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Ameliorative Effect of *Terminalia muelleri* Leaves Extract on Kidney Tissue in Streptozotocin – Induced Diabetic Rats

Samya Mahmoud Ahmed[1], Amany M. Gad [2] and Marwa S.M. Diab [3]

[1]Biochemistry Department, Egyptian Drug Authority (EDA)-Formerly NODCAR, Giza, Egypt.
[2]Pharmacology Department, Egyptian Drug Authority, EDA, Formerly NODCAR, Giza, Egypt Department of Pharmacology and Toxicology, Faculty of Pharmacy, Sinai University, Kantara Branch, Ismailia, Egypt.
[3]Molecular Drug Evaluation Department, Egyptian Drug Authority (EDA)-Formerly NODCAR, Giza, Egypt.

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ABSTRACT

The current study aimed to explore the potential renal-protective effect of *Terminalia Muelleri* leaves extract (TME) in streptozotocin (STZ)–induced diabetic rats. Experimental diabetes mellitus was created by subcutaneous injection of STZ at initial dose (27.5mg/kg), followed by three successive doses (11.25 mg/kg). Following the induction of diabetes, the diabetic rats were divided into three groups as the following, STZ group, two groups of diabetic rats treated with a daily oral dose of TME (200mg /kg b.wt) or Pioglitazone (PIO) drug (1.58mg /kg b.wt) for four weeks (STZ+ TME and STZ + PIO. In the same time, two groups of healthy rats were employed the first group was conducted as control non treated group and the rats of the second one were administered a daily oral dose of TME (200mg /kg b.wt., TME group) for four weeks. The induction of kidney injury in diabetic rats was manifested by the recorded changes in kidney function-related parameters in serum; creatinine, urea, total protein and albumin, cystatin C (CysC), kidney injury molecule-1 (KIM-1), oxidative stress and antioxidant biomarkers (MDA level, GSH content and CAT activity) in renal tissue, with the assessment of inflammatory– associated biomarkers ((NF-κB, TNF-α and IL-1β) in renal tissue. Pathological and histochemical examination of renal tissue of diabetic rats revealed many histological changes with significant formation of amyloid positive protein with decreasing of total protein content. The obtained results revealed that, TME or PIO administration significantly ameliorated kidney injury with the tendency to lower the incidence of histological and changes in renal tissue of diabetic rats. The obtained results showed that, TME elicited a pronounced improving effect in comparison with PIO that was used as a reference antidiabetic drug. In conclusion: Our results suggested that TME could be a better alternative therapy in ameliorating diabetic-associated kidney disorders.

Keywords: Diabetes, kidney disorder, inflammation, oxidative stress, *antioxidant,* amyloid protein*, Terminalia mulleri*

1. Introduction

Diabetes mellitus (DM) is a prodigious degenerative disease linked to aberrant disruption in insulin secretion with consequent hyperglycemia along with the abnormalities in the metabolism of carbohydrates, lipids, and proteins (Garcia, 2017). DM and its associated acute or chronic consequences are now widespread disorders that pose a threat to our health and lives, as it is considered as one of the main causes of secondary pathophysiological changes in various organ systems (Hill-Briggs *et al*., 2021). DM is accompanied by macrovascular and microvascular complications that adversely disturb the functions of several organs as kidney failure, heart disease, retinopathy, and neuropathy (Ojo, 2016). Among the most common vascular effects, chronic renal failure and end-stage renal disorders are frequently associated with the changes in metabolism and hemodynamics. Metabolic pathways such as

Corresponding Author: Samya M. Ahmed, Biochemistry Department, Egyptian Drug Authority (EDA)-Formerly NODCAR, Giza, Egypt. E-mail: samya.mahmoud3010@gmail.com

glycolysis and lipolysis produced methyglyoxal that binds to proteins or nucleic acids forming advanced glycation end products which results in the production of free radicals and enhancement of oxidative stress (Cha *et al.,* 2018). Hyperglycemia has been shown to promote the generation of excess reactive oxygen species (ROS) due to the mitochondria's high rate of glucose oxidation (Gonzalez *et al.,* 2023). Chronic hyperglycemia-induced oxidative stress plays a major role in the onset and progression of diabetes and its related vascular complications (Wu *et al.,* 2023). In addition to oxidative stress, inflammatory mechanisms have been reported to play a crucial role in the pathogenesis of kidney injury in diabetes (Rayego-Mateos *et al.,* 2020). Controlling blood pressure or plasma glucose levels strictly is the cornerstone of managing diabetic renal complications, as it delays the onset and progression of the kidney disease; yet, these ways are insufficient (Yokoyama *et al.,* 2016).This shortcoming highlights the necessity for more advanced therapeutic agents that may be able to target the oxidative stress – inflammatory cytokine signaling cascade and postpone the onset and development of kidney complications in diabetes (Hartman *et al.,* 2020).Therefore, in search of more innovative therapeutic agents, many studies have been conducted on medicinal plants, which thought to represent a significant natural source of drug development (Gao *et al.,* 2022; Samy *et al.,* 2023) . Among these plants that have been investigated recently in Egypt and worldwide is *Terminalia* plant (Elmalah *et al.,* 2022). The genus *Terminalia* (family: *Combretaceae*) includes more than 100 species distributed in the tropical area (Zhang *et al.,* 2019). It has high content of several phytochemical compounds as flavonoids, cyclic triterpenes, tannins that have significant phytopharmacological and medicinal activities as antioxidant, antimicrobial, anti-inflammatory, anticancer, hepatoprotective and antidiabetic (Gupta *et al.,* 2021; Khan *et al.,* 2022; Vijayalakshmi *et al*., 2023; Japhet *et al.,* 2024). *Terminalia* species are traditionally used because of pharmaceuticals and nutritional benefits (He *et al.,* 2023). For these reasons, Terminalia *muelleri* Benth was chosen for its medicinal qualities (Fahmy *et al.,* 2016; Fahmy *et al.,* 2017; Rashed *et al.,* 2018; Ahmed and Masoud, 2022; Eltablawy *et al.,* 2023). Therefore, the present study was conducted to assess the potential renal-protective effects of *Terminalia mulleri* leave extract (TME) against STZ – induced kidney injury in diabetic rats.

2. Materials and Methods

2.1. Plant Materials and extraction

The leaves of *Terminalia Muelleri* Benth. (TM) were gathered from Giza Zoo botanical garden and the plant was taxonomistically verified by the El-Orman Botanical Garden herbarium specialist in Giza, Egypt. The shade-dried powdered leaves (250 g) were extracted with ethanol (80% v/v) at room temperature (3×1L). After the extracts were filtered, the pooled filtrates were concentrated in a rotary vacuum evaporator at a lower pressure, producing a dried residue (30 g). The solid residue was kept at 4°C and suspended in saline when used in the later experimental studies (Fahmy *et al.*, 2016).

2.2. Chemicals and drugs

Streptozotocin (STZ) was purchased from Sigma Aldrich (St. Louis, Mo, USA.). Pioglitazone HCl (PIO) was kindly provided by Amoun Pharmaceutical Industries Co., Cairo, Egypt. PIO recommended dose for rats is 1.58 mg/kg b.wt., according to Ghosh (2005) and it was suspended in distilled water for oral administration. Kits used in this study were purchased by authenticated local provider from several manufacturers. All the other chemicals utilized in this study were of highest purity analytical grade.

2.3. Experimental Animals

Adult male albino rats, weighing 200 ± 30g, obtained from the laboratory stock colony of NODCAR were included in the present study. The rats were kept under well-regulatory laboratory conditions (temperature 22±3 ºC and 12 h light /dark cycle), housed in stainless steel cages, and were allowed free access to balanced food and water *ad libitum*. Rats were acclimatized for one week before the experiment. Experimental protocol used in this study was performed according to the ethical criterion approved by the research ethics committee for experimental and clinical studies at NODCAR (NODCAR/I/48/19).

2.4. Induction of diabetes mellitus

Experimental diabetes was induced in overnight fasted rats by subcutaneous injection of freshly prepared STZ, dissolved in 0.1 M citrate buffer (pH 4.5), with an initial dose of 27.5mg/kg b.wt., booster

injections of three successive doses (11.25 mg/kg b.wt.) were given through two weeks (Eltablawy *et al.,* 2015). After each injection and for one day, the drinking water was replaced by 5% glucose to prevent hypoglycemia and mortality. Blood glucose was determined at 48h after each injection to assess the induction of diabetes. Diabetes was affirmed by the elevation of fasting blood glucose (FBG) levels determined at 48h and on 5th day after injection. Only rats that had permanent fasting blood glucose levels around 250 mg /dl were included in this study.

2.5. Experimental design

Following the induction of diabetes, Normal and hyperglycemic rats were randomly divided into five groups (ten animals each), as the following:

1. **Normal control**: normal rats received saline (1ml/kg b.w) daily via gastric gavage for four weeks
2. **TME group**: normal rats received a daily oral dose of TME (200mg /kg b.wt)via gastric gavage for four weeks (Fahmy *et al.,* 2016).
3. **STZ group**: diabetic rats received saline daily via gastric gavage for four weeks and served as diabetic control group.
4. **STZ + TME group**: diabetic rats received a daily oral dose of TME (200mg /kg b.wt)via gastric gavage for four weeks.
5. **STZ+ PIO group**: diabetic rats received a daily oral dose of PIO (1.58mg /kg b.wt) via gastric gavage for four weeks

At the end of the treatment period, after an overnight fasting, blood samples were collected from the retro-orbital venous plexus in test tubes and allowed to be clot 20 min at 37°C then centrifuged at 3000 rpm for 20 min to separate the serum and stored at -20°C for the determination of fasting blood glucose, creatinine, urea, total protein, albumin, CysC and KIM-1. Afterwards, rats of all groups were euthanized by decapitation and the kidneys of the different groups were rapidly excised and washed thoroughly with ice-cold saline. Kidneys were divided into two parts; one part was placed in 10% (v/v) buffered formalin solution for histopathological and histochemical studies while the other part was weighted and homogenized with phosphate-buffered saline to prepare kidney tissue homogenate (10% homogenate). The homogenates were centrifuged and the supernatants were stored at -80°C until use in the assessment of oxidative stress markers as malodialdehyde (MDA), reduced glutathione (GSH) content, catalase activity (CAT), and inflammatory markers as nuclear factor kappa-B (NF-κB), tumor necrosis factor- alpha (TNF-α), interleukin -1β (IL-1β).

2.6. Biochemical assays

2.6.1. Estimation of fasting blood glucose (FBG) and kidney function indices

Serum concentrations of FBG and renal functions as creatinine, urea, total protein and albumin were estimated by using *in vitro* diagnostic commercial kits purchased from Bio- diagnostic (Bio-diagnostic, Cairo, Egypt) according to manufacturer's instructions.

2.6.2. Estimation of kidney-specific biomarkers

Quantitative detection of CysC and KIM-1levels in serum was performed by Enzyme-Linked ImmunosorbentAssay (ELISA) technique using MyBioSource, Inc. (USA) and Aviva Systems Biology (USA) kits, respectively according to the manufacturer's protocol.

2.6.3. Estimation of oxidant-antioxidant status in renal tissue homogenate

The levels of MDA, GSH, and CAT activity were measured in kidney tissues homogenate using *in vitro* diagnostic commercial kits purchased from Bio-diagnostic (Bio-diagnostic, Cairo, Egypt), according to the manufacturer's instructions.

2.6.4. Estimation of inflammatory markers

The quantitative evaluation of pro-inflammatory cytokines as TNF-α, IL-1βand inflammatory mediator as NF-κB concentrations were assayed in renal tissues homogenate according to manufacturer's instructions by using ELISA kits purchased from RayBiotech® Co. Ltd. (RayBiotech, Inc., USA).

2.6.5. Histological Examination

For microscopic examination, Kidney tissue fragments of rats in different groups were fixed in 10% neutral-buffered formalin. Tissue samples were irrigated and dehydrated with various alcohol grades, scrubbed in xylene, and lastly embedded in paraffin wax. Paraffin–embedded sections of kidneys were set up sectioning at 4-5µm thickness, stained with hematoxylin /eosin (H&E) solution (Banchroft and Gamble, 2008). Then sections examined under light microscope.

2.6.6. Histochemical analysis

The optical density of histochemically, Congo red stained kidney sections of control and treated groups for amyloid positive protein detection (Valle, 1986) were recorded using Image J software. The mean optical density was used to compare the amyloid positive protein content of the different groups. The comparison was established as the mean of the treated group value with the control group.Also, Mercuric bromophenol blue (Mazia *et al*. 1953) stained sections of kidney were examined for total protein content in control and treated groups.

2.7. Statistical analysis

Statistical analysis was performed using Statistical package for social science (SPSS) software (version 19, IBM, Armonk, NY, USA). All experimental results are depicted as mean ± standard error (SE). One-way analysis of variance (ANOVA) succeeded by Duncan's multiple comparisons test that was used to measure significant differences between groups. P less than 0.05 was significant.

3. Results

3.1. Effect of TME treatment on blood glucose levels

In the present model, STZ-treated rats showed a significant (P<0.05) elevation in FBG level by 2.3-fold as compared to normal control rats. On the other hand, diabetic rats treated with TME or Pioglitazone (PIO) exhibited considerable reduction in FBG levels by 35% and 41 % respectively as compared to the STZ group. Notably, TME treated rats displayed no change in FBG (Figure 1).

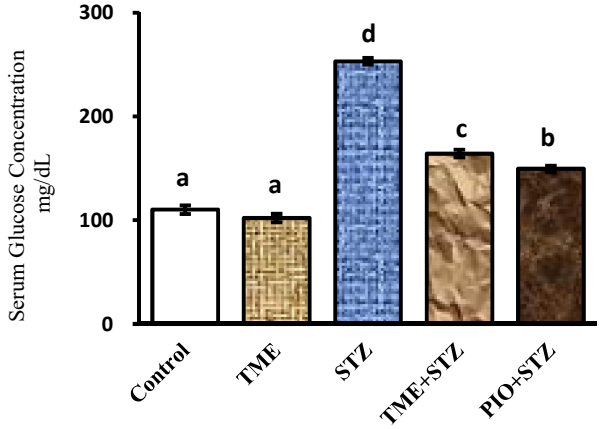

Fig. 1: The concentration of glucose in serum of all studied groups after 4 weeks of treatment. Values are represented as mean± SEM; n=6.One-way ANOVA test was applied following by Duncan's multiple comparisons between groups; different superscript letters on the bar means the presence of significant difference between the mean values (P<0.05).

3.2. Effect of TME treatment on kidney function markers

As shown in Table 1, STZ-treated rats exhibited a significant deterioration of kidney function as demonstrated by marked increase in the serum levels of creatinine and urea by 82.6% and 49.6%respectively, along with reduction in total protein and albumin levels by 15% and 16.8% respectively as compared to normal control rats. Contrary, the daily treatment with TME or PIO

ameliorated STZ–induced changes in kidney function as revealed by the reduction of creatinine by 28.6 % and 36.5, urea by 16.2% and 22.4% respectively, in addition to significant (P<0.05) elevation in total protein by 27.3 % and 19.8%, albumin by 16.9% and 15.3% respectively as compared to STZ -treated rats. Meanwhile, the levels of these parameters showed non significant differences in rats treated with TME extract only.

Table 1: Effect of TME treatment on kidney function biomarkers in the different treated groups.

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Normal control	$0.69^{ab} \pm 0.04$	$40.54^{a} \pm 2.78$	$7.49^{bc} \pm 0.16$	$3.92^{b} \pm 0.13$
TME	$0.61^{a} \pm 0.06$	$37.37^{a} \pm 1.60$	$7.25^{b} \pm 0.17$	$3.67^{b} \pm 0.06$
STZ	$1.26^{d} \pm 0.03$	$60.63^{c} \pm 2.30$	$6.37^{a} \pm 0.15$	$3.26^{a} \pm 0.08$
TME+STZ	$0.90^{c} \pm 0.06$	$50.80^{b} \pm 1.87$	$8.11^{c} \pm 0.33$	$3.81^{b} \pm 0.07$
PIO+STZ	$0.80^{bc} \pm 0.05$	$47.03^{b} \pm 1,48$	$7.63^{bc} \pm 0.31$	$3.75^{b} \pm 0.08$

Values are represented as mean± SEM; n=6.One-way ANOVA test was applied following by Duncan's multiple comparisons between groups; different superscript letters on the bar means the presence of significant difference between the mean values (P<0.05).

3.3. Effect of TME treatment on early kidney injury biomarkers (Cys C and KIM-1)

Compared with control rats, serum Cys C and KIM-1 levels were significantly (P<0.05) elevated by 97.8% and 131.6 % respectively in STZ- treated rats. In contrast, treatment of diabetic rats with TME or PIO significantly (P<0.05) reduced serum Cys C level by 26.7 % and 33% respectively, KIM-1 levels by 32% and 46.5% respectively as compared to STZ-diabetic rats (Figure 3).

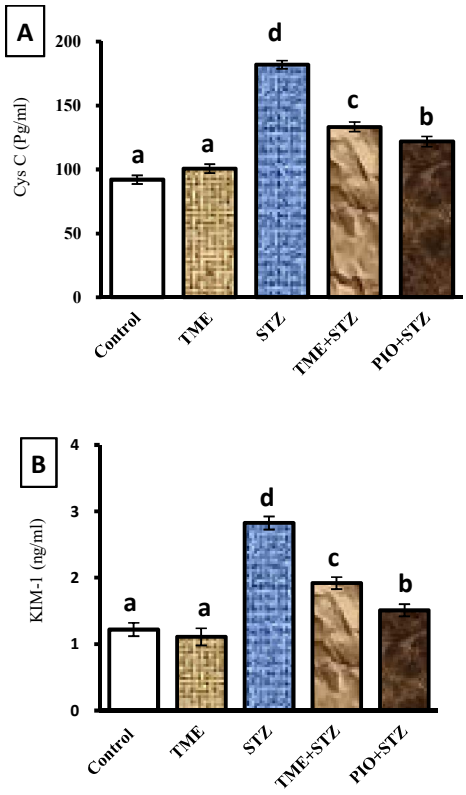

Fig. 2: The levels of Cys C (A) and KIM-1 (B) in serum of all studied groups. Values are represented as mean± SEM; n=6.One-way ANOVA test was applied following by Duncan's multiple comparisons between groups; different superscript letters on the bar means the presence of significant difference between the mean values (P<0.05).

3.4. Effect of TME treatment on MDA level, GSH content and CAT activity in kidney tissue

Diabetic rats demonstrated pronounced elevation in oxidative stress as evidenced by168.2% elevation in renal MDA level that coincident with marked reduction in GSH content by 30.7 % and CAT activity by 66.1% as compared to the normal rats. On contrary, treatment of diabetic rats with TME or PIO effectively decreased MDA level by 34.5 % and41.9% respectively, associated with marked elevation in GSH content by 77.6 % and 110.5% respectively, and CAT activity by 336.4 % and 275 % respectively as compared to STZ-treated group. Treatment with the extract nearly normalized GSH content and afforded better CAT activity than the PIO drug in diabetic rats (Figure 2).

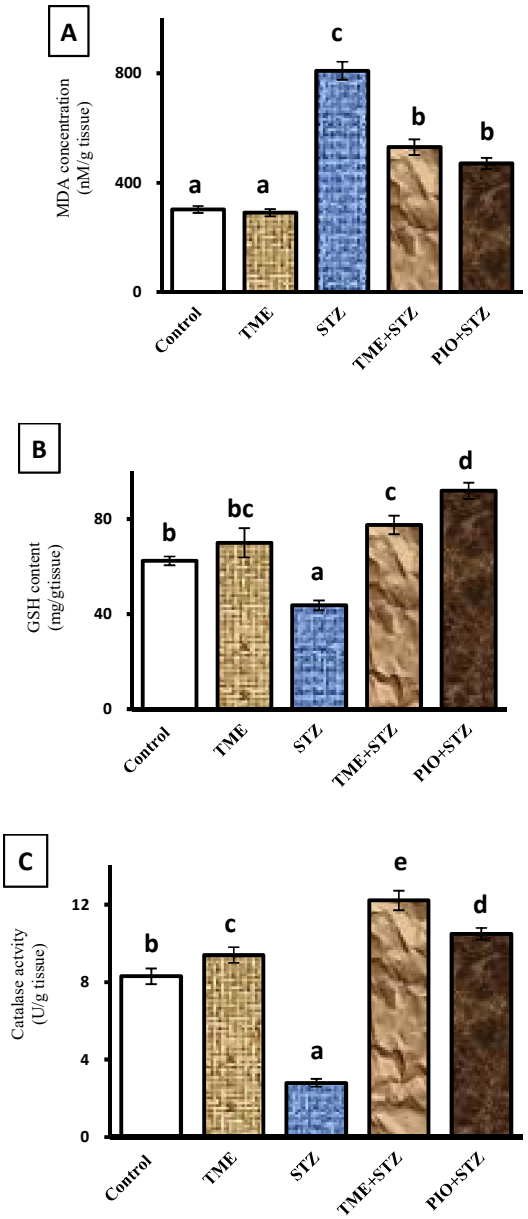

Fig. 3: The levels of MDA (A), GSH content (B) and catalase activity(C) in renal tissue homogenate of all studied groups. Values are represented as mean± SEM; n=6.One-way ANOVA test was applied following by Duncan's multiple comparisons between groups; different superscript letters on the bar means the presence of significant difference between the mean values (P<0.05).

3.5. Effect of TME on renal inflammatory markers

As illustrated in Figure 4, STZ treated group exhibited a marked elevation in renal TNF-α, IL-1β and NF-kB levels by 3.5, 4.2, and 5.6-fold respectively as compared to control group. Oral administration of TME or PIO resulted in a considerable reduction inTNF-α level by 36.3% and 44.7% respectively, IL-1β level by 39.7% and 49.1% respectively, and in NF-kB by 50.6% and 61.1% respectively as compared to STZ- treated group.

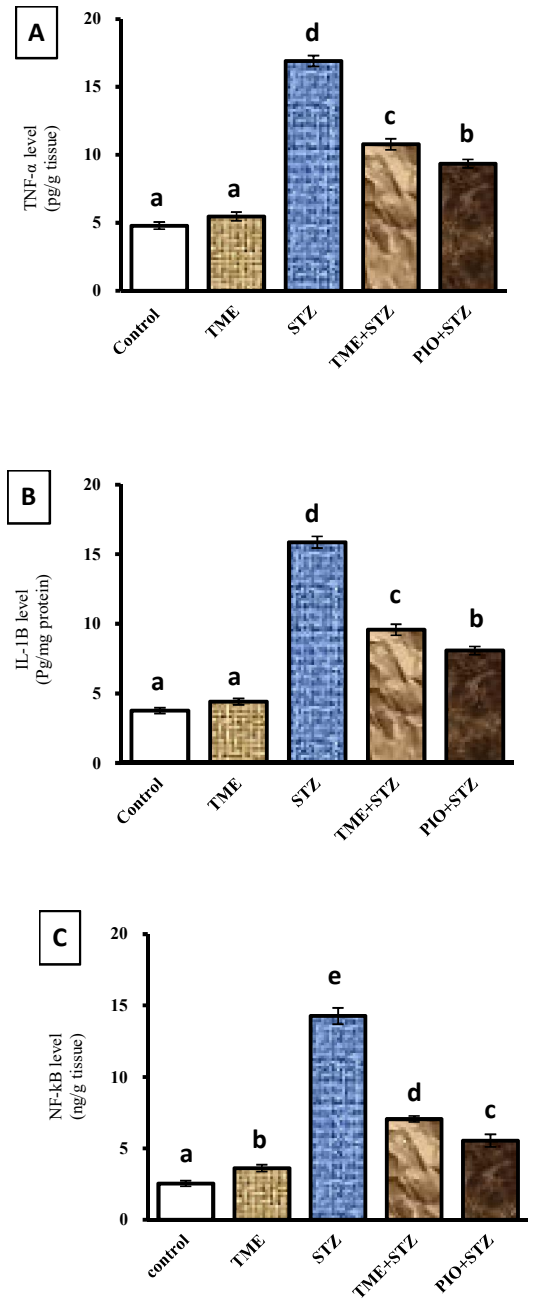

Fig. 4: The levels of TNF-α (A), IL-1β (B)and NF-kB (C) in kidney tissue of all studied groups. Values are represented as mean± SEM; n=6.One-way ANOVA test was applied following by Duncan's multiple comparisons between groups; different superscript letters on the bar means the presence of significant difference between the mean values (P<0.05).

3.6. Histopathological observations

As shown in Figure 5, H&E stained sections of kidney in control and TME animal groups (5A and 5B) showed normal kidney histological architecture. The glomeruli were well demonstrated with normal bowman space. The renal tubules filling the bulk of the kidney parenchyma were clearly observed. Diabetic kidney (Figure 5 C) showed atrophy of the glomeruli and the tubules were fairly preserved. Treatment of diabetic animals with TME or PIO improved cellular regeneration which is quite prominent as shown in Figure 5D and 5E.

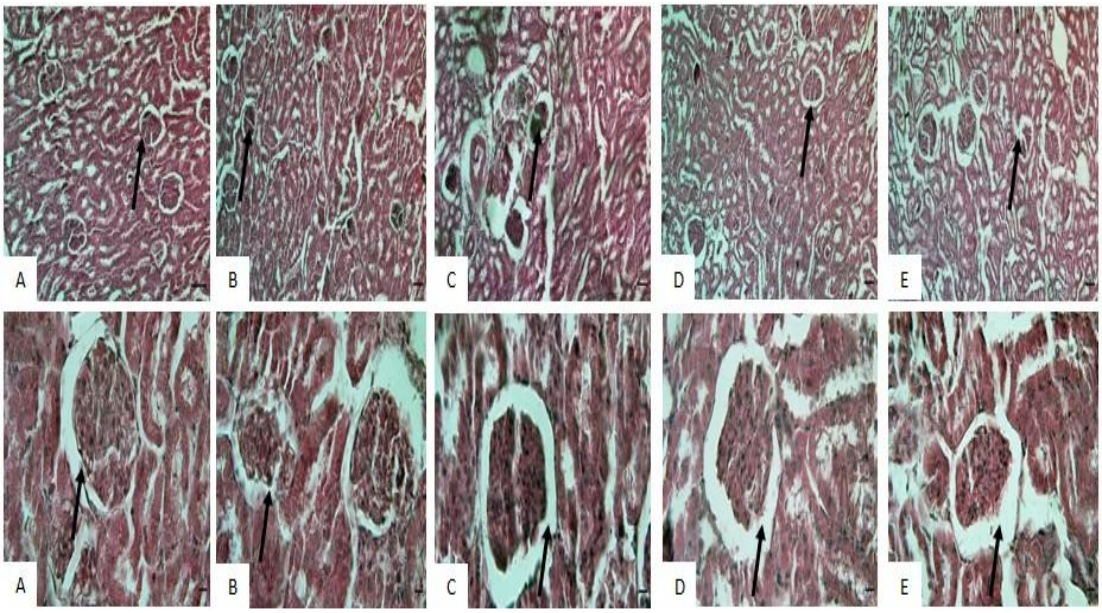

Fig. 5: Photomicrographs of rat kidney tissue of the examined groups stained by H&E. Figures A (normal control) and B (TME group) show normal renal tissue architecture with normal glomerular tuft (arrow). There was atrophy of the glomerulus and the tubules were fairly preserved in the diabetic animals (Figure 5C). The diabetic rats treated with TME or PIO (Figures 5D or 5 E) reveals renal tissue regenerations which shows a remarkable reversible cellular injury (H&E, ⎯ = 50 um and 10um).

3.7. Histochemical observations (Amyloid aggregation):

Amyloid plaques were counted in 20 random high microscopic field (40X) in Congo red-stained sections to stand the presence of amyloid fibrils (Fig 6 A-E). The images were quantified in Image J software. The obtained data were then statistically analyzed as shown in Table (2).

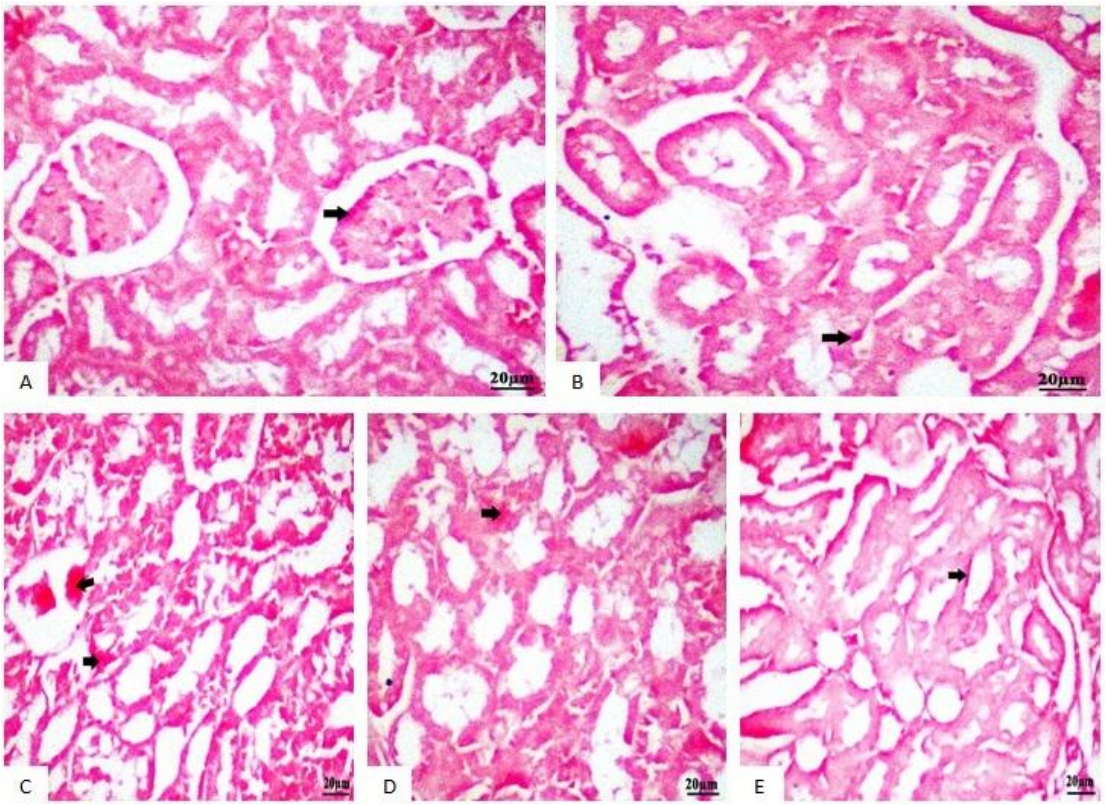

Fig. 6: (A-E): photomicrographs of rat kidney sections stained by Congo red for detecting amyloid protein Deposit in Glomerulus (arrow). In groups A and B: control and TME (little amyloid protein plaques in stained sections can be seen) while in diabetic group (C) highly positive red amyloid protein deposite in the kidney cortex was appeared (arrow). Groups 4 and 5(D&E) elicited reduction in the amyloid deposits (moderate quantity). (40X).

Table 2: The optical density of amyloid protein deposite in the kidney cortex of normal, diabetic and diabetic -treated rats.

Groups	Amyloid β- protein Mean ± SE
Normal control	0.219± 0.005
TME	0.2175±0.005
STZ	0.3991±0.013[a]
TME + STZ	0.2498±0.011
PIO + STZ	0.2614±0.012

The values are considered significant at $p \leq 0.05$, data are presented as means ± SE. **a** means significant compared to normal control.

3.8. Histochemical observations (Mercuric Bromophenol Blue, BPB):

Protein content was counted in 20 random high microscopic field (40X) in BPB-stained sections. Figures 7 (A-E) illustrated the changes in total protein content in sections of the kidney cortex of the control and the different experimental groups. Sections of control rat (7A) showing intensely stained cytoplasm, nucleus and cell membrane of tubule cells and the glomeruli. Contractually, the STZ group exhibited weak staining reflecting a significant decrease in total protein content in the cuboidal cells of the convoluted tubules and the lobulated and atrophied glomeruli while degenerated glomerulus was negatively stained (Fig. 7C). The kidney cortex of rats of STZ treated groups with TME and PIO showed moderate content of total protein (Fig. 7 D&E) respectively as compared to the control group (Fig. 7A).

Kidney cortex of rats administrated TME exhibited highly protein content (Fig. 7B) which as control group.

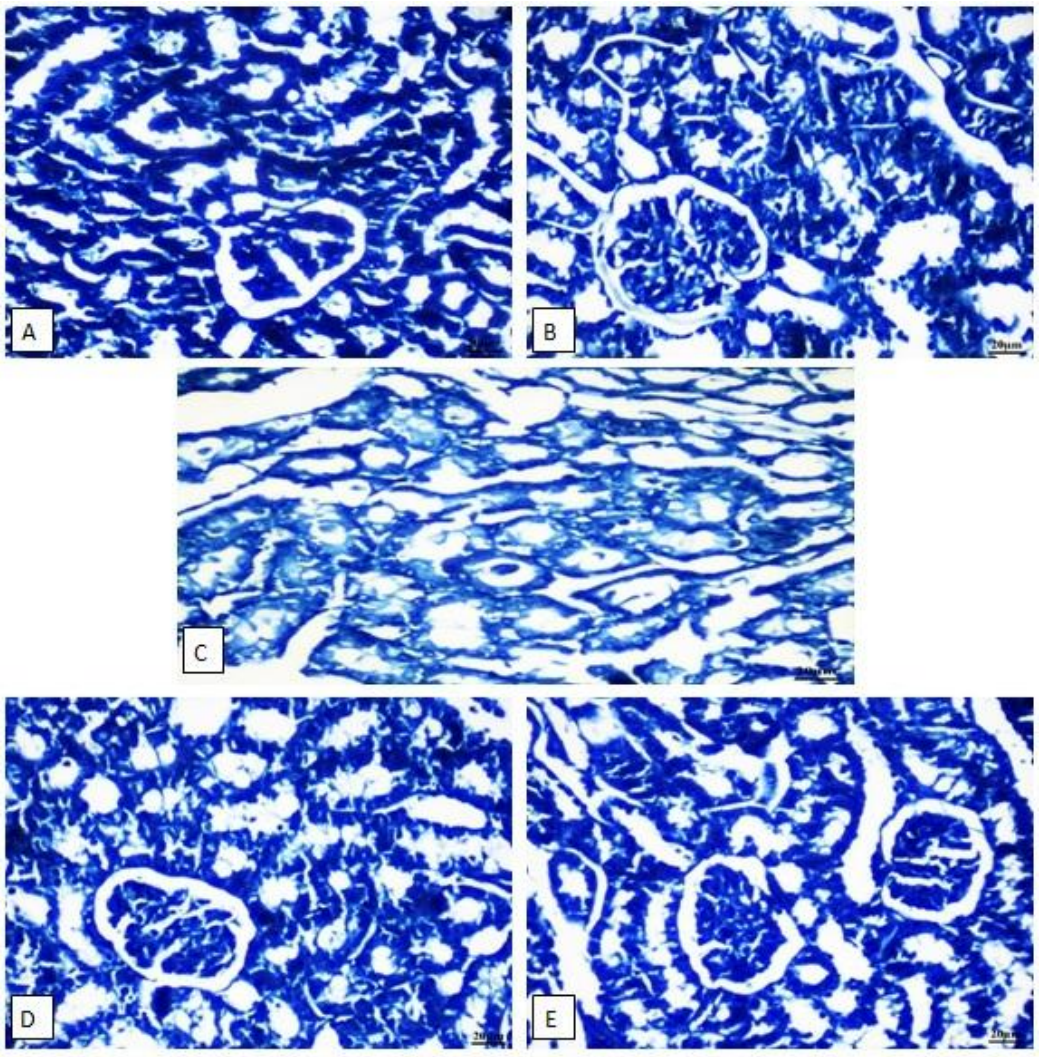

Fig. 7: (A-E): photomicrographs of rat kidney sections stained by mercuric bromophenol blue (BPB) for detecting protein content in kidney. In groups A and B: control and TME (showing high protein content in kidney) while in diabetic group (C) mild protein content can be seen. Treated STZ groups by TME or PIO (D&E) showing moderate quantity protein content (40X).

4. Discussion

The present study elicits the efficiency of TME in alleviating kidney dysfunction in STZ-induced diabetic rat. The therapeutic effect of TME on diabetic rats was associated with the mitigation of NF-κB driven inflammation and oxidative stress mediated kidney disorder. The potential of TME therapeutic efficacy was compared to the effect of PIO that was employed in the current study as a reference anti-diabetic drug. In comparison to normal rats, results of the present study revealed that STZ caused a significant increase in blood glucose level during the experimental period. This increase in glucose level reflected the destroying effect of STZ on insulin producing pancreatic endocrine cells selectively, which leads to the induction of hyperglycemia and its related complications especially the development of diabetic renal complications (Noshahr *et al*., 2020; Guo *et al*., 2021; Arif *et al*., 2022; Rahmani *et al*., 2023). In the current study the obtained and persistent elevation in glucose level is associated with significant impairment of kidney function, increase of Cys C and KIM-1 levels,

oxidative stress and inflammation. Glomerulus and the tubules atrophy were developed in diabetic animals with the formation of amyloid protein. These findings indicate the successful production of diabetic nephropathy in diabetic animal model. Kidney abnormalities progress by altering renal hemodynamics, leading to glomerulosclerosis, renal dysfunction and proteinuria (Ricciardi and Gnudi, 2021). An imbalance of nitrogen in combination with impaired protein synthesis initiates the production of non-protein nitrogenous compounds such as creatinine and urea during diabetic nephropathy conditions (Pandya *et al*., 2016). In this study the increased levels of serum urea and creatinine along with the significant decrease in serum total protein and albumin indicate the diminished renal function (Othman *et al*., 2021), that have been linked to the decline in the kidney's structural integrity. Owing to being influenced by a number of non-renal factors, the use of creatinine as the primary indicator for kidney dysfunction is limited (Hilton, 2011). Recently, Cys C and KIM-1 are used as other more reliable biomarkers for kidney function evaluation. They are peptides whose release into circulation is specific to tubular injury (Trutin *et al*., 2022). Notably; elevated levels of these kidney injury biomarkers indicate renal dysfunction. Cys C, a cysteine protease inhibitor that has been continuously produced by all nucleated cells, is established as an endogenous low-molecular weight marker of glomerular filtration rate, GFR, (Zhou *et al*., 2016). KIM-1, a transmembrane glycoprotein that is expressed on renal proximal tubule epithelial cells, is believed to play a role in renal tubule-interstitial damage (Yin and Wang, 2016). It was detected as sensitive and specific biomarkers for early prediction of acute and chronic kidney injury in experimental animals (Jin *et al*., 2017).

In the present study, it was demonstrated that glomerular filtration marker Cys C and tubular injury marker KIM-1 significantly increased in diabetic control rats. In agreement with our results, previous studies shown that diabetic nephropathy was associated with alteration in renal function (Omoboyowa *et al*., 2020; Omoboyowa *et al*., 2021). Glomerular filtration rate and tubular impairment are hallmark of kidney disease including diabetic nephropathy. This impairment is mediated by oxidative stress and inflammation (Charlton *et al*., 2021). Oxidative stress can be considered as a causative agent in diabetes progress as well as the diabetes associated inflammatory cascade that progress renal damage from genesis to progression (Hung *et al*., 2021; Singh *et al*., 2022). In concordance with this, renal tissue of diabetic rats showed a marked elevation in MDA, reduction in glutathione content and CAT activity. This disturbance in redox state of kidney tissue initiates the release of various pro-inflammatory cytokines as TNF-α and IL-1β via the activation of NF-κB, resulting in inflammation and subsequent immunological response (Kumari *et al*., 2021). Overproduction of these mediators leads to inflammatory changes in the kidney. NF-kB signaling and inflammatory cytokines have been reported to be closely related to the pathophysiology of diabetic nephropathy (Ahmed *et al.,*2016; Kumari *et al*., 2021 and Rahmani *et al.*,2023). During glomerular injury and tubulointerstitial diseases, NF-κB activation in podocytes, mesangial cells, and tubular cells has been reported (Song *et al*., 2019). TNF-α induces overproduction of free radicals, apoptotic damage of renal cell and increase the permeability of albumin in renal glomeruli (Sun and Kanwar, 2015). In addition to this, IL-1 β is activated by ROS, which the further amplified the cytokine and chemokine release that involved in the pathogenesis of DM and its related diabetic complications (Lei *et al*., 2019). In the current study the histological examination shows atrophy rather than hypertrophy in the glomeruli of diabetic animals in association with positive amyloid deposit. Amyloid fibrils are present extracellularly and deposit locally at specific sites in the body, often the site of amyloid protein production, or systemically with involvement of multiple organs. Amyloid depositions impair the appropriate supply of nutrition and oxygen to tissues or induce inflammatory reactions that cause tissue damage (Stefani and Rigacci,2013). Therefore, the obtained increase in the serum levels of creatinine, urea Cys C and the renal proinflammatory factors contribute to the formation and deposition of amyloid in the kidneys impairing their functions. It has been reported that renal amyloidosis causes increased glomerular secretion, stress, and subsequent epithelial degeneration of tubular cells with consequent progressive functional renal impairment, which suggests its association with the development and severity of renal disease progression (Gong *et al*., 2007; Díez *et al*., 2014; Huang *et al*., 2016). Also, Garber (1980) reported that the decrease in serum albumin and total protein content of the diabetic rats may be due to decreased amino acids uptake and greatly decreased concentration of a variety of essential amino acids (Brosnan *et al*., 1984), reduction in protein synthesis which in turn may be due to the decrease in the amount and availability of mRNA (Wool *et al*., 1986) and a reduction in ribosomal protein synthesis as a result of insulin deficiency

(Jefferson *et al*., 1983) and this findings give an explanation for the current results of BPB stained sections of STZ (Mahmoud *et al* ., 2019 ; Abd El-Gawwad, 2020).

Pioglitazone (PIO) is anti-diabetic agent that functions predominantly as insulin sensitizers in peripheral and hepatic tissues by binding to and activating nuclear peroxisome proliferator-activated receptor γ (PPARγ) that is widely found in several tissues and has an important role in renal physiology. It is expressed in glomerular, mesangial and tubular segments (especially the renal medullary collecting duct), as well as in renal microvasculature (Pino and DeFronzo, 2019). Results of the present study revealed that, administration of PIO to diabetic rats resulted in a significant decrease in glucose level, marked improvement of renal function, reduction of oxidative stress, with the attenuation of kidney inflammation response. This finding provided evidence that in addition to glycemic control, PIO could directly activate endogenous renal PPARγ, resulting in the suppression of inflammatory molecules. Several studies have shown that PIO can reduce oxidative stress by improving some antioxidant enzymes at the kidney with decreasing the level of MDA, indicating the restoration of anti-oxidative capacity in the injured kidney (Kong *et al*., 2011; Kuru Karabas *et al*., 2013; Sun *et al*., 2016). In the present study the administration of PIO inhibited the activation of renal NF-κB and significantly reduced the secretion of the pro-inflammatory cytokines TNF-α, and IL-1β. This finding proved the anti-inflammatory properties of PIO and corroborated with the previous studies reported the beneficial effects of PPAR-γ agonists on the kidney (Agrawal *et al*., 2016; Mousleh *et al*., 2018; Medić *et al*.,2019; Ye *et al*., 2024). A meta-analysis performed by Zhou *et al.,* (2020) concluded that, PIO should be considered and cautiously used in patients high risk of type 2 diabetes mellites for the prevention of cardiovascular endpoints. The use of complementary and/or alternative medicine and especially the consumption of botanicals have been increasing worldwide, because of supposedly less frequent side effects when compared to therapeutic agents (Ghaedi *et al*., 2017).

Terminalia muelleri Benth. was chosen for its medicinal potentials. (Fahmy *et al*.,2016; Fahmy *et al*., 2017; Rashed *et al*., 2018; Ahmed and Masoud, 2022; Eltablawy *et al*., 2023). It has been reported that TM leaf possess a valuable amount of cinnamic acid, gallic acid, rutin, quercetin, methyl gallate and cafoylacetylhexoside (Elmalah *et al*., 2022). Administration of TME to diabetic rats significantly reduced blood glucose level, indicating the anti-diabetic effect of TME that could be reflected its ability to act as an insulin sensitizer. This finding is in the same line with the previous studies that explained the ability of TM phytoconstituents to increase glucose transport and metabolism and /or stimulate insulin secretion (Rashed *et al*., 2018; Savych et al., 2021; Ansari *et al*., 2022; Wong *et al*., 2023). In addition to the observed hypoglycemic effect of TME, it significantly reduced the production of free radical generation as manifested by the obtained decrease in renal MDA level in concomitant with the significant increase in GSH content and Catalase activity. This enhancement in the antioxidant status in kidney tissue reflects the ability and efficacy of TM polyphenolic compounds to scavenge the reactive oxygen species directly with the inhibition of lipid peroxidation cascade proving the antioxidant properties of TME and confirmed the previous studies of Fahmy *et al*. (2016); Ahmed and Masoud, (2022). Results of the present study revealed that, the inflammatory response is an essential pathophysiological process during diabetes-induced renal injury. It has been reported that TNF-α is the upstream of the inflammatory cascade and initiates the up-regulation of chemokines and cytokines meanwhile, IL-1β is one the downstream of the inflammatory cascade that can damage renal cells directly (Okusa, 2002). Therefore, the inhibition of this response is an important approach to prevent the progression of renal impairment. TME extract administration to diabetic rats significantly attenuated the obtained increase in the levels of renal TNF-α, IL-1β and NF-κB. This finding demonstrated that the curative effect of TM in STZ-induced diabetic renal impairment occurred via the mitigation of the pro-inflammatory cytokine involved in the NF-κB pathway, such as TNF-α and IL-1β. This finding proved the efficient anti-inflammatory role of natural flavonoids against the diabetic renal impairment. Results of the present study clearly demonstrated that treatment with TME extract resulted in a marked inhibition of oxidative stress with the reduction of hyperglycemia leading the blocked of proinflammatory cascade in the kidney of diabetic rats thereby resulting in attenuation renal inflammation. Therefore, the glycemic control may be a vital mechanism by which TME extract inhibited diabetic kidney disease. It is worthy to say that the glycemic control exerted by TME extract closely similar to that by which PIO exerted with a slight difference that referred the priority of PIO. This difference could be attributed to the poor bioavailability of flavonoids.

5. Conclusion

The findings of the present study revealed the anti-diabetic, antioxidant, anti-inflammatory and renal protective effects of TME in STZ-induced diabetic rats. TME enhanced antioxidant status and maintained kidney architecture. To sum up, the outcomes of this study may support the potential effect of TME as an alternative therapy for controlling the diabetic -related renal complications. However, more studies are needed to determine the clinical dosage, dosage formulation, administration method and the key mechanism to enhance the beneficial effect of TME extract in combating diabetic renal impairment.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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