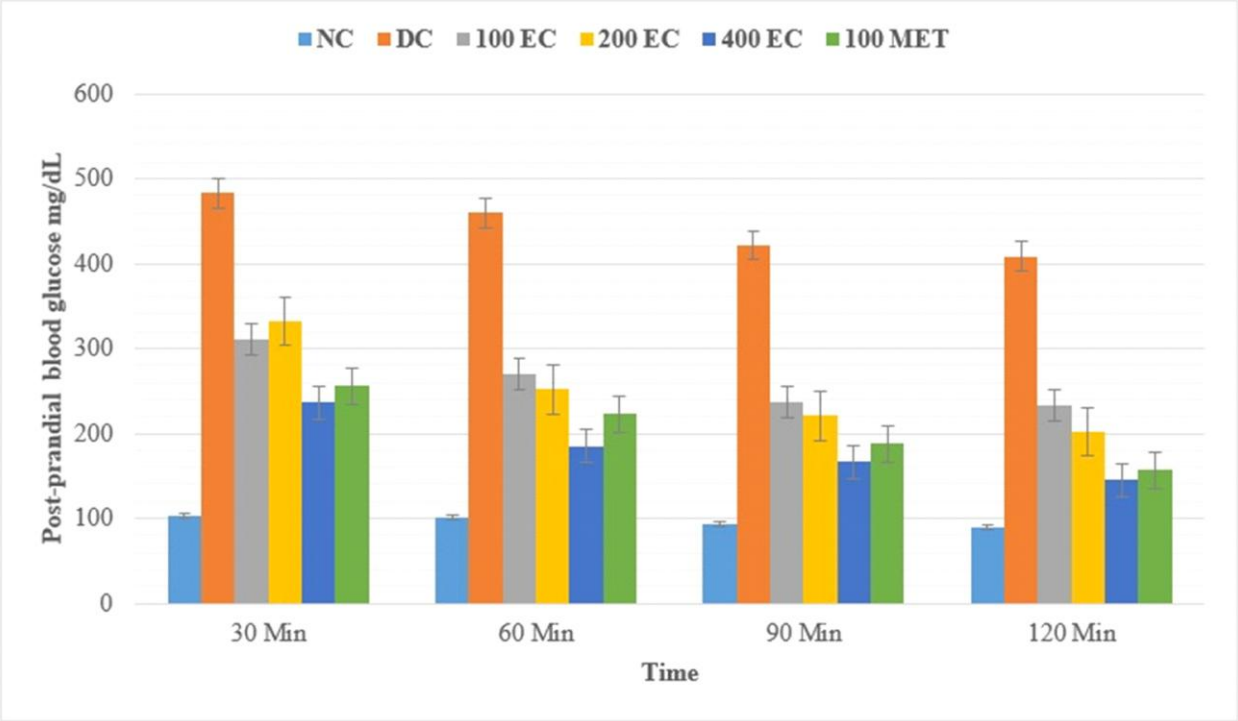

Figure 1: Post-prandial blood glucose levels of diabetic and non-diabetic rats treated with residual aqueous fraction of *Ethulia conyzoides L*. (EC) and Metformin (MET), NC= normal control, DC= diabetic control, n=5, error bars indicate standard error of the mean.

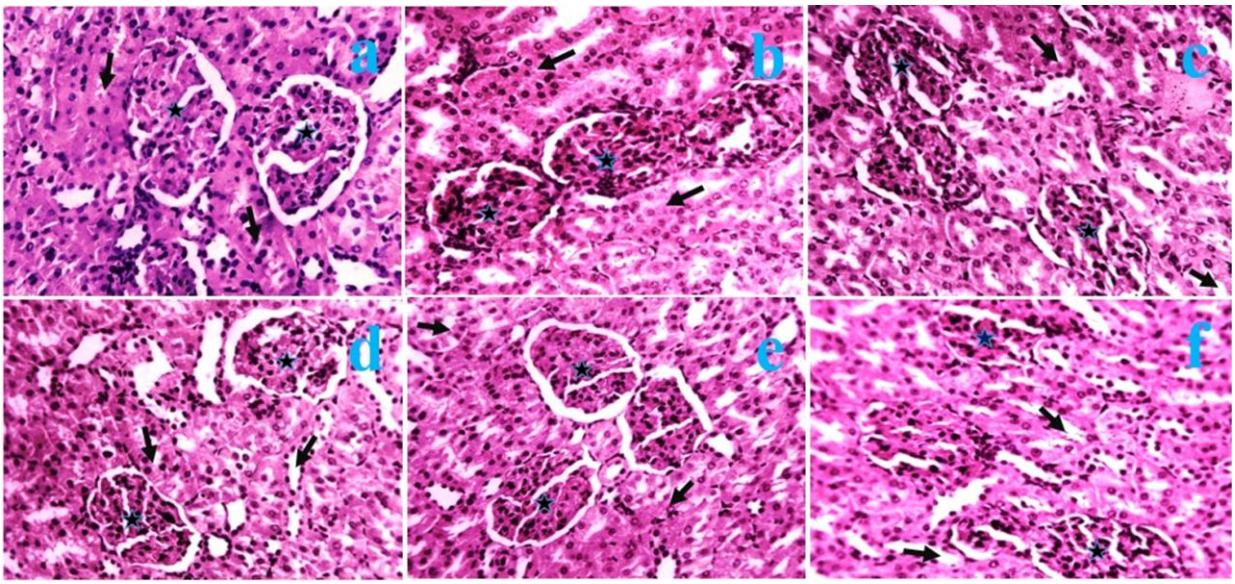

Figure 2: Composite photomicrographs of kidney of non-diabetic and diabetic rats showing normal glomerulus and tubules in **a**, compacted glomerulus and tubules in **b,** compacted glomerulus and distorted tubules in **c,** normal glomerulus and distorted tubules in **d,** normal glomerulus and tubules in **e,** and compacted glomerulus with distorted tubules in **f**. H&E x250.
a= kidney of normal control rats, **b**= kidney of diabetic control rats, **c**= kidney of diabetic rats treated with 100 mg/kg *Ethulia conyzoides L.,* **d**= kidney of diabetic rats treated with 200 mg/kg *Ethulia conyzoides L.,* **e**= kidney of diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.,* **f**= kidney of diabetic rats treated with Metformin.

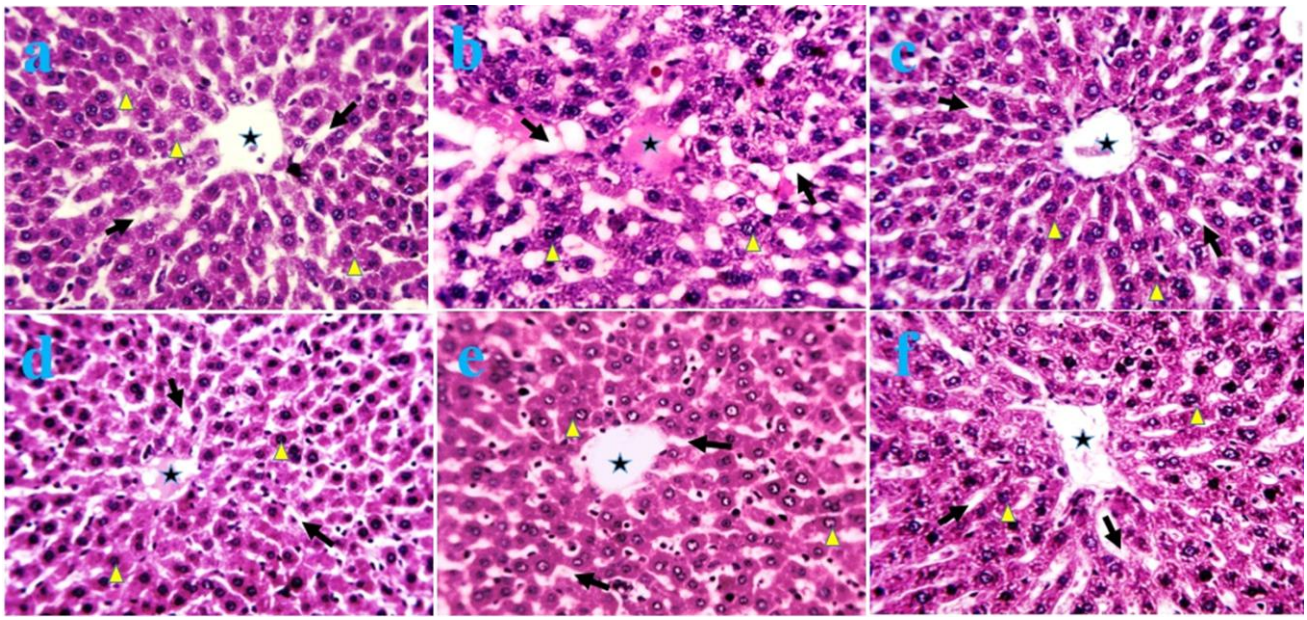

Figure 3 Composite photomicrographs of liver of non-diabetic and diabetic rats showing normal central vein, hepatocytes and sinusoids in **a**, occluded central vein and numerous hepatic vacuoles in **b,** normal central vein and hepatocytes with few vacuoles in **c,** normal central vein, hepatocytes and sinusoids in **d & e,** and enlarged sinusoids with distorted central vein in **f**. H&E x250. **a**= liver of normal control rats, **b**= liver of diabetic control rats, **c**= liver of diabetic rats treated with 100 mg/kg *Ethulia conyzoides L.,* **d**= liver of diabetic rats treated with 200 mg/kg *Ethulia conyzoides L.,* **e**= liver of diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.,* **f**= liver of diabetic rats treated with Metformin.

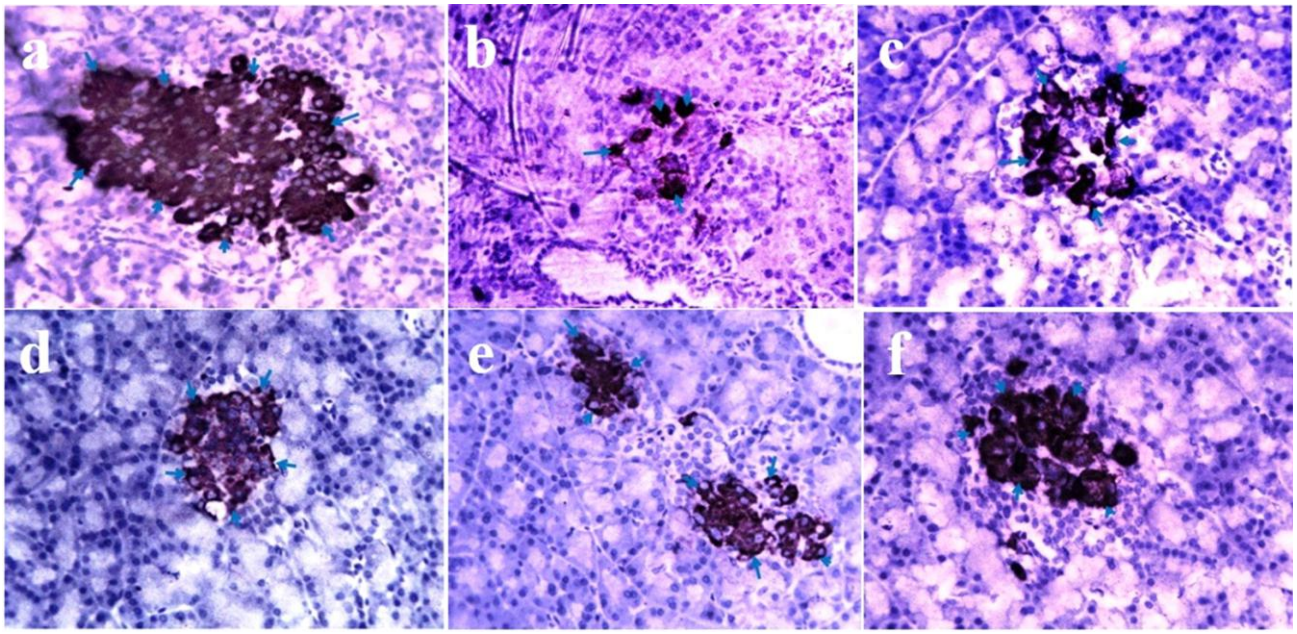

Figure 4 Composite photomicrographs of pancreatic islet of non-diabetic and diabetic rats showing normal homogenous positive staining of insulin/β-cells by insulin antibody in **a**, few areas of positive staining of insulin/β-cells by insulin antibody in **b,** near-normal positive staining of insulin/β-cells by insulin antibody in **c, d & e,** and normal positive staining of insulin/β-cells by insulin antibody in **f**. Insulin antibody counterstained with hematoxylin x250. **a**= pancreatic islet of normal control rats, **b**= pancreatic islet of diabetic control rats, **c**= pancreatic islet of diabetic rats treated with 100 mg/kg *Ethulia conyzoides L.,* **d**= pancreatic islet of diabetic rats treated with 200 mg/kg *Ethulia conyzoides L.,* **e**= pancreatic islet of diabetic rats treated with 400 mg/kg *Ethulia conyzoides L.,* **f**= pancreatic islet of diabetic rats treated with Metformin.

Discussion

The significant reduction in the blood glucose level of rats treated with a residual fraction of *Ethulia conyzoides L.* that was observed in the present study showed that 200 mg/kg and 400 mg/kg residual fraction of *Ethulia conyzoides L.* can be used to treat hyperglycemia. Coumarins are considered as one of the active compounds in *Ethulia conyzoides L.*[25] Therefore, the anti-hyperglycemic activity of *Ethulia conyzoides L.* might be due to its coumarin content. Umbelliferone is a form of coumarin that was reported to prevent hyperglycemia in diabetic rats.[26,27] Synthesized coumarin analogs were reported to maintain a normal blood glucose level in diabetic rats.[17] Tert-butyl coumarin was reported to decrease plasma glucose levels in streptozotocin-induced diabetic mice.[28,29] The role of coumarin in maintaining blood glucose level might be the cause of near-normal positive staining of insulin/β-cell by insulin antibody that was noticed in the pancreatic islet of diabetic rats treated with *a* residual fraction of *Ethulia conyzoides L.* extract in the present study.

A previous study suggests that a decrease in plasma albumin level is a prognostic sign of Type 2 diabetes in humans.[30] Hence, the decrease in albumin of diabetic rats in the present study. An earlier report indicated a relationship between serum albumin levels and the incidence of Type 2 diabetes[31]. Albumin is the most abundant serum/plasma protein which accounts for about 60% of the total protein concentration.[32] Therefore, the significant decrease in serum albumin concentration that was observed in the present study might be the cause of a decrease in total protein concentration and then resulting in the elevated levels of ALT and AST in diabetic rats that was observed in the present study. The elevated ALT and AST levels are in agreement with a study conducted by Music *et al.*[33] Low serum albumin have been reported to be associated with inflammation and liver disease.[34] Elevated ALT level was also reported to play a role in the development of non-alcoholic fatty liver disease (NAFLD) in type 2 diabetic patients.[35] Increase in serum albumin was reported to protect against early onset and progression of Type 2 diabetes.[36] Hence the normal hepatocytes central vein and sinusoids that was observed in the liver of diabetic rats treated with 200 mg/kg and 400 mg/kg *Ethulia conyzoides L.* in the present study. Serum ALT and AST were reported to be significantly higher in diabetic conditions.[37,38] In the present study 400 mg/kg residual fraction of *Ethulia conyzoides L.* was able to stabilize ALT, AST, and albumin levels and also protect the hepatocytes of diabetic rats.

Urea and creatinine levels are used for accessing kidney function.[39] The increase in urea and creatinine levels of diabetic rats observed in the present study demonstrates that the increased serum urea and creatinine levels are complications associated with T2D. This is in agreement with an earlier report by Fadhilah and Wahyuni.[40] Serum albumin was reported to be associated with diabetic kidney disease in Chinese population.[41] Hence, the decrease in serum albumin of diabetic rats that was observed in the present study might be the cause of the increase in serum urea and creatinine. A high urea-to-creatinine ratio was reported to be an indicator of increased mortality rate in patients with acute kidney injury.[42] Elevated urea and creatinine levels were reported as a prognostic sign of diabetic nephropathy.[40] This might be the cause of glomerular and renal tubular distortion in diabetic control rats that was observed in the present study. Plasma creatinine level was reported to be significantly higher in diabetic individuals when compared with those of healthy individuals.[43] The present study indicated that 200 mg/kg and 400 mg/kg residual fraction of *Ethulia conyzoides L.* prevented renal tubular damage and glomerular distortion in diabetic rats.

The liver plays a critical role in lipid metabolism. Therefore, liver injury may occur as a result of altered lipid metabolism.[44] The significant increase in triglycerides, cholesterol, and low-density lipoprotein of diabetic rats that was observed in the present study might be the cause of distorted hepatocytes and hepatic vacuoles in the liver of diabetic rats. Hypertriglyceridemia is associated with NAFLD, steatosis, fibrosis, and Type 2 diabetes.[45,46] High serum triglyceride was reported to be a prognostic sign of type 2 diabetes.[47] Type 2 diabetes is also associated with low levels of HDL and elevated LDL levels.[45,48] Fatty liver was reported to play a role in the development and complications of Type 2 diabetes.[49] This might be the cause of hepatic vacuoles that was observed in the liver of diabetic control rats in the present study. The normal liver and kidney histology observed in Metformin and *Ethulia conyzoides L.* (200mg/kg and

400mg/kg) treated diabetic rats, coupled with the normal homogenous positive staining of insulin/β-cell by insulin antibody in diabetic rats treated with Metformin and a residual aqueous fraction of *Ethulia conyzoides L.* at 200mg/kg and 400mg/kg that was observed in the present study suggest that; both Metformin and a residual aqueous fraction of *Ethulia conyzoides L*. could prevent β-cell degeneration and liver/kidney damage. Hence, a residual aqueous fraction of *Ethulia conyzoides L.* is effective in the prevention and control of T2D. The coumarin content of *Ethulia conyzoides L.* might be responsible for its β-cell and liver/kidney protection in a diabetic state. Therefore, a residual aqueous fraction of *Ethulia conyzoides L.* could be used in the prevention and treatment of T2D in other mammals including humans.

Conclusions

Type 2 diabetes is characterized by hypertriglyceridemia, an increase in ALT, AST, urea, and creatinine. Treatment with a residual aqueous fraction of *Ethulia conyzoides L.* at 200mg/kg and 400mg/kg prevented liver and kidney damage in type 2 diabetic rats by improving liver and kidney functions, maintaining lipid metabolism. It also maintains the normal pool of pancreatic β-cells by preventing pancreatic islet damage.

Conflict of Interests: The authors declare that they have no competing interest.

Authors' contributions: Conception and design: All authors; Administrative support: WM & HOO; Provision of study materials: WM & HOO; Collection and assembly of data: All authors; Data analysis and interpretation: All authors; Initial draft of the manuscript: WM & HOO; Critical review of the manuscript: NID; Final approval of manuscript: All authors.

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