



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/2221-1691.393580

Agmatine ameliorates diabetes type 2–induced nephropathy in rats

Fatemah O. Kamel¹, Ohoud Shagroud¹, Mai A.Alim A.Sattar Ahmad¹, Gamal S Abd El–Aziz², Abdulhadi S. Burzangi¹, Duaa Bakhshwin¹, Maha Jamal¹✉, Shahid Karim¹✉¹Department of Clinical Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia²Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ABSTRACT

Objective: To assess the nephroprotective potential of agmatine in a rat model of streptozotocin-induced diabetic nephropathy.

Methods: A single dose of streptozotocin (40 mg/kg) coupled with a fructose diet induced diabetes in Wistar rats. Agmatine (40 and 80 mg/kg) was administered to rats for 12 weeks. The body weight and fasting blood glucose were measured weekly. Insulin level, urine output, total protein, albumin, blood urea nitrogen, creatinine, and cystatin-C were also determined at the end of the experiment. Furthermore, superoxide dismutase, glutathione, interleukin-1 β , interleukin-6, and tumor necrosis factor-alpha were evaluated in kidney tissue. Histopathological study was also performed using hematoxylin and eosin staining.

Results: Agmatine at both doses significantly increased final body weight, and lowered fasting blood glucose, urine output, insulin, total protein, albumin, blood urea nitrogen, creatinine, and cystatin-C levels compared with the diabetic group ($P < 0.05$). Inflammatory markers and antioxidant effect were significantly improved in agmatine-treated rats. Moreover, the histopathological changes in renal structure were ameliorated by agmatine treatment.

Conclusions: Agmatine alleviates diabetic nephropathy by improving renal functions and reducing inflammation and oxidative stress. The molecular mechanisms of its nephroprotective actions need to be investigated in future study.

KEYWORDS: Agmatine; Type 2 diabetes; Nephropathy; Oxidative stress; Nephroprotection

1. Introduction

Diabetes mellitus (DM) has the potential to impact multiple organs within the body, with the extent and severity of its effects being contingent upon its duration and intensity. This can result in

the manifestation of diverse organ failures[1]. DM is the primary etiological factor for chronic kidney disease (CKD) on a global scale. It has been reported that approximately 40% of individuals with diabetes may develop CKD throughout their lifespan[2]. Diabetic nephropathy (DN), which is also referred to as diabetic kidney disease (DKD), is distinguished by structural modifications in the kidney. Specifically, there is an expansion of mesangial cells and thickening of the basement membrane in the glomeruli, as well as hypertrophy of the tubules[3].

The kidneys of diabetic patients are affected by several metabolic abnormalities, including hyperaminoacidemia, glomerular hyperfiltration and hyperperfusion, and hyperglycemia. These abnormalities are known to cause inflammation and eventual fibrosis[4].

Maintaining intensive glycemic control is of utmost importance in the early stages of the disease for preventing DKD. Several studies

Significance

The incidence of diabetic nephropathy continues to rise, necessitating the implementation of supplementary therapeutic interventions for preventing or mitigating the progression of this illness. Our study shows that agmatine can protect against diabetic nephropathy by modulating anti-inflammatory and antioxidant activities. Further study is needed to investigate the mechanism of its nephroprotective actions.

✉To whom correspondence may be addressed. E-mail: skaled@kau.edu.sa (S. Karim); mjamal@kau.edu.sa (M. Jamal)

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2024 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow.

How to cite this article: Kamel FO, Shagroud O, Ahmad MA, Abd El-Aziz GS, Burzangi AS, Bakhshwin D, et al. Agmatine ameliorates diabetes type 2-induced nephropathy in rats. Asian Pac J Trop Biomed 2024; 14(1): 8-16.

Article history: Received 6 September 2023; Revision 26 September 2023; Accepted 20 November 2023; Available online 23 January 2024

have indicated that intensive glucose control may not effectively decrease the likelihood of CKD advancement or cardiovascular mortality in advanced stages of DKD[5]. According to the guidelines established by the Kidney Disease: Improving Global Outcomes, it is recommended that the target range for HbA1c should fall between <6.5% to <8.0%. The determination of the precise target should be based on the degree of hypoglycemia risk that is present in each patient[6].

Agmatine is a naturally occurring byproduct of *L*-arginine metabolism that has been found to exhibit antioxidant, anti-apoptotic, and anti-inflammatory characteristics[7,8]. Numerous subsequent studies have examined the physiological and pharmacological impact of agmatine on mammals. Agmatine has demonstrated protective effects against various disorders, including cardio-protection, nephro-protection, gastro-protection, neuro-protection, and gluco-protection[9]. The experimental findings of Lortie *et al.* indicate that agmatine can enhance the glomerular filtration rate (GFR) by stimulating endothelial nitric oxide synthase (eNOS)[10]. Therefore, the objective of the current study was to assess the protective effect of two different dosages of agmatine against streptozotocin (STZ)-induced DN in rats.

2. Materials and methods

2.1. Chemicals and drug solutions

STZ (S0130), agmatine (A7127) and fructose (1286504) were purchased from Merck, Germany. Glucose solution was purchased from the local pharmacy. All chemicals used in the study were of analytical grade. Solution of STZ was prepared in sodium citrate buffer (0.1 M, pH-4.4), agmatine and fructose solutions were prepared in normal saline and water, respectively.

2.2. Animals

Male adult Wistar rats ($n = 30$), weighing approximately 200-250 g, were procured from the College of Pharmacy Animal House at King Abdulaziz University, and used in this study. The animals were kept in separate standard cages at standard animal room temperature (23 ± 1)°C and 12 hours of light/dark cycle were maintained during the whole study period. They had free access to Purina rat chow and water.

2.3. Ethical statement

This research was approved by the Research Ethics Committee at Faculty of Pharmacy, King Abdulaziz University (PH-1442-44). This research was conducted by implementing regulations of the Law of Ethics of Research on living creatures in the Kingdom of Saudi Arabia.

2.4. Induction of diabetes

The experimental induction of DM in rats involved the administration of drinking water containing a weight-per-volume concentration of 10% fructose for the entirety of the study. Following 14 days during which fructose water was consumed, a single administration of STZ at a concentration of 40 mg/kg b.w. was administered[11]. Rats that received STZ injections were administered a 5% glucose solution for 24 h in order to counteract the reduction in blood glucose levels induced by the drug. Glucose concentration was measured in rats after 72 h using the Accu-Chek test strip glucometer (Roche Diagnostics GmbH, Mannheim, Germany) by puncturing the tail vein. Rats having a blood glucose level exceeding 250 mg/dL were deemed to be afflicted with diabetes and were subsequently incorporated into the study. The initiation of treatments occurred on the fifth day after STZ injection, and this was designated as the first day of treatment.

2.5. Experimental design

After acclimatization for one week, the animals are randomly divided into four groups ($n = 6$). Group 1 (control): rats received only a vehicle sodium carboxyl methyl cellulose (0.5% Na-CMC). Group 2 (diabetic control): the experimental diabetes was induced; Group 3 (AGM 40): diabetic rats received fructose 10% + low dose agmatine (40 mg/kg daily)[12]. Group 4 (AGM 80): diabetic rats received fructose 10% + high dose agmatine (80 mg/kg daily)[13].

Groups 1 and 2 were given 0.5% Na-CMC five times weekly. Groups 3 and 4 were given agmatine 40 and 80 mg/kg, respectively five times weekly at the same time for 12 weeks. All drugs were freshly prepared in 0.5% Na-CMC and given by oral gavage.

At the end of the experiment, rats were kept in metabolic cages for 24-hour urine collection. Then the rats were deprived of food for 12 h before they were anesthetized using ether. After anesthesia, blood was withdrawn from the retro-orbital plexus to estimate glucose level and other biochemical markers. Rats were euthanized *via* cervical dislocation and their abdomens were opened where the right and left kidneys were removed, weighed, and divided sagittally into two halves; the right halves were frozen in -80 °C for homogenate preparation.

2.6. Determination of body weight, kidney weight, and kidney weight/body weight ratio

The initial and weekly body weights of the rats were recorded throughout the experiment. The left and right kidneys of each rat were also weighed. The kidney to body weight ratio was determined by computing the mean weight of the bilateral kidneys, dividing it by the body weight, and subsequently multiplying the quotient by 100 to obtain a percentage value.

KW/BW ratio=(mean weight of the bilateral kidneys)/(body weight)×100

2.7. Blood sample collection

The rodents were anesthetized *via* an intraperitoneal injection of ketamine 40 mg/kg and xylazine 5 mg/kg, then the blood samples were obtained from the retro-orbital plexus utilizing yellow gel venipuncture tubes. Following collection, the samples were promptly subjected to centrifugation at 3000 rpm for 5 min. The resultant serum was subsequently separated and stored at -80°C for biochemical analysis.

2.8. Preparation of renal homogenate

Following euthanasia, the right kidney of each rat was collected and washed with a cold isotonic saline solution. Subsequently, the kidneys were cryopreserved at -80°C until further use. The homogenate was generated through the process of thawing the kidneys and subsequently measuring 0.1 g from each specimen. The allotted quantity was meticulously diced with a disinfected scalpel and subsequently introduced into 200 mL of chilled modified phosphate-buffered saline. The process of homogenization was accomplished through the utilization of the Ultra-Turrax[®] T 25. Subsequently, the homogenate was subjected to centrifugation at 3000 rpm for 15 min and the resultant supernatant was procured and preserved at -80°C for subsequent analysis of the desired analytes.

2.9. Determination of diabetic biomarkers

2.9.1. Determination of fasting blood glucose (FBG) and insulin

The blood glucose levels were assessed with ELISA Kit (MBS7233226), while the plasma insulin levels were determined using the Rat Elisa kit (MBS045315).

2.9.2. Homeostasis model assessment of insulin resistance (HOMA-IR)

IR was calculated using the HOMA-IR equation:

$$\text{HOMA-IR} = \text{Fasting insulin (mU/mL)} \times \text{FBG (mg/dL)} / 405 [14].$$

2.10. Determination of renal function and renal biomarkers

2.10.1. Urinary output

Polyuria is a clinical manifestation of DM, characterized by a urinary output that exceeds 2.5 L per day or more than 40 mL/kg within 24 h. As per the definition, the condition occurs when the amount of urine excreted exceeds the normal range [15]. In order to quantify polyuria in rats, they were subjected to metabolic cages for 24 h [16].

2.10.2. Total urinary protein and albumin

Total urinary protein was estimated using the commercial kit (Cat No. MBS268472) and ELISA kit was used for estimation of urinary albumin (Cat No. MBS564099).

2.10.3. Blood urea nitrogen (BUN), serum creatinine (Cr), and cystatin-C (Cys-C)

BUN, serum Cr, and Cys-C were determined using myBiosource ELISA Kits (Cat No. MBS2611086, Cat No. MBS2749827 and Cat No. MBS042119, respectively).

2.11. Determination of oxidative stress in renal tissue

Commercial kit was used to assess oxidative stress markers glutathione (GSH) (MBS749827) and superoxide dismutase (SOD) (MBS042119) in renal tissue. Instructions provided by the manufacturer were followed for measuring the concentration of stress markers.

2.12. Determination of inflammatory markers in renal tissue

A commercial kit was used to assess inflammatory markers interleukin-1 β (IL-1 β) (MBS264984), interleukin-6 (IL-6) (MBS726707), and tumor necrosis factor-alpha (TNF- α) (MBS355371) in renal tissue. Instructions provided by the manufacturer were followed for measuring the concentration of stress markers.

2.13. Histopathological examination

The kidney samples were subjected to standard histological processing, which involved a 48-h fixation in 10% neutral buffered formalin, followed by dehydration, clearing, and paraffin embedding. The examination of 4- μm -thick sections stained with hematoxylin and eosin (H&E) was conducted using a electric light microscope (Carl Zeiss AxioStar Plus, Oberkochen, Germany). The renal components were examined for indications of injury by a pathologist who was blinded to the treatment groups. The tubular injury was evaluated in tubules of a specific segment that exhibited distinct tubular alterations, such as tubular dilatation/flattening, loss of brush border, vacuolated cells, cellular detachment, localized necrosis, intraluminal nuclei, and debris.

2.14. Statistical analysis

All the data are presented as mean \pm standard deviation (SD). The statistical analysis of the data was performed using SPSS 20 (SPSS Corp., Armonk, NY, USA). One-way ANOVA was used to compare the data followed by Tukey's multiple comparisons. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Effect of oral administration of agmatine on body weight in diabetes-induced nephropathy in rats

The diabetic group exhibited a noteworthy reduction in body weight with a significant elevation in the kidney-to-body weight ratio in comparison to the control group, as depicted in Table 1 ($P <$

0.05). However, treatment with 40 and 80 mg/kg agmatine markedly increased body weight and decreased kidney to body weight ratio compared with the diabetic group ($P < 0.05$).

3.2. Agmatine modulates diabetic parameters in STZ-induced DN in rats

As illustrated in Figure 1A, there was a significant increase in blood

Table 1. Effect of oral administration of agmatine on body weight in diabetes-induced nephropathy in rats.

Parameters	Normal control	Diabetic control	Agmatine (40 mg/kg)	Agmatine (80 mg/kg)
Initial body weight (g)	227.75 ± 12.66	223.87 ± 15.08	222.75 ± 12.71	223.25 ± 11.29
Final body weight (g)	360.37 ± 11.97	292.75 ± 15.61 [#]	324.87 ± 14.15 ^{#*}	342.00 ± 10.87 [*]
Kidney to body weight ratio	0.35 ± 0.01	0.42 ± 0.03 [#]	0.33 ± 0.02 [*]	0.33 ± 0.01 [*]

Values are expressed as mean ± SD ($n = 6$). Statistical analysis is carried out by one-way ANOVA and Tukey's *post hoc* test. [#] $P < 0.05$ compared with the normal control group, ^{*} $P < 0.05$ compared with the diabetic control group.

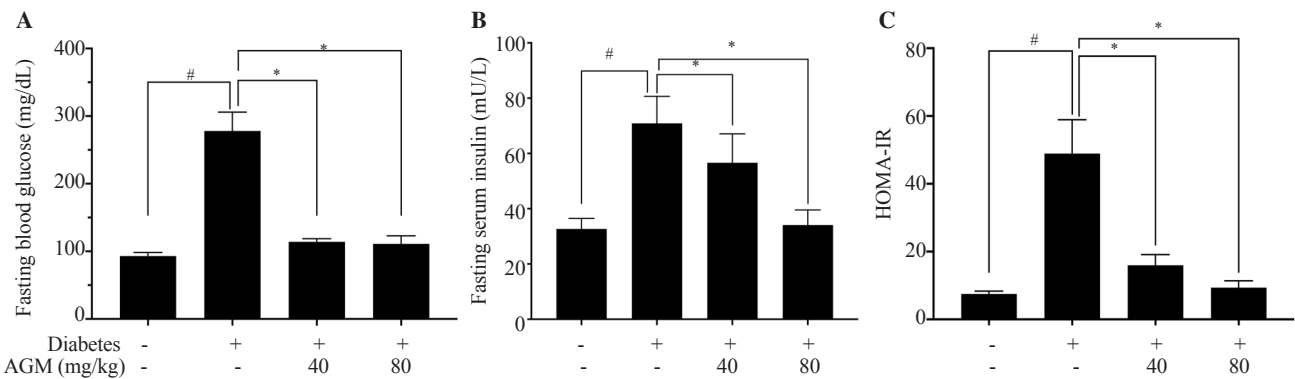


Figure 1. Agmatine improves (A) fasting blood glucose, (B) fasting serum insulin, and (C) HOMA-IR in rats with streptozotocin-induced diabetic nephropathy. Values are expressed as mean ± SD ($n = 6$). Statistical analysis is carried out by one-way ANOVA and Tukey's *post hoc* test. [#] $P < 0.05$ compared with the normal control group, ^{*} $P < 0.05$ compared with the diabetic group. HOMA-IR: homeostasis model assessment of insulin resistance; AGM: agmatine.

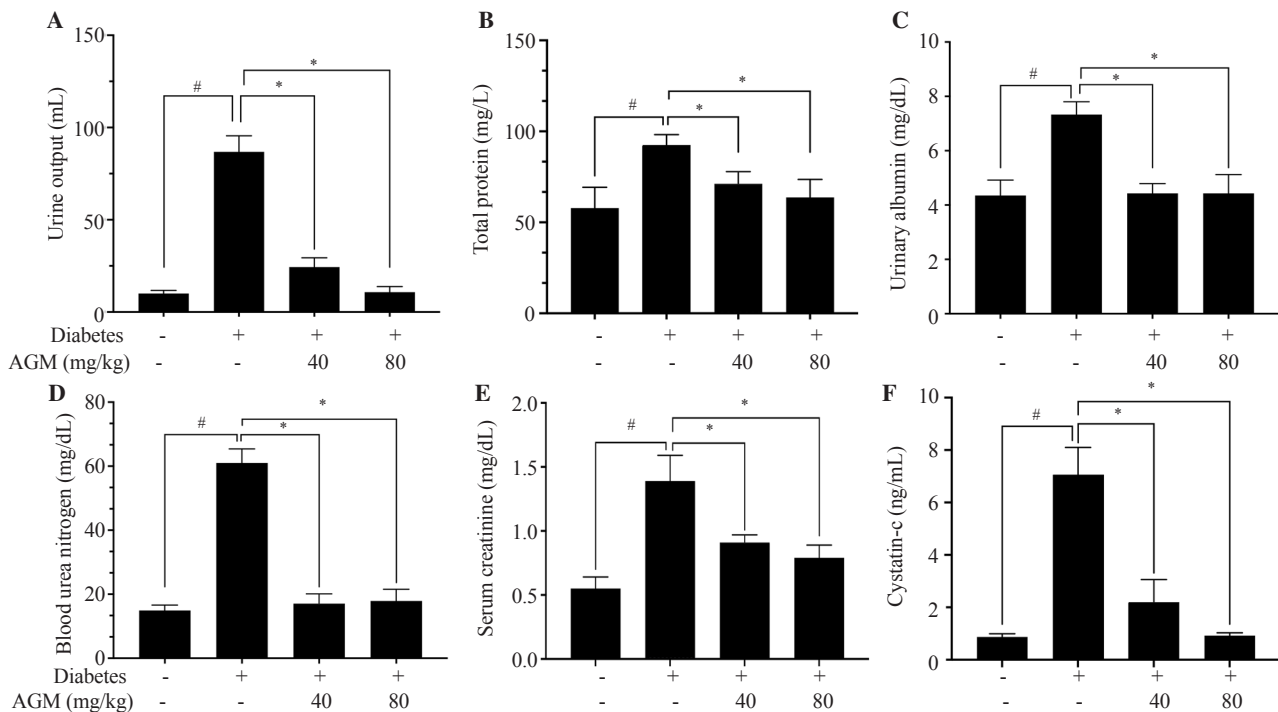


Figure 2. Agmatine improves renal function in diabetes-induced nephropathy. (A) Urine output, (B) total protein, (C) urinary albumin, (D) blood urea nitrogen, (E) serum creatinine, and (F) cystatin-c. Values are expressed as mean ± SD ($n = 6$). Statistical analysis is carried out by one-way ANOVA and Tukey's *post hoc* test. [#] $P < 0.05$ compared with the normal control group, ^{*} $P < 0.05$ compared with the diabetic group.

glucose levels ($P < 0.05$) of the diabetic group as compared to the control group. Conversely, a significant reduction in blood glucose levels was observed in the groups treated with 40 and 80 mg/kg agmatine when compared to the diabetic group ($P < 0.05$).

Moreover, the insulin level and HOMA-IR in the diabetic group were significantly higher ($P < 0.05$) compared to the control group. Treatment with agmatine at doses of 40 and 80 mg/kg resulted in a notable decrease in both HOMA-IR and fasting insulin levels when compared with diabetic group (Figure 1B and C).

3.3. Agmatine improves renal function in STZ-induced DN in rats

As shown in Figure 2, the urine output and the levels of total protein, urinary albumin, BUN, Cr, and Cys-C in the diabetic group were markedly higher in comparison with the control group ($P < 0.05$). Agmatine treatment at 40 and 80 mg/kg pronouncedly lowered these increased parameters ($P < 0.05$).

3.4. Agmatine reduces oxidative stress in DN

Figure 3A shows a marked decline in the SOD activity in STZ-

induced diabetic rats as compared to the control group ($P < 0.001$). Agmatine treatment at 40 and 80 mg/kg significantly increased the SOD activity as compared to the diabetic control group. Furthermore, the GSH level in the diabetic group was significantly lower in comparison with all other test groups ($P < 0.05$) (Figure 3B). However, 40 and 80 mg/kg agmatine markedly enhanced the GSH level ($P < 0.05$).

3.5. Agmatine attenuates inflammation in diabetic renal tissues

As shown in Figure 4, IL-1 β , IL-6, and TNF- α levels were significantly raised in the diabetic group as compared to control ($P < 0.05$). Rats treated with both doses of agmatine showed a significant decline in these inflammatory marker levels as compared to the diabetic group ($P < 0.05$).

3.6. Agmatine ameliorates the tubular damages induced by DN

The photomicrograph of kidney tissue from the control group revealed the normal histological structure of renal corpuscles

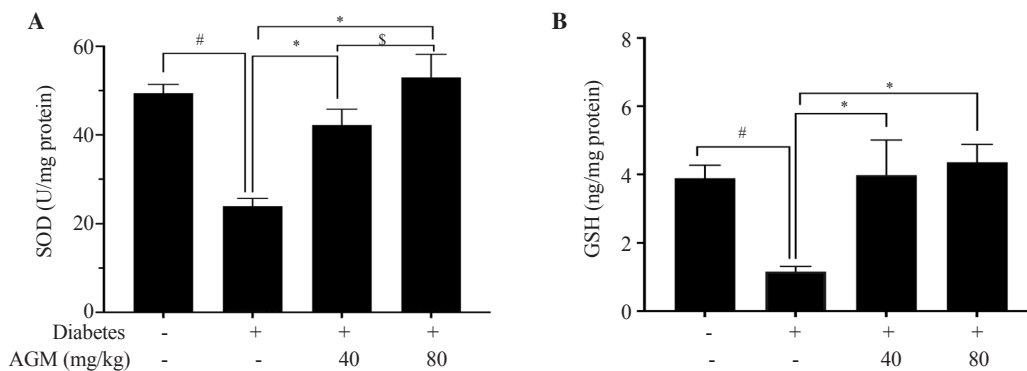


Figure 3. Agmatine reduces oxidative stress in streptozotocin-induced diabetic nephropathy. (A) SOD; (B) GSH. Values are expressed as mean \pm SD ($n = 6$). Statistical analysis is carried out by one-way ANOVA and Tukey's *post hoc* test. # $P < 0.05$ compared with the normal control group, * $P < 0.05$ compared with the diabetic group, $^sP < 0.05$ compared with the diabetic group treated with 40 mg/kg agmatine. SOD: superoxide dismutase; GSH: glutathione.

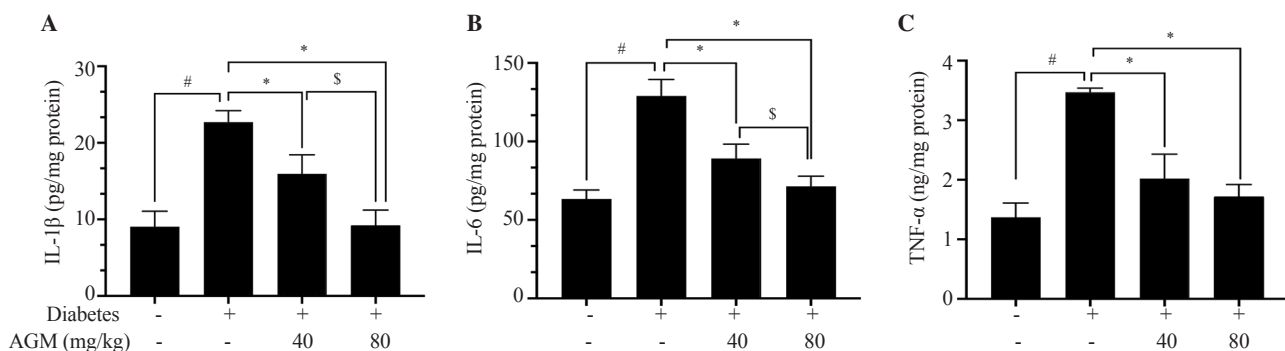


Figure 4. Agmatine attenuates inflammation in renal tissue of streptozotocin-induced diabetic rats. (A) IL-1 β , (B) IL-6, (C) TNF- α . Values are expressed as mean \pm SD ($n = 6$). Statistical analysis is carried out by one-way ANOVA and Tukey's *post hoc* test. # $P < 0.05$ compared with the normal control group, * $P < 0.05$ compared with the diabetic group, $^sP < 0.05$ compared with the diabetic group treated with 40 mg/kg agmatine.

and tubules (Figure 5A). Each renal corpuscle was formed of a glomerulus and Bowman's capsule. The glomerulus was a tuft of capillaries and had a basement membrane.

In contrast, the diabetic group (Figure 5B) showed some morphological changes such as atrophic glomeruli, while in others, the glomeruli appeared hypertrophied with a reduction in capsular space. In addition, some glomeruli showed dilated peripheral capillary loops with vacuolization. Eosinophilic hyaline depositions were found between the capillary loops, which might be diffused or nodular. Mild to moderate inflammatory cell infiltration with areas of inter-tubular hemorrhage could also be seen in some sections. Regarding proximal convoluted tubules (PCTs), some tubules showed swollen vacuolated lining cells that bulged into the lumen while other tubules showed atrophied lining with the disappearance of the brush borders. Meanwhile, widening of the lumen of distal convoluted tubules (DCTs) with flattening of their epithelial lining was seen with some pyknotic nuclei.

As illustrated in Figure 5C, the diabetic group co-treated with 40 mg/kg of agmatine showed improved renal structure when compared to the diabetic group. Nevertheless, some glomeruli still showed atrophic changes with a localized deposition of eosinophilic hyaline material and dilatation of the peripheral capillary loops. Moreover, fewer areas of intertubular hemorrhage were present. Moreover, some of the PCTs and DCTs appeared nearly normal while others showed evidence of damage in the form of small vacuolation or loss of brush borders. For the diabetic group co-treated with 80 mg/kg of agmatine (Figure 5D), more improved structural changes were observed. The majority of renal cortex, PCTs, and DCTs appeared similar to control findings. However, some congested blood vessels and dilated interstitial spaces could be seen.

4. Discussion

DN is a significant complication that exerts a considerable influence

on global populations[17]. The pathogenesis of DN encompasses a spectrum of functional alterations, including augmented intraglomerular pressure, activation of the renin-angiotensin system, oxidative stress, and fibrotic changes, which are compounded by an inherent genetic predisposition[18]. Moreover, it has been observed that individuals suffering from DN exhibit elevated levels of circulating inflammatory mediators such as adhesion molecules and chemokines, along with intrarenal immune cell infiltration[19].

Elevated blood glucose levels, or hyperglycemia, have been associated with an increase in the production of advanced glycation end products and the promotion of a proinflammatory environment. These factors have been correlated with a gradual decline in glomerular filtration rate (GFR). Elevated levels of adhesion molecules, infiltrating cells, reactive oxygen species (ROS), cytokines, and metalloproteinases have been identified as indicative markers of the aforementioned condition[19,20]. The hemodynamics of the kidneys are negatively impacted by IR, as evidenced by previous research[19–21].

This study examined the potential protective properties of agmatine at different dosages (40 and 80 mg/kg/day) based on earlier studies[7,13] against DN in rats. In this study, histopathological parameters were employed along with assessments of renal function, inflammatory markers, and oxidative stress. Elevated blood levels of BUN and Cr have been associated with renal impairment across various conditions and instances of chemical poisoning[22]. Following STZ treatment, it was observed that there was a significant increase in both serum biochemical parameters. The correlation between significant reductions in body weight and kidney hypertrophy index in DM has been linked to hyperglycemia, hypoinsulinemia, and the degradation of muscle proteins[23]. The administration of agmatine at both doses resulted in a significant increase in body weight and prevented the loss of muscle tissue caused by hyperglycemia in STZ-induced DN.

The present study confirmed that agmatine treatment at both low and high doses resulted in a noteworthy decrease in FBG and insulin

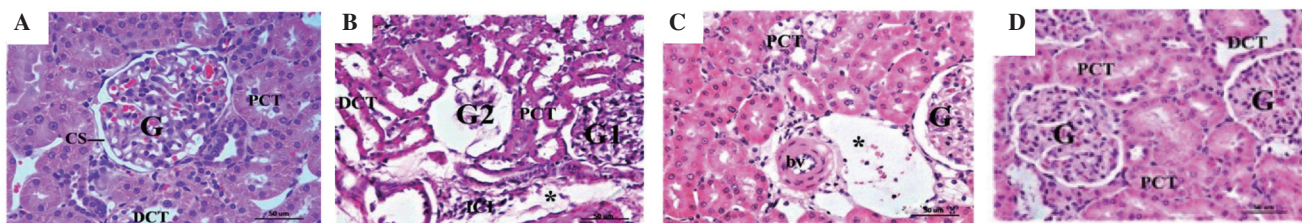


Figure 5. Representative photomicrographs of kidney sections (H&E, $\times 400$). (A) The control group shows that the renal corpuscle is formed of a glomerular tuft of capillaries (G), surrounded by Bowman's capsule and separated by capsular space (CS). (B) The diabetic group shows hypertrophied (G1) and atrophied (G2) glomeruli with wide interstitial spaces (*). Some proximal convoluted tubules (PCTs) and distal convoluted tubules (DCTs) appeared dilated with flattening of the lining cells. (C) The diabetic group treated with 40 mg/kg of agmatine shows improved glomerulus (G). However, interstitial spaces (*), thickened blood vessels (bv), and some damaged PCTs are noticed. (D) The diabetic group treated with 80 mg/kg of agmatine shows significantly improved glomeruli (G).

level, and HOMA-IR, which was comparable to the normal control group. The impaired kidney functions in rats with STZ-induced diabetes were indicated by elevated levels of BUN, total protein, and Cr, as well as changes in the histomorphological architecture of the kidneys, characterized by tubular necrosis, severe glomerular morbidity, and intertubular hemorrhage. These findings align with the results found in previous studies[24,25]. Agmatine treatment exhibited a mitigating effect on the progression of the DN, which was evidenced by a reduction in indicators of renal function as well as the attenuation of renal injuries caused by oxidative stress, fibrosis, and inflammation[26].

The occurrence of DN is attributed to the impact of hyperglycemia-induced oxidative and osmotic stress, as reported in previous studies[24,27]. Several *in vitro* studies have demonstrated that hyperglycemic conditions lead to an elevation of ROS, resulting in kidney damage[27]. Under hyperglycemic conditions, cortical glomerulus cells, such as endothelial, mesangial, and tubular epithelial cells, exhibited a significant increase in the accumulation of ROS. The kidneys may experience an increase in size due to the accumulation and excessive production of TNF- α [28,29]. Renal hypertrophy can arise due to an elevation in the pace of protein synthesis or a reduction in the degradation of extracellular organelles within the renal system. The study demonstrated that agmatine treatment resulted in the reduction of kidney hypertrophy markers, leading to the reversal of renal hypertrophy in DN rats[28,30]. Changes in the levels of cellular GSH and SOD are considered as reliable markers of oxidative stress.

Elevated levels of lipid peroxidation and nitric oxide can lead to heightened oxidative stress, resulting in the depletion of enzymes responsible for antioxidant defense. Previous studies have established a correlation between GSH and SOD with diabetes-induced DN[31,32]. The aforementioned results offer additional evidence for the effectiveness of agmatine therapy in treating DN rats, as well as their ability to act as antioxidants. The administration of agmatine at doses of 40 and 80 mg/kg to DN rats resulted in a significant decrease in oxidative stress, as evidenced by the restoration of normal levels of antioxidant defense enzymes.

The Nrf2/HO-1 pathway functions as a significant intracellular defense against oxidative stress in the fight against oxidative stress[32,33]. The cellular pathway is responsible for regulating the activity of phase II detoxifying enzymes, which in turn helps to mitigate the harmful effects of ROS and maintain optimal cellular redox homeostasis. The upregulation of HO-1, GPx, and NAD(P)H NQO1 expression is observed in response to oxidative stress, facilitated by a regulatory antioxidant element[34,35]. Agmatine exhibits antioxidant and antihyperglycemic properties that have the potential to stabilize membrane lipids and reduce the likelihood of lipid oxidation, thereby mitigating oxidative stress. Agmatine therapy resulted in a reduction of FBG, insulin and HOMA-IR.

The inflammatory response is a pivotal factor in the development of numerous pathological conditions, which are characterized by an elevation in cytokines and inflammatory mediators. The progression of DN is attributed, at least in part, to the presence of inflammatory cytokines such as IL-1 β , IL-6, and TNF- α [19,20]. TNF- α was found to induce cell death in various types of cells including epithelial, glomerular, and mesangial cells, and is known to cause direct renal damage by generating free radicals. Furthermore, increased IL-6 results in alterations in the extracellular matrix dynamics and elevation of endothelial permeability, in addition to enhancing mesangial cell proliferation and fibronectin level[36,37]. IL-1 β has been associated with intraglomerular hemodynamic abnormalities, which are involved in the production of prostaglandins[38]. The findings of this study demonstrate that agmatine exhibits anti-inflammatory properties by significantly reducing inflammatory marker levels in renal tissue.

The prioritization of targeting the nuclear factor kappa B (NF- κ B) is recommended for the reduction of oxidative stress and inflammation. DN is characterized by the induction of oxidative stress, which subsequently contributes to the progression of renal dysfunction by upregulating NF- κ B activity[39]. The activity of NF- κ B is necessary for the phosphorylation and degradation of inhibitors of NF- κ B (I κ B). The imbalance in cytokine levels and oxidative stress is triggered by the activation of NF- κ B[40]. The NF- κ B activation during DN may be attributed to the heightened production of pro-inflammatory cytokines[36,38,39]. The experimental results indicate that treatment with agmatine significantly reduced the high levels of inflammatory cytokines, which may be attributed to NF- κ B activation and needs further investigation.

Nevertheless, the present study has certain limitations pertaining to the investigation of molecular processes associated with antioxidant defence systems. Additionally, the study did not examine the protein and gene expressions of pro-inflammatory markers including IL-6, TNF- α , cyclooxygenase-2, and NF- κ B (p65).

In conclusion, the results of our experiments demonstrate that agmatine has a nephroprotective impact by lowering inflammatory and oxidative stress levels. Further studies need to be carried out to elucidate the molecular mechanisms of the nephroprotective effect of agmatine.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

The authors acknowledge the Deanship of Scientific Research at

King Abdulaziz University, Jeddah, Saudi Arabia for the technical and financial support.

Funding

The Deanship of Scientific Research at King Abdulaziz University, Jeddah, Saudi Arabia has funded this project, under grant no. (KEP MSc-42-140-1443).

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

Material preparation, data collection and analysis were performed by FOK, MAA and OS. Study design and conception were done by MAA, FOK, GSAE and SK. The first draft of the manuscript was written by ASB, OS and DB. The previous version and revised version of the manuscript was commented by MJ, SK and FOK. The final manuscript was read and approved by MAA, FOK, OS, ASB and SK.

References

[1] Daryabor G, Atashzar MR, Kabelitz D, Meri S, Kalantar K. The effects of type 2 diabetes mellitus on organ metabolism and the immune system. *Front Immunol* 2020; **11**. doi: 10.3389/fimmu.2020.01582.

[2] Jager KJ, Kovesdy C, Langham R, Rosenberg M, Jha V, Zoccali C. A single number for advocacy and communication—worldwide more than 850 million individuals have kidney diseases. *Kidney Int* 2019; **96**(5): 1048-1050.

[3] Poloni JAT, Rotta LN. Diabetic kidney disease: Pathophysiological changes and urinalysis contribution to diagnosis—a narrative review. *J Lab Precis Med* 2022; **7**: 3.

[4] Grabias BM, Konstantopoulos K. The physical basis of renal fibrosis: Effects of altered hydrodynamic forces on kidney homeostasis. *Am J Physiol Renal Physiol* 2014; **306**(5): F473-F485.

[5] Kirkman MS, Mahmud H, Korytkowski MT. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes mellitus. *Endocrinol Metab Clin North Am* 2018; **47**(1): 81-96.

[6] Rossing P, Caramori ML, Chan JCN, Heerspink HJL, Hurst C, Khunti K, et al. KDIGO 2022 clinical practice guideline for diabetes management in chronic kidney disease. *Kidney Int* 2022; **102**(5): S1-S127.

[7] El-Kashef DH, El-Kenawi AE, Rahim MA, Suddek GM, Salem HA. Agmatine improves renal function in gentamicin-induced nephrotoxicity in rats. *Can J Physiol Pharmacol* 2016; **94**(3): 278-286.

[8] Kim JM, Lee JE, Cheon SY, Lee JH, Kim SY, Kam EH, et al. The anti-inflammatory effects of agmatine on transient focal cerebral ischemia in diabetic rats. *J Neurosurg Anesthesiol* 2016; **28**(3): 203-213.

[9] Piletz JE, Aricioglu F, Cheng JT, Fairbanks CA, Gilad VH, Haenisch B, et al. Agmatine: Clinical applications after 100 years in translation. *Drug Discov Today* 2013; **18**(17-18): 880-893.

[10] Lortie MJ, Novotny WF, Peterson OW, Vallon V, Malvey K, Mendonca M, et al. Agmatine, a bioactive metabolite of arginine. Production, degradation, and functional effects in the kidney of the rat. *J Clin Invest* 1996; **97**(2): 413-420.

[11] El-Kashef DH, El-Kenawi AE, Rahim MA, Suddek GM, Salem HA. Agmatine improves renal function in gentamicin-induced nephrotoxicity in rats. *Can J Physiol Pharmacol* 2016; **94**(3): 278-286.

[12] El-Sherbeeney NA, Nader MA, Attia GM, Ateyya H. Agmatine protects rat liver from nicotine-induced hepatic damage *via* antioxidative, antiapoptotic, and antifibrotic pathways. *Naunyn-Schmiedeberg's Arch Pharmacol* 2016; **389**(12): 1341-1351.

[13] Miski SF, Ahmad MAAAS, Esmat A. Effect of agmatine on non-alcoholic fatty liver disease induced by type 2 diabetes in rats. *J Pharm Res Int* 2021; **33**(31A): 127-134.

[14] Breneman CB, Tucker L. Dietary fibre consumption and insulin resistance—the role of body fat and physical activity. *Brit J Nutr* 2013; **110**(2): 375-383.

[15] Oelke M, De Wachter S, Drake MJ, Giannantoni A, Kirby M, Orme S, et al. A practical approach to the management of nocturia. *Int J Clin Pract* 2017; **71**(11): e13027.

[16] Kennedy BW. Metabolic caging for animal research. *Lab Anim (NY)* 2012; **41**(6): 171-176.

[17] Hoogveen EK. The epidemiology of diabetic kidney disease. *Kidney Dial* 2022; **2**(3): 433-442.

[18] Agarwal R. Pathogenesis of diabetic nephropathy. *ADA Clinical Compendia* 2021; **2021**(1): 2-7.

[19] Hojs R, Ekart R, Bevc S, Hojs N. Markers of inflammation and oxidative stress in the development and progression of renal disease in diabetic patients. *Nephron* 2016; **133**(3): 159-162.

[20] Donate-Correa J, Luis-Rodríguez D, Martín-Núñez E, Tagua VG, Hernández-Carballo C, Ferri C, et al. Inflammatory targets in diabetic nephropathy. *J Clin Med* 2020; **9**(2): 458.

[21] Muskiet MHA, Smits MM, Morsink LM, Diamant M. The gut-renal axis: Do incretin-based agents confer renoprotection in diabetes? *Nat Rev Nephrol* 2014; **10**(2): 88-103.

[22] van Meer L, Moerland M, Cohen AF, Burggraaf J. Urinary kidney biomarkers for early detection of nephrotoxicity in clinical drug development. *Br J Clin Pharmacol* 2014; **77**(6): 947-957.

[23] Mestry SN, Dhodi JB, Kumbhar SB, Juvekar AR. Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by *Punica granatum*

- Linn. leaves extract. *J Tradit Complement Med* 2017; **7**(3): 273-280.
- [24]Shahin DHH, Sultana R, Farooq J, Taj T, Khaizer UF, Alanazi NSA, et al. Insights into the uses of traditional plants for diabetes nephropathy: A review. *Curr Issues Mol Biol* 2022; **44**(7): 2887-2902.
- [25]Sun W, Liu X, Zhang H, Song Y, Li T, Liu X, et al. Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy via disabling KEAP1. *Free Radic Biol Med* 2017; **108**: 840-857.
- [26]Alaofi AL. Sinapic acid ameliorates the progression of streptozotocin (STZ)-induced diabetic nephropathy in rats via NRF2/HO-1 mediated pathways. *Front Pharmacol* 2020; **11**. doi: 10.3389/fphar.2020.01119.
- [27]Wu T, Ding L, Andoh V, Zhang J, Chen L. The mechanism of hyperglycemia-induced renal cell injury in diabetic nephropathy disease: An update. *Life* 2023; **13**(2). doi: 10.3390/life13020539.
- [28]Qiu Y, Tang L. Roles of the NLRP3 inflammasome in the pathogenesis of diabetic nephropathy. *Pharmacol Res* 2016; **114**: 251-264.
- [29]Kiritoshi S, Nishikawa T, Sonoda K, Kukidome D, Senokuchi T, Matsuo T, et al. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells. *Diabetes* 2003; **52**(10): 2570-2577.
- [30]Forman HJ, Zhang H. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nat Rev Drug Discov* 2021; **20**(9): 689-709.
- [31]Mazumder PM, Rathinavelusamy P, Sasmal D. Role of antioxidants in phytomedicine with special reference to antidiabetic herbs. *Asian Pac J Trop Dis* 2012; **2**: S969-S979.
- [32]Zhu X, Xu X, Du C, Su Y, Yin L, Tan X, et al. An examination of the protective effects and molecular mechanisms of curcumin, a polyphenol curcuminoid in diabetic nephropathy. *Biomed Pharmacother* 2022; **153**. doi: 10.1016/j.biopha.2022.113438.
- [33]Ayinde KS, Olaoba OT, Ibrahim B, Lei D, Lu Q, Yin X, et al. AMPK allostery: A therapeutic target for the management/treatment of diabetic nephropathy. *Life Sci* 2020; **261**. doi: 10.1016/j.lfs.2020.118455.
- [34]Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: Harms and benefits for human health. *Oxid Med Cell Longev* 2017; **2017**. doi: 10.1155/2017/8416763.
- [35]Hammad M, Raftari M, Cesário R, Salma R, Godoy P, Emami SN, et al. Roles of oxidative stress and Nrf2 signaling in pathogenic and non-pathogenic cells: A possible general mechanism of resistance to therapy. *Antioxidants* 2023; **12**(7). doi: 10.3390/antiox12071371.
- [36]Ranasinghe R, Mathai M, Zulli A. Cytoprotective remedies for ameliorating nephrotoxicity induced by renal oxidative stress. *Life Sci* 2023; **318**. doi: 10.1016/j.lfs.2023.121466.
- [37]Milosevic K, Stevanovic I, Bozic ID, Milosevic A, Janjic MM, Laketa D, et al. Agmatine mitigates inflammation-related oxidative stress in BV-2 cells by inducing a pre-adaptive response. *Int J Mol Sci* 2022; **23**(7). doi: 10.3390/ijms23073561.
- [38]Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol* 2008; **19**(3): 433-442.
- [39]Sahakyan G, Vejux A, Sahakyan N. The role of oxidative stress-mediated inflammation in the development of T2DM-induced diabetic nephropathy: Possible preventive action of tannins and other oligomeric polyphenols. *Molecules* 2022; **27**(24). doi: 10.3390/molecules27249035.
- [40]Yuan Q, Tang B, Zhang C. Signaling pathways of chronic kidney diseases, implications for therapeutics. *Signal Transduct Target Ther* 2022; **7**(1): 182.

Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Liang Q, Tan BJ