ISSN:0976-0172

Dr.M.Kishore Babu, JBio sci Tech, Vol 13(4),2025,13-29

Journal of Bioscience And Technology
www.jbstonline.com

The impact of various flaxseed, melatonin, gum acacia, and betaine combinations on diabetic rats with chronic renal damage caused by adenine

M Kishore Babu[1], K.Srirama Krishna[2], M.Rajan[3], K Balaram Kumar[4]

1. Professor , Department of pharmaceutics , QIS College of pharmacy , Ongole , A.P

2. Assistant Professor , Department of Pharmacognosy , QIS College of pharmacy , Ongole , A.P

3. Professor , Department of Pharmacognosy , QIS College of pharmacy , Ongole , A.P

4.Associate Professor , Department of Pharmacognosy , QIS College of pharmacy , Ongole , A.P

Abstract

Chronic kidney disease (CKD) and diabetes mellitus (DM) are linked to high rates of morbidity and death. Apoptosis, oxidative stress, and inflammation are the main factors influencing their development. The effects of nine distinct pairings or trios of gum acacia (GA), melatonin, betaine, and flaxseed on adenine-induced chronic kidney disease (CKD) in streptozotocin (STZ)-induced diabetic rats were investigated in this work. Significant hyperglycemia and CKD symptoms, including increased plasma levels of cystatin C and indoxyl sulfate, raised urine levels of N-acetyl-β-D-glucosaminidase (NAG) and the NAG/creatinine ratio, and decreased creatinine clearance, were seen in rats treated with adenine and STZ. Urine osmolality and renalase activity also significantly decreased, whereas IL-1β, IL-6, and TNF-α significantly increased.

levels as well as a drop in IL-10. Significant impairments were also seen in oxidative stress indicators such as glutathione reductase, superoxide dismutase, total antioxidant capacity, and catalase activities. CKD-related histopathology alterations corroborated these results. Most of these alterations were mitigated to varied degrees by treatment with combinations of two or three drugs. Notably, the combination of GA, melatonin, and betaine showed the greatest improvement in all metrics while maintaining the structural integrity of the kidney tissue. Along with potential synergistic molecular, pharmacokinetic, and pharmacodynamic interactions, the improved glycemic control this combination achieves may help to explain these gains. These results provide credence to the idea that this combination may slow the advancement of CKD in those with diabetes. To confirm its effectiveness and safety for clinical usage, further mechanistic research, pharmacokinetic profiles, and long-term toxicity data are required.

KEYWORDS

diabetes, chronic kidney disease, gum acacia, melatonin, betaine, flaxseed

- Corresponding Author
  Kishore babu
  kishorebabu@gmail.com

1 Introduction

Over 537 million persons between the ages of 20 and 79 globally suffer from diabetes mellitus (DM), a serious global health issue (Sun et al., 2022). Nephropathy, neuropathy, and retinopathy are among the many micro- and macrovascular consequences that are linked to it; these conditions all dramatically raise morbidity, death, and medical expenses. Damage to glomerular capillaries causes diabetic nephropathy, which may develop into chronic kidney disease (CKD) and need dialysis or kidney transplantation (Arora and Singh, 2013). Regardless of its cause, chronic kidney disease (CKD) affects about 200 million people globally and adds considerably to the global disease burden. Due to a lack of access to treatment, millions of people die from CKD every year (Jha and Modi, 2018).

The pathophysiology and development of both DM and CKD are significantly influenced by inflammation, oxidative stress, and apoptosis (Akchurin and Kaskel, 2015; Tsalamandris et al., 2019). Low-grade but chronic inflammation is common in patients with DM and/or CKD, and it raises the risk of disease complications and death (Duncan et al., 2003). Increased levels of oxidative and inflammatory indicators have been connected to both the micro- and macro-vascular problems of diabetes and are adversely correlated with renal function (Qian, 2017). Important inflammatory mediators, such as C-reactive protein, tumor necrosis factor, and other cytokines, as well as indicators of oxidative and nitrosative stress, have comparable harmful effects in animal models of chronic kidney disease (CKD) (Kurose et al., 2013). These results provide credence to the possible use of antioxidants and anti-inflammatory drugs as supplemental treatments for DM and CKD patients. Since there is currently no cure for diabetes mellitus, pharmaceutical therapy attempts to either improve insulin sensitivity (e.g., thiazolidinediones and the biguanide metformin) or increase endogenous insulin production (e.g., sulphonylureas). Patients may underestimate the severity of their diabetes mellitus due to the chronic nature of the illness and the often delayed development of complications. Poor adherence to recommended medicines and a greater propensity to investigate alternative treatments, such as herbal medicine, are often caused by this (Cefalu et al., 2011; Forbes and Cooper, 2013).

Despite developments across the world, plant-based therapies are still often utilized to treat a variety of pathological disorders, either in addition to or instead of traditional pharmaceuticals. Despite the lack of strong evidence for their efficacy or safety, many patients include herbal extracts in their regimens because they believe they are “natural” and “do no harm” (Cefalu et al., 2011; Rutebemberwa et al., 2013). This highlights the need of carefully planned clinical, animal, and laboratory research to determine the effectiveness and, more crucially, safety of these drugs as well as to clarify their mode of action. Numerous substances, including betaine, melatonin, gum acacia (GA), and flaxseed, have shown promise in preclinical and clinical research involving individuals with diabetes and/or chronic kidney disease.

Traditional folk medicine has used flaxseed (Linum usitatissimum) to cure a variety of illnesses (Mueed et al., 2022). Of its 32% to 45% oil content, 51% to 55% is α-linolenic acid. Furthermore, flaxseed has a lot of phenolic chemicals.

including phenolic acids, flavonoids, and lignans. Among these, secoisolariciresinol diglucoside has been shown to exhibit antioxidant, hypolipidemic, and hypoglycemic properties (Mueed et al., 2022). Preclinical studies have shown that flaxseed may attenuate the progression of experimentally induced CKD in diabetic rat models (Al Za’abi et al., 2021b), supporting its potential as a therapeutic agent in DM and CKD.

Melatonin, a hormone produced by the pineal gland, functions as an antioxidant and has been shown to support immune function, scavenge free radicals, and exert cardioprotective and neuroprotective effects (Samanta, 2022). It has also been demonstrated to mitigate cisplatin-induced nephrotoxicity, both as monotherapy and in combination with other agents (Ali et al., 2020).

Betaine (trimethylglycine) is a naturally occurring amino acid present in a variety of microorganisms, plants, and animals, including seafood, wheat germ, and spinach (Dobrijević et al., 2023). It plays a key role in methylation reactions and contributes to homocysteine reduction (McRae, 2013). In addition to its antioxidant and anti-inflammatory properties, betaine has demonstrated therapeutic potential in conditions such as obesity, cancer, and Alzheimer’s disease (Zhao et al., 2018). Furthermore, it has been shown to enhance renal function and protect kidneys against tetrachloride-induced nephrotoxicity (Ozturk et al., 2003). Notably, the combination of betaine and melatonin has been reported to provide greater protection against cisplatin-induced nephrotoxicity than either agent alone (Al Za’abi et al., 2021a).

GA is a prebiotic, water-soluble dietary fiber and complex heteropolysaccharide derived from *Acacia senegal* or *Acacia seyal* trees. Multiple experimental and clinical studies have demonstrated the beneficial effects of GA in CKD (Ali et al., 2010; 2013a). In adenine-induced CKD models, GA has been shown to improve various biochemical, physiological, and behavioral parameters (Ali et al., 2015).

Recent evidence suggests that combining nephroprotective agents may offer enhanced efficacy compared to monotherapy (Casanova et al., 2020). Therefore, in this study, we aimed to examine the efficacy and/or potential toxicity of different combinations of GA, melatonin, betaine, and flaxseed in a rat model of adenine-induced CKD with experimentally induced diabetes.

2 Materials and methods

2.1 Animals

Male Wistar rats were obtained from the small animal house, Sultan Qaboos University (SQU), Oman, and housed in a room with a regulated environment (temperature 22 °C ± 2 °C, relative humidity approximately 60%, and 12 h light–dark cycle, with light at 6.00 a.m.), and they were fed a standard additive-free diet and tap water *ad libitum.* The use of rats was approved by the SQU Ethical Committee for Animal Use in Research (SQU/EC-AUR/ 2023–2024/2). All procedures involving the animals and their care were carried out in accordance with the guidelines of the national and international laws and policies.

2.2 Experimental design

A sample size of six rats per group was chosen based on the standard practices in preclinical studies and published guidelines (Charan and Kantharia, 2013). Rats (n = 66) were randomly assigned into 11 equal groups and treated as follows:

- G1 (control): received distilled water.
- G2 (adenine + streptozotocin): received an intraperitoneal dose of 50 mg/kg of streptozotocin, dissolved in 0.1 M citrate buffer (pH 4.5), to induce DM and then adenine 0.25% w/w in the feed for 4 weeks to induce CKD.
- G3 (M + B): similar to G2 and additionally received melatonin (suspended in 0.9% saline at a dose of 10 mg) and betaine (suspended in 0.9% saline at an oral dose of 200 mg/kg/day).
- G4 (G + B): similar to G2 and additionally received GA in drinking water at a concentration of 15% w/v and betaine (suspended in 0.9% saline at an oral dose of 200 mg/kg/day).
- G5 (F + B): similar to G2 and additionally received flaxseed (15% w/w) and betaine (suspended in 0.9% saline at an oral dose of 200 mg/kg/day).
- G6 (M + G): similar to G2 and additionally received melatonin (suspended in 0.9% saline at an oral dose of 10 mg) and GA in drinking water at a concentration of 15% w/v.
- G7 (M + F): similar to G2 and additionally received melatonin (suspended in 0.9% saline at an oral dose of 10 mg) and flaxseed (15% w/w).
- G8 (F + G): similar to G2 and additionally received flaxseed (15% w/w) and GA in drinking water at a concentration of 15% w/v.
- G9 (F + G + M): similar to G2 and additionally received flaxseed (15% w/w), GA in drinking water at a concentration of 15% w/v, and melatonin (suspended in 0.9% saline at an oral dose of 10 mg).
- G10 (F + M + B): similar to G2 and additionally received flaxseed (15% w/w), melatonin (suspended in 0.9% saline at an oral dose of 10 mg), and betaine (suspended in 0.9% saline at a dose of 200 mg/kg/day).
- G11 (G + M + B): similar to G2 and additionally received GA in drinking water at a concentration of 15% w/v, melatonin (suspended in 0.9% saline at an oral dose of 10 mg), and betaine (suspended in 0.9% saline at an oral dose of 200 mg/kg/day).

The doses were selected based on previous studies from our group and others, which demonstrated their efficacy and safety in similar animal models (Al Za'abi et al., 2018; Al Za'abi et al., 2021a; Al Za'abi et al., 2021b).

One day before the end of the treatment period, rats were individually placed in metabolic cages, and urine was collected over a 24-h period. At the end of the treatment period, rats were anesthetized via intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). Blood was collected from the abdominal aorta in heparinized tubes, and the plasma was harvested by centrifugation at 900 g at 4 °C for 15 min. The rats were then euthanized by anesthetic overdose, and the two kidneys were excised. A small section of the right kidney was fixed in 10% formalin for histological analysis. The remainder of the right kidney and the left kidney were dipped in liquid nitrogen and frozen at −80 °C for further analysis.

2.3 Drugs and chemicals

Streptozotocin, betaine, adenine, and GA were purchased from Sigma-Aldrich (Saint Louis, MO, United States). Melatonin was purchased from Glentham Life Sciences (Unit 5 Leafield Way, Corsham, UK). Flaxseed was purchased from Badia Spices Inc. (Doral, FL, United States). The plasma urea, creatinine, calcium, phosphorous, uric acid, and urine albumin were measured using the fully automated chemistry analyzer BS-120, Mindray (Shenzhen, CHINA). Urine N-acetyl-β-D-glucosaminidase (NAG), plasma neutrophil gelatinase- associated lipocalin (NGAL), cystatin C, interleukin-6 (IL-6), interleukin-1 beta (IL-1β), interleukin-10 (IL-10), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and tumor necrosis factor alpha (TNF-α) were measured using ELISA kits from Elabscience Bionovation Inc. (Houston, Texas, United States). Plasma 8- isoprostane, advanced glycation end products (AGEs), indoxyl sulfate, and renalase were measured using ELISA kits from Assay Genie Ltd. (Windsor Place, Dublin, Ireland). Glutathione reductase (GR) was measured using colorimetric assay kits from Bio-vision (Milpitas, CA, United States). superoxide dismutase (SOD), total antioxidant capacity

(TAC), and catalase were measured using colorimetric assay kits from Elabscience Bionovation Inc. (Houston, Texas, United States). Urine osmolality was measured using a freezing point osmometer (Gonotec, GmbH, Berlin, Germany).

2.4 Histological analysis

Paraffin-embedded renal tissue sections were stained with hematoxylin and eosin (H&E) and Picrosirius red (ab150681, Abcam). Renal tubular necrosis was assessed following the study by Ali et al. (2013b) using a scoring method on a scale of 0–4, where 0 = normal, no necrosis; 1 < 10%; 2 = 10%–25%; 3 = 26%–75%; 4 > 75%. Three 40X microscopic fields were analyzed from each kidney section of each animal of the 11 groups, and the score was calculated according to the mean percentage. Fibrosis was assessed using the Picrosirius red stain.

2.5 Statistical analysis

Data are given as the mean ± SEM and were analyzed by one- way analysis of variance followed by Bonferroni's multiple comparison tests (GraphPad Prism version 5.03, San Diego, CA, United States).

3 Results

3.1 Physiological parameters

The effects of melatonin, betaine, GA, and flaxseed on the physiological parameters in rats with diabetes and adenine-induced CKD are summarized in Table 1. As shown in the table, the initial body weights were comparable across the groups. Treatment with adenine and STZ caused a significant reduction in body weight change and a

significant increase in relative kidney weights, liver weights, water intake, urine flow, feed intake, and fecal output compared with the control group. Treatment with the different combinations significantly ameliorated the induced changes in relative kidney weight and urine flow. The flaxseed–betaine, flaxseed–melatonin, flaxseed–GA, flaxseed–melatonin–betaine, and GA–melatonin–betaine combinations significantly ameliorated the induced changes in body weight. The GA–betaine, GA–melatonin, flaxseed–melatonin, flaxseed–GA, flaxseed–melatonin–betaine, and flaxseed–melatonin–GA combinations significantly ameliorated the induced changes in liver weight. Adenine- and STZ-induced changes in feed intake were significantly reversed by the melatonin–GA, melatonin–flaxseed, GA–flaxseed–melatonin, and GA–melatonin–betaine combinations. All the combinations except GA–melatonin–betaine significantly ameliorated the induced changes in the fecal output.

3.2 Blood glucose levels

The effects of melatonin, betaine, GA, and flaxseed on various plasma renal parameters and blood glucose levels in rats with diabetes and adenine-induced CKD are summarized in Table 2. The adenine and STZ group had significantly higher fasting blood glucose levels (32.7 ± 0.30 mmol/L) than the control group (4.2 ± 0.11 mmol/L). All the treatment combinations except the GA–betaine combination significantly reduced these levels. The combination of GA–melatonin–betaine yielded the most substantial reduction (7.5 ± 0.48 mmol/L) compared with the other treatment combinations.

3.3 Biochemical and urinary parameters

Adenine and STZ caused a significant decrease in plasma calcium levels and significantly increased phosphorus, uric acid, urea, and creatinine plasma levels (Table 2). All the treatment combinations significantly mitigated the changes in calcium and uric acid levels. On the other hand, only the flaxseed–GA–melatonin and GA–melatonin–betaine combinations significantly attenuated the changes in phosphorus levels. All the treatment combinations, except melatonin–betaine, GA–betaine, and flaxseed–betaine, attenuated the changes in urea and creatinine plasma levels. The melatonin–flaxseed, flaxseed–GA–melatonin, and GA–melatonin–betaine combinations yielded the most pronounced and significant reductions in urea levels, whereas the GA–melatonin–betaine combination yielded the most notable and significant reduction in creatinine levels compared to the other treatment combinations.

Adenine and STZ induced significant changes in

the urinary creatinine levels, NAG levels, albumin/creatinine ratios, creatinine clearance, NAG/creatinine ratios, and urine osmolality compared with the control group (Table 3). The GA–melatonin–betaine combination improved all these changes, except the albumin/ creatinine ratios. Treatment with other different combinations, except GA–betaine, significantly improved the NAG/creatinine ratios and osmolality.

3.4 Renal plasma indices

Adenine and STZ induced a significant increase in NGAL activity and cystatin C and indoxyl sulfate concentration, and a significant reduction in the renalase activity (Figure 1). All the treatment groups significantly reduced indoxyl sulfate concentrations. In addition, all the treatment combinations, except melatonin–betaine and GA–betaine, significantly improved cystatin C and NGAL levels. Finally, all the treatment groups, except melatonin–betaine, GA–betaine, and melatonin–GA, significantly increased renalase levels. The GA–melatonin–betaine combination yielded the most notable improvement across all the parameters compared with the other treatment combinations.

3.5 Inflammatory indices

Adenine and STZ induced a significant increase in IL-1β, IL-6, and TNF-α and significant suppression of IL-10 levels compared with the control group (Figure 2). All treatment combinations, expect melatonin–betaine, significantly reduced IL-1β and IL-6 levels. The GA–melatonin–betaine combination produced the most notable and significant mitigation in IL-1β compared with other treatment combinations. Furthermore, all treatment combinations, except melatonin–betaine and GA–betaine, significantly ameliorated the induced changes in IL-10 and TNF-α levels.

3.6 Oxidative stress indices

Adenine and STZ induced a significant increase in 8-OHdG, 8- isoprostane, and AGEs (Figure 3), an effect that was significantly attenuated by all the treatment combinations, but it was more pronounced with the GA–melatonin–betaine combination.

Figure 4 presents the results of the different treatment groups on various oxidative stress indices. Adenine and STZ significantly decreased SOD, GR, TAC, and catalase activities compared with the control group. All treatment combinations, except GA–betaine, significantly attenuated the changes in SOD activity, with the effects generally comparable across groups. Treatment with flaxseed–betaine, melatonin–flaxseed, flaxseed–GA, flaxseed–melatonin–betaine, and GA–melatonin–betaine significantly increased GR activity. All the treatment groups except melatonin–betaine, GA–betaine, and flaxseed–GA significantly increased TAC activity, with the GA–melatonin–betaine combination producing the most marked and significant increase compared to the other combinations. Finally, treatment with melatonin–flaxseed, flaxseed–GA–melatonin, flaxseed–melatonin–betaine, and GA–melatonin–betaine significantly increased the catalase activity.

3.7 Histopathological changes

Microscopic analysis of the renal cortex of the rats in the control group exhibited a normal renal architecture, which was characterized by intact glomeruli and tubules (lesion score: 0) (Figure 5A). The

TABLE 3 Effect of treatment with melatonin (M), betaine (B), gum acacia (G), and flaxseed (F) on some urine parameters in rats with both diabetes (D) and adenine (A)-induced chronic kidney disease (CKD).

Parameters/ treatments	Group 1 Control	Group 2 A + D	Group 3 A + D + M + B	Group 4 A + D + G + B	Group 5 A + D + F + B	Group 6 A + D + M + G	Group 7 A + D + M + F	Group 8 A + D + F + G	Group 9 A + D + F + G + M	Group 10 A + D + F + M + B	Group 11 A + D + G + M + B
Creatinine (µmol/L)	8514.0 ± 775.8	364.7 ± 99.1[a]	619.5 ± 96.4[a]	417.1 ± 46.8[a]	952.8 ± 434.9[a]	600.5 ± 95.6[a]	1045.1 ± 226.0[a]	1097.0 ± 462.2[a]	871.3 ± 66.2[a]	871.6 ± 92.4[a]	3493.9 ± 180.7[abcdfghi]
NAG (ng/mL)	3.71 ± 0.40	14.47 ± 1.30[a]	9.39 ± 1.21[ab]	9.10 ± 0.47[ab]	6.68 ± 0.52[b]	6.37 ± 0.92[b]	6.44 ± 1.23[b]	4.83 ± 0.49[bcd]	4.91 ± 0.42[bcd]	5.05 ± 0.41[bcd]	4.37 ± 0.35[bcd]
Albumin/creatinine ratio (mg/µmol)	0.54 ± 0.08	2.43 ± 0.53[a]	2.24 ± 0.23[a]	1.25 ± 0.15	2.32 ± 0.63[a]	0.92 ± 0.10[bce]	1.13 ± 0.25[be]	1.66 ± 0.30	1.37 ± 0.13	1.22 ± 0.32	1.22 ± 0.08
Creatinine clearance (mL/ minute)	3.74 ± 0.41	0.49 ± 0.13[a]	0.61 ± 0.17[a]	0.41 ± 0.07[a]	1.31 ± 0.55[a]	1.04 ± 0.25[a]	1.57 ± 0.35[a]	1.88 ± 0.70[a]	0.89 ± 0.08[bc]	1.23 ± 0.17[a]	3.13 ± 0.28[bcdfghi]
NAG/creatinine ratio (ng/µmol)	0.47 ± 0.09	57.37 ± 15.75[a]	17.52 ± 3.59[b]	23.99 ± 3.98[a]	12.84 ± 3.12[b]	13.83 ± 4.21[b]	8.16 ± 2.82[b]	7.27 ± 2.20[b]	5.86 ± 0.78[b]	6.18 ± 0.90[b]	1.27 ± 0.13[bd]
Osmolality (mOsmol/kg)	1710.2 ± 142.7	656.0 ± 37.7[a]	810.0 ± 33.6[a]	759.2 ± 56.4[a]	981.0 ± 60.8[a]	947.8 ± 45.2[a]	1074.3 ± 111.7[ab]	1179.8 ± 175.3[abd]	1052.3 ± 46.2[ab]	1165.3 ± 42.7[abd]	1726.2 ± 127.0[bcdfghij]

Values in the table are means ± SEM (n = 6). Diabetes (D) was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg) on the first day of the experiment; after diabetes confirmation, CKD was induced by the inclusion of adenine (A) in the feed at a concentration of 0.25%w/w for 35 days, and M (10 mg/kg) and B (200 mg/kg) were administered concurrently to rats by oral gavage. G (15%w/v) was added in drinking water. F (15%w/w) was administered in a powdered form in the food. On the 35th day of treatment, the rats were placed in metabolic cages for urine collection. NAG: N-acetyl-β-D-glucosaminidase.

Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Statistical significance was set at $p < 0.05$. Superscripts indicate the following comparisons:

acontrol group vs. all other groups.

bA + D (untreated) group vs. all other A + D treated groups. cA + D + M + B group vs. all other A + D treated groups. dA + D + G + B group vs. all other A + D treated groups. eA + D + F + B group vs. all other A + D treated groups.

fA + D + M + G group vs. all other A + D treated groups. gA + D + M + F group vs. all other A + D treated groups. hA + D + F + G group vs. all other A + D treated groups.

iA + D + F + G + M group vs. all other A + D treated groups.

jA + D + F + M + B group vs. all other A + D treated groups.

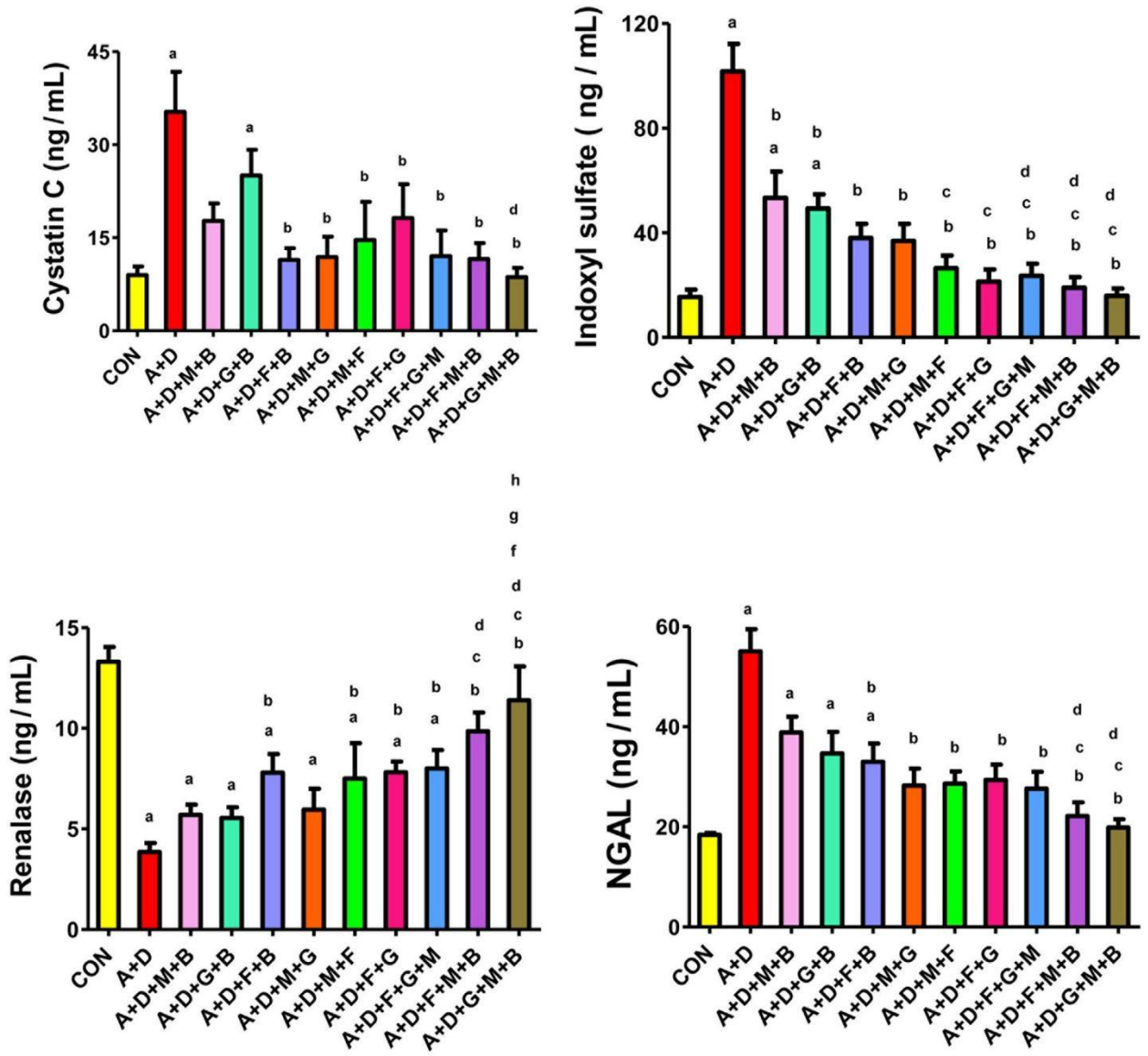

FIGURE 1
Effect of different combinations of flaxseed (F, 15%$^{w/w}$), betaine (B, 200 mg/kg), melatonin (M, 10 mg/kg), and gum acacia (G, 15%$^{w/v}$) on the plasma activity of cystatin C, concentrations of neutrophil gelatinase-associated lipocalin (NGAL) and indoxyl sulfate, and renalase activity in control (CON) rats and rats treated with both adenine (A, 0.25%) and diabetes (D, 55 mg/kg). Each vertical column with bar represents the mean ± SEM (n = 6). Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Statistical significance was set at $p < 0.05$. Letters above the bars indicate statistically significant differences as follows: (a) control group vs. all other groups. (b) A + D (untreated) group vs. all other A + D treated groups. (c) A + D + M + B group vs. all other A + D treated groups. (d) A + D + G + B group vs. all other A + D treated groups. (e) A + D + F + B group vs. all other A + D treated groups. (f) A + D + M + G group vs. all other A + D treated groups. (g) A + D + M + F group vs. all other A + D treated groups. (h) A + D + F + G group vs. all other A + D treated groups. (i) A + D + F + G + M group vs. all other A + D treated groups. (j) A + D + F + M + B group vs. all other A + D treated groups.

The group that received STZ and adenine showed significant renal tubule basophilia and severe cystic dilatation, together with a large number of cellular casts (lesion score: 4) (Figure 5B). A lesion score of three was awarded to kidney tissues from rats treated with melatonin–betaine, flaxseed–GA, flaxseed–GA–melatonin, and GA–melatonin–betaine. These tissues had notable tubular dilatation and basophilia while maintaining intact glomeruli (Figures 5C,H,I). It is noteworthy that kidney tissues from rats given the flaxseed–GA–melatonin combination showed tubular regeneration in the tissues under examination. Lesion scores of 2 were assigned to kidney tissues from rats treated with GA–betaine, flaxseed–betaine, melatonin–GA, melatonin–flaxseed, and flaxseed–melatonin–betaine. These tissues showed mild-to-moderate tubular dilatation while keeping intact glomeruli (Figures 5D–G,J). Furthermore, Picrosirius red stain revealed significant peritubular fibrosis in the kidney tissues of the groups who received just adenine and STZ (Figure 5L).

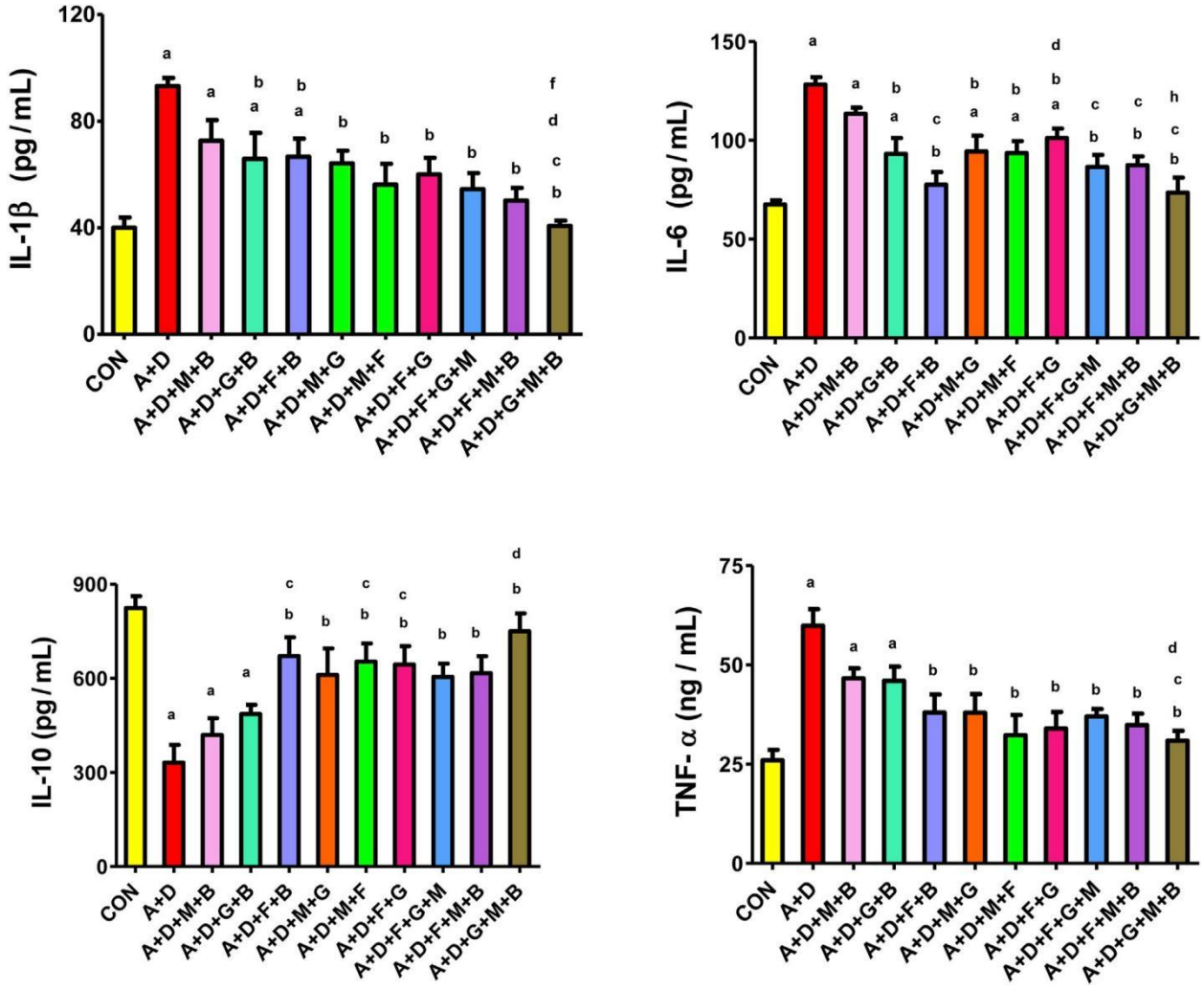

FIGURE 2
Effect of different combinations of flaxseed (F, 15%$^{w/w}$), betaine (B, 200 mg/kg), melatonin (M, 10 mg/kg), and gum acacia (G, 15%$^{w/v}$) on the on the plasma concentration of tumor necrosis factor (TNF-α), interleukin 1-beta (IL-1β), interleukin-6 (IL-6), and interleukin-6 (IL-10) in control (CON) rats and rats treated with both adenine (A, 0.25%) and diabetes (D, 55 mg/kg). Each vertical column with bar represents the mean ± SEM (n = 6). Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Statistical significance was set at $p < 0.05$. Letters above the bars indicate statistically significant differences as follows: (a) control group vs. all other groups. (b) A + D (untreated) group vs. all other A + D treated groups. (c) A + D + M + B group vs. all other A + D treated groups. (d) A + D + G + B group vs. all other A + D treated groups. (e) A + D + F + B group vs. all other A + D treated groups. (f) A + D + M + G group vs. all other A + D treated groups. (g) A + D + M + F group vs. all other A + D treated groups. (h) A + D + F + G group vs. all other A + D treated groups. (i) A + D + F + G + M group vs. all other A + D treated groups. (j) A + D + F + M + B group vs. all other A + D treated groups.

4 Discussion

A major cause of chronic kidney disease (CKD) and a major health problem, diabetes mellitus (DM) significantly impairs quality of life, increases the risk of severe cardiovascular events, increases the rate of premature death, and places a heavy strain on health care systems (Francis et al., 2024). According to a recent global agreement, new treatments are necessary to stop kidney disease from developing and worsening as well as to lessen some of the health and financial costs that come with it (Francis et al., 2024).

In diabetics, a variety of metabolic, hemodynamic, inflammatory, and fibrotic variables involving complex signaling pathways promote the clinical evolution of kidney disease.

all work together to cause the kidney structure and function to gradually deteriorate (Sinha and Nicholas, 2023). Effective treatment plans should target and reverse the pathogenic pathways that propel the advancement of the illness in addition to maintaining glycemic control. It has been shown that a number of phytochemicals that target important pathological pathways in diabetes and chronic kidney disease (CKD) may reverse some of the underlying processes linked to the deterioration in renal function associated with diabetes mellitus (DM) (Al Za'abi et al., 2018; Al Za'abi et al., 2021a; Al Za'abi et al., 2021b). As a result, these substances could be useful as supplements to existing prescribed drugs, which might enhance patient results.

In this work, we used a rat model of experimentally generated diabetes and chronic kidney disease to assess the effects of various flaxseed, GA, melatonin, and betaine combinations. These organic

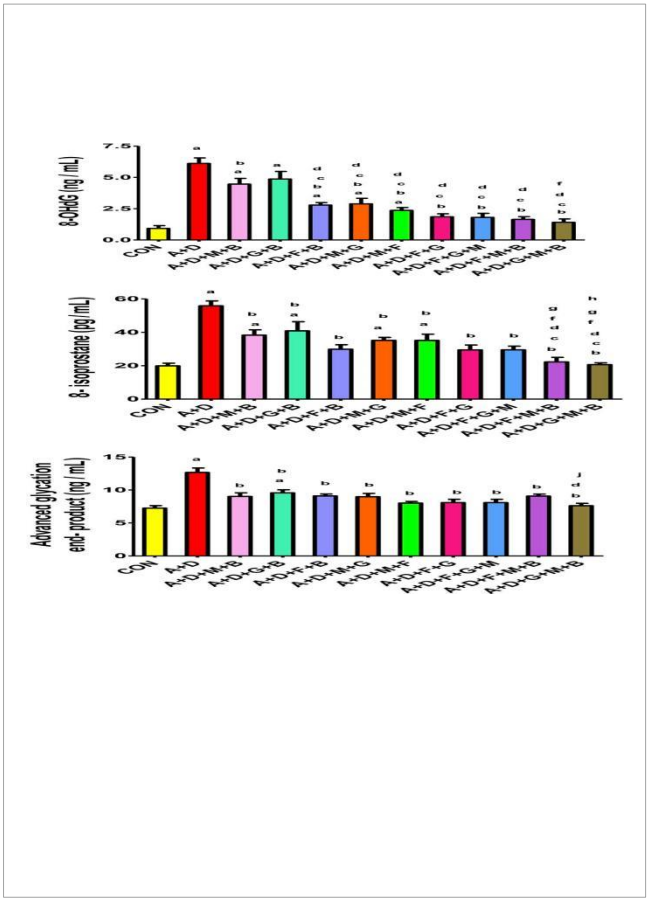

FIGURE 3 (Continued)
groups. (c) A + D + M + B group vs. all other A + D treated groups. (d) A + D + G + B group vs. all other A + D treated groups. (e) A + D + F + B group vs. all other A + D treated groups.(f) A + D + M + G group vs. all other A + D treated groups. (g) A + D + M + F group vs. all other A + D treated groups. (h) A + D + F + G group vs. all other A + D treated groups. (i) A + D + F + G + M group vs. all other A + D treated groups. (j) A + D + F + M + B group vs. all other A + D treated groups.

products have previously been investigated for their potential to improve the glucose metabolism and/or renal function and are known to possess potent antioxidant and anti-inflammatory properties (Al Za'abi et al., 2018; Al Za'abi et al., 2021a; Al Za'abi et al., 2021b).

The induction of diabetes and CKD using STZ and adenine, respectively, in the experimental animals resulted in significant hyperglycemia, weight loss, polyuria, impaired renal function and structure, and increased oxidative stress accompanied by a compromised antioxidant defense system and dysregulated inflammatory markers. These features closely mirror the clinical and pathophysiological features of CKD in DM. Treatment with the two-drug and three-drug combinations was associated with the attenuation of most of these pathological changes to varying degrees. Notably, the GA–melatonin–betaine combination was linked to the most significant glycemic control and overall improvement across the majority of the measured indices, including urinary

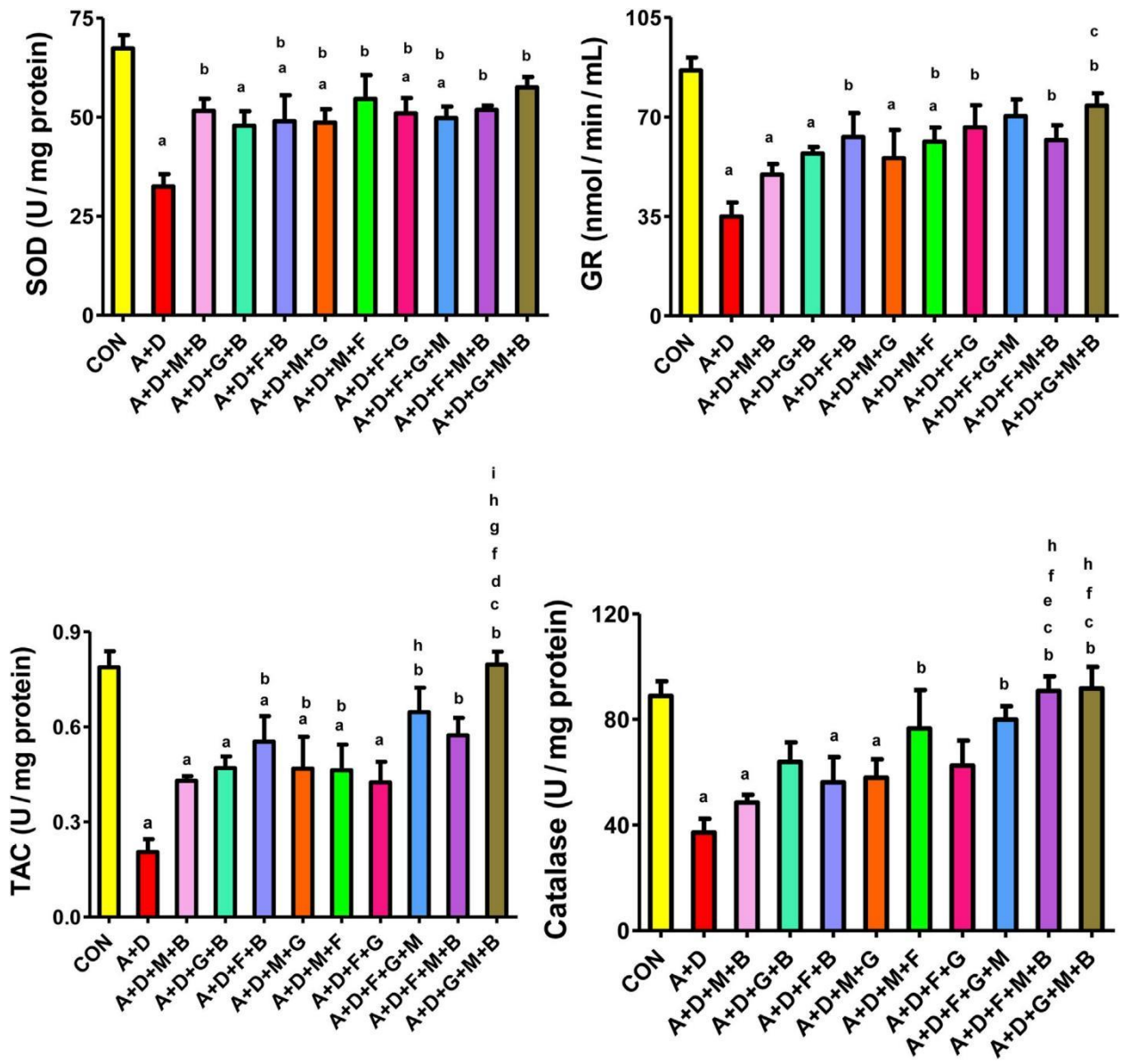

FIGURE 4
Effect of different combinations of flaxseed (F, 15%$^{w/w}$), betaine (B, 200 mg/kg), melatonin (M, 10 mg/kg), and gum acacia (G, 15%$^{w/v}$) on the plasma concentration of superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), and glutathione reductase (GR) in control (CON) rats and rats treated with both adenine (A, 0.25%) and diabetes (D, 55 mg/kg). Each vertical column with bar represents the mean ± SEM (n = 6). Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Statistical significance was set at $p < 0.05$. Letters above the bars indicate statistically significant differences as follows: (a) control group vs. all other groups. (b) A + D (untreated) group vs. all other A + D treated groups. (c) A + D + M + B group vs. all other A + D treated groups. (d) A + D + G + B group vs. all other A + D treated groups. (e) A + D + F + B group vs. all other A + D treated groups. (f) A + D + M + G group vs. all other A + D treated groups. (g) A + D + M + F group vs. all other A + D treated groups. (h) A + D + F + G group vs. all other A + D treated groups. (i) A + D + F + G + M group vs. all other A + D treated groups. (j) A + D + F + M + B group vs. all other A + D treated groups.

the combination. Second, considering the pathological role of intestinal dysbiosis in the pathogenesis of CKD (Stavropoulou et al., 2021), it is possible that this prebiotic property of GA independently contributed to improving the renal outcomes by positively modulating the gut–kidney axis. In support of this, CKD progression was ameliorated and uremic toxins were blunted in experimental rats with gut microbiota modified using lactulose (Sueyoshi et al., 2019).The observed improvements in the renal indices may partly be explained by the stronger glycemic control observed with the GA–melatonin–betaine combination, which may have contributed to the attenuation of some of the pathological processes driving renal function deterioration. GA is a viscous, water-soluble fiber that likely exerts hypoglycemic effects by reducing postprandial glucose absorption (Ibrahim et al., 2023).

There is also evidence that GA regenerates β-cells and exerts insulin-

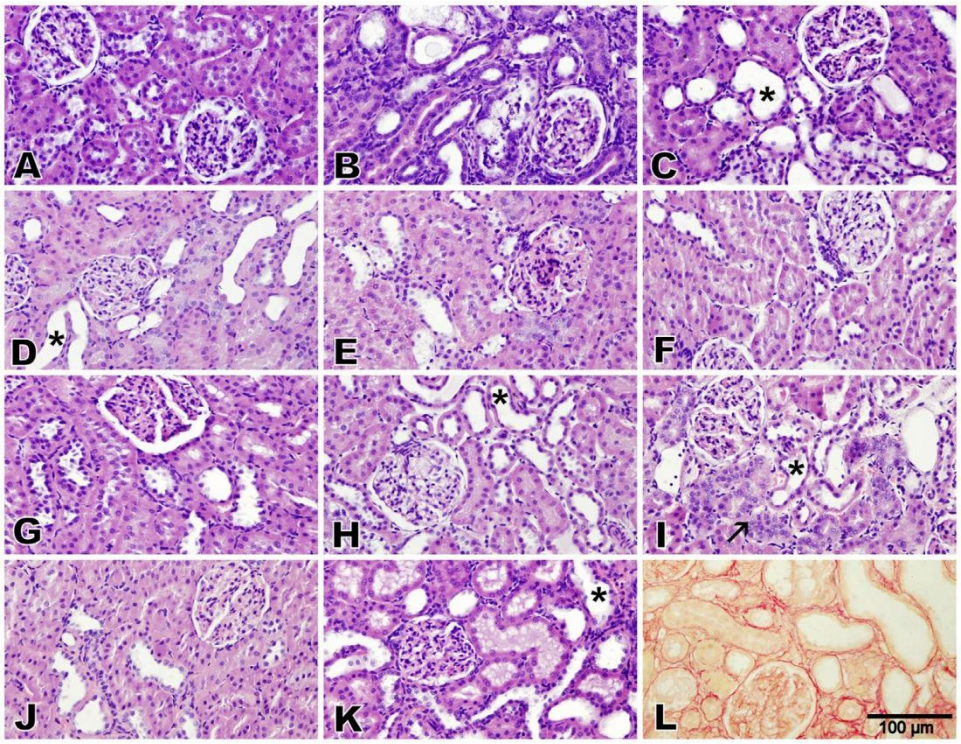

FIGURE 5
Photomicrographs of the renal cortex (Bar = 100 µm, H&E stain, except for L: Picrosirius red). The control group exhibited normal renal architecture with intact glomeruli and tubules (lesion score 0) (A). Adenine and diabetes group exhibited severe cystic dilatation and pronounced basophilia of the renal tubules, numerous casts (lesion score 4), and marked peritubular fibrosis (B,L). Groups treated with melatonin–betaine, flaxseed–GA, and flaxseed–GA–melatonin exhibited marked tubular dilatation and basophilia while maintaining intact glomeruli (lesion score 3), with the GA–melatonin-treated group showing notable tubular regeneration in the examined tissues (C,H,I). Groups treated with GA–betaine, flaxseed–betaine, GA–melatonin, flaxseed–melatonin, flaxseed–melatonin–betaine, and GA–melatonin–betaine exhibited mild-to-moderate tubular dilatation while maintaining intact glomeruli (lesion score 2) (D,E,F,G,J,K). The asterisks indicate cystic dilatation of renal tubules, and the arrow indicates regenerative renal tubules.

similar activity by blocking hepatic gluconeogenesis and increasing the absorption of glucose by muscles and adipose tissue (Ibrahim et al., 2023). The significance of GA in blood glucose management is further supported by clinical studies that demonstrate that GA lowers carbohydrate consumption and improves blood glucose levels in individuals with metabolic syndrome (Jarrar et al., 2021).

In addition to these benefits, this study and others have shown that GA has anti-inflammatory and antioxidant qualities (Ibrahim et al., 2023; Shanab et al., 2023). The strong antioxidant qualities of GA's lysine, tyrosine, and histidine amino acid residues (Xu et al., 2017; Ibrahim et al., 2023) and its abundance of phenolic acids, which function as electron donors and free radical scavengers (Shanab et al., 2023), are thought to be the source of its antioxidant capacity. Although GA's immunomodulatory processes are not entirely understood, it is thought that its derivative butyrate, which controls the production of inflammatory mediators via the NFκB pathway, is the main driver (Al-Jubori et al., 2023).

The pineal gland produces the endogenous hormone melatonin, which controls the circadian cycle and is often used as a supplement to treat insomnia (Delpino et al., 2021). By promoting glucose transport to skeletal muscle cells via the insulin receptor substrate-1/phosphoinositide 3 (IRS1/PI3)-kinase pathway, it has been shown to control glucose homeostasis (Zhu

et al. (2023). Melatonin supplementation has been shown in clinical studies to enhance glycemic variability, HbA1c, insulin sensitivity, and fasting blood glucose (Delpino et al., 2021; Martorina and Tavares, 2023). In the present work, we show that when melatonin is given in conjunction with GA and betaine, its glucose-lowering impact is much increased in comparison to other combinations. This implies that melatonin either improves the experimental animals' glycemic control on its own or in concert with GA and betaine. Through three main intracellular signaling pathways—the phospholipase C/inositol triphosphate route, the adenylyl cyclase/cAMP pathway, and the cGMP pathway—melatonin receptors control the release of insulin from pancreatic β cells (Sharma et al., 2015). Patel et al. (2022) recently shown that in STZ-induced diabetic mice, melatonin also decreases apoptosis and promotes β-cell regeneration.

Across a variety of experimental models of membranous nephropathy, lupus nephritis, hypertensive nephrosclerosis, acute ischemic kidney injury, unilateral ureteral obstruction-induced renal injury, treatment-induced nephrotoxicity, and contrast-induced AKI, the current findings further support the well-established renoprotective role of melatonin (Stacchiotti et al., 2002; Ozbek et al., 2009; Patschan et al., 2012; Wu et al., 2012; Cheng et al., 2014; dos Santos et al., 2018; Al Za'abi et al., 2021a). Melatonin's anti-inflammatory, anti-apoptotic, and antioxidant qualities have been primarily implicated in its renoprotective actions. With an indole ring and side chains that provide broad-spectrum scavenging action against a variety of reactive oxygen and nitrogen species, melatonin is a strong antioxidant (Tan et al., 2015). Additionally, its metabolites maintain their ability to neutralize free radicals, enhancing melatonin's total antioxidant effectiveness (Tan et al., 2015). Additional data indicates that melatonin controls the expression of genes for a number of antioxidant enzymes (Emamgholipour et al., 2016).

Additionally, there are other ways that melatonin reduces inflammation, such as by inhibiting pro-inflammatory cytokines including TNF, IL-1, IL-6, and IL-8; downregulating 5-lipoxygenase; and decreasing leukocyte adhesion (Cho et al., 2021). For many inflammatory disorders, these results have been shown in both human clinical trials and animal research (Cho et al., 2021). Compared to other combinations, melatonin given in conjunction with GA and betaine had the most anti-inflammatory impact in this study. Interestingly, although other melatonin-containing combinations only had mild benefits, the melatonin–betaine combination did not substantially reduce any of the inflammatory indicators. In contrast, a prior research showed that melatonin–betaine had strong anti-inflammatory effects in mice with nephrotoxicity caused by cisplatin (Al Za'abi et al., 2021a). Melatonin and betaine seem to have mild anti-inflammatory effects in the setting of diabetic kidney disease, but the addition of GA seems to greatly increase the anti-inflammatory benefits of this combination.

In addition to being produced naturally in the liver from choline, betaine is a methyl derivative of glycine that is mostly acquired from food (Alvarenga et al., 2022). Diabetes, metabolic syndrome, dyslipidemia, and cardiovascular risk factors have all been connected to betaine insufficiency (Lever and Slow, 2010). In contrast, studies have shown that betaine supplementation improves intestinal barrier integrity, glycemic management, insulin resistance, renal function, liver damage, adipose dysfunction, and inflammatory markers (Arumugam et al., 2021; Alvarenga et al., 2022).

Stored in the kidney and liver, betaine performs three vital physiological roles: it acts as a methyl donor in the methionine cycle, which detoxifies homocysteine to methionine, a process essential for many cellular processes; it also acts as an osmolyte that controls cell volume and as a chemical chaperone that guards against protein degradation (Lever and Slow, 2010; Dobrijević et al., 2023). Betaine's osmoregulatory action helps shield renal cells from electrolyte imbalances and osmotic stress (Alvarenga et al., 2022; Dobrijević et al., 2023). In this investigation, the GA–melatonin–betaine

combination more significantly normalized phosphorus, urea, and uric acid in rats treated with adenine and STZ than the GA–melatonin combination. The osmoregulatory qualities of betaine may be partly responsible for this improved impact. Furthermore, it has been shown that betaine directly reduces hyperuricemia by modifying a number of renal uric acid and organic anion transporters (Liu et al., 2013).

There is growing evidence that homocysteine has a high link with chronic kidney disease (CKD) and renal damage, despite its frequent association with cardiovascular disorders (Shih et al., 2022; Chen et al., 2023). The cytotoxic effects of homocysteine, the activation of profibrotic transcriptional factors, the decreased glomerular filtration linked to hyperhomocysteinemia, and the elevated generation of free radical species are some of the hypothesized processes (Shih et al., 2022; Chen et al., 2023). Therefore, the observed improvements in renal function with the GA–melatonin–betaine combination may have been facilitated by the homocysteine detoxifying effect of betaine. Additionally, this combination's antioxidant and anti-inflammatory profile outperformed that of the GA–melatonin combination by itself. Despite not being a direct scavenger of free radicals, experimental in vitro data indicates that betaine's antioxidant effects are mediated by the development of physical barriers around cells and the upregulation of endogenous non-enzymatic antioxidant defense systems (Zhang et al., 2016). Additionally, the nuclear factor-kappa B (NF-κB) signaling pathway, which controls the production of many pro-inflammatory effector molecules such as TNF-α and IL-1β, is suppressed by betaine (Zhao et al., 2018).

A popular dietary supplement, flaxseed is high in lignans, fiber, flavonoids, and alpha-linoleic acids (Mueed et al., 2022). It has previously been shown to have therapeutic promise in reducing diabetes-induced chronic kidney disease in experimental animals (Al Za'abi et al., 2021b). Clinical investigations supported this conclusion by showing that flaxseed improved glycemic response, fasting blood glucose, and many inflammatory biomarkers (Kavyani et al., 2023; Musazadeh et al., 2024). Flaxseed-containing combinations, however, did not provide outcomes in the present trial that were on par with the other combinations. One explanation is that the other components' absorption could have been hampered by flaxseed's laxative qualities. On the other hand, flaxseed lignans may have decreased the bioavailability of the other ingredients in the treatment combinations by inducing cytochrome P450 enzymes (Defries et al., 2021). Clarifying these possible relationships will need further research.

When combined, our results imply that GA, melatonin, and betaine may provide a new complimentary treatment strategy for reducing the pathophysiology aspects of CKD and diabetes. It is still unknown, however, whether this research is applicable to human treatment. Even though our results point to a possible synergistic impact, further mechanistic confirmation is needed for the underlying processes, which were not thoroughly investigated. Therefore, future research should look into the molecular pathways that this combination influences, look into possible pharmacokinetic interactions, and assess clinical effectiveness and long-term results in human patients.

In conclusion, the effects of experimentally produced CKD and diabetes in rats treated with adenine and STZ were mitigated to varying degrees by the administration of diverse combinations of GA, melatonin, betaine, and flaxseed. The GA–melatonin–betaine combination resulted in the most notable drop in blood glucose levels among all combinations, along with improvements in oxidative stress, inflammation, renal damage indicators, and overall renal function. We speculate that the improved glycemic control brought about by this combination, in conjunction with complementing molecular, pharmacokinetic, and pharmacodynamic processes, may be the cause of the observed improvement in the development of CKD. Our results highlight the GA-melatonin-betaine combination's therapeutic potential in providing comprehensive protection against CKD and diabetes. To confirm these results, investigate the precise processes behind these interactions, and assess the combination's long-term therapeutic effectiveness and safety in the treatment of diabetic CKD in people, further study is necessary.

References

Kaskel, F., and O. M. Akchurin (2015). An update on chronic kidney disease inflammation. 39, 84–92. doi:10.1159/000368940 Blood Purif.

Manoj, P., Nemmar, A., Ali, B. H., Al Salam, S., Al Suleimani, Y., and Al Za'abi, M. (2018). Gum acacia reduces oxidative and nitrosative stress, systemic inflammation, and enhances renal function in Rats with adenine-induced chronic renal disease developed diabetes due to streptozotocin. doi:10.1159/000488176 Cell. Physiology Biochem. 45, 2293–2304.

Ali, H., Al Sabahi, M., Ali, B. H., and Al Za'abi, M. (2021a). the beneficial effects of betaine and melatonin, either separately or together, on mice's cisplatin-induced nephrotoxicity. 394, 1693–1701; Schmiedeb. Arch. Pharmacol. doi:10.1007/ s00210-021-02097-z

Ali, H., Ali, B. H., and Al Za'abi, M. A. (2021b). Flaxseed's impact on oxidative stress and systemic inflammation in diabetic rats with or without chronic renal disease. doi:10.1371/journal.pone.0258800 PLOS ONE 16, e0258800

Al-Jubori, Y., Sirasanagandla, S. R., Madden, J., Das,

S., Ahmed, N. T. B., and Albusaidi, R. (2023). A comprehensive analysis of evidence-based clinical studies examining the effectiveness of gum Arabic in illness management. 10.3390/biom13010138 Biomolecules 13, 138

Al-Masroori, N., Al-Harthi, T., Kayed, R. R., Al-Salam, S., Al Husseni, I., Ali, B. H., et al. (2010). Gum Arabic's effects on rats suffering from chronic renal failure brought on by adenine. doi:10.1258/ebm. Exp. Biol. Med. (Maywood) 235, 373–382. 2009.009214

Beegam, S., Al-Shukaili, A., Nemmar, A., Schierling, S., Ali, B. H., Al-Husseni, I., et al. (2013a). Gum Arabic's impact on inflammation and oxidative stress in rats with adenine-induced chronic renal failure. doi:10.1371/journal.pone.0055242 PLOS ONE 8, e55242. Ali, B. H., Ramkumar, A., Beegam, S., Waly, M. I., Al-Salam, S., Al Za'abi, M., et al. (2013b). Adenine-induced chronic renal failure in mice: a novel paradigm and its impact on gum acacia treatment: a comparison with rats. 384–393 in J. Pharmacol. Toxicol. Methods, doi:10.1016/j.vascn.2013.05.001

Al-Husseini, I., Mandel, P., Nemmar, A., Schupp, N., Al Balushi, K., Ali, B. h., et al. (2015). In rats with adenine-induced chronic renal failure, gum acacia reduces genetic damage. doi:10.1111/eci.12501 Eur. J. Clin. Investigation 45, 1221–1227

Ali, B. H., Nemmar, A., Ali, H., Abdelrahman, A., Al Suleimani, Y., Manoj, P., et al. (2020). Impact of concurrent curcumin and melatonin therapy on rats' cisplatin-induced nephrotoxicity. doi:10.1016/j. biopha.2020.110761 Biomed. & Pharmacother. 131, 110761.

S. Alobaidi. 2024. A narrative evaluation of the therapeutic potential of Arabic gum (Acacia Senegal) in the treatment of chronic renal disease. doi:10.3390/jcm13195778 J. Clin. Med. 13, 5778

Ferreira, M. S., Kemp, J. A., Alvarenga, L., and Mafra, D. (2022). A narrative evaluation of betaine's function in individuals with chronic renal disease. 11, 395–406; doi:10.1007/s13668-022-00426-z; Curr. Nutr. Rep.

Singh, U. K., and M. K. Arora (2013). An update on the molecular pathways underlying the etiology of diabetic nephropathy. 58, 259–271. doi:10.1016/j.vph.2013. 01.001 Vasc. Pharmacol.

Paal, M. C., Donohue, T. M., Ganesan, M., Osna, N. A., Kharbanda, K. K., and Arumugam, M. K. (2021). A thorough analysis of betaine's positive benefits. The biological doi:10.3390/biology10060456; 10, 456

Prieto, M., Vicente-Vicente, L., Casanova, A. G., Hernández-Sánchez, M. T., López-Hernández, F. J., Martínez-Salgado, C., et al. (2020). The effectiveness of clinically tested protectants against cisplatin nephrotoxicity was reviewed and meta-analyzed. 76, 23–33. doi:10.1007/s00228-019-02771-5 Eur. J. Clin. Pharmacol

Stephens, J. M., Ribnicky, D. M., and Cefalu, W. T. (2011). "Diabetes and Herbal Medicine," in Herbal Medicine: Clinical and Biomolecular Aspects Editors: I. F. F. Benzie and S. Wachtel-Galor (CRC Press/Taylor & Francis, Boca Raton, FL). http://www.ncbi.nlm.nih.gov/books/NBK92755/ is the online version. (retrieved 15 January 2025).

Kantharia, N. D., and J. Charan (2013). How is sample size determined in animal research? 303–306 in J. Pharmacol. Pharmacother; doi: 10.4103/0976-500X.119726

Ji, P., Liu, Y., Wan, H., Zhang, J., Chen, W., Feng, J., and Ji, P. (2023). A comprehensive review and meta-analysis of the relationship between hyperhomocysteinemia and chronic renal disease in the general population. 24 247. doi:10.1186/s12882- 023-03295-y BMC Nephrol.

Huang, L.-T., Tain, Y.-L., Wu, T.-H., and Cheng, M.-C. (2014). Young spontaneously hypertensive rats with L-NAME: melatonin's renoprotective benefits. Pediatr. & Neonatol. 55, 189–195. doi:10.1016/j.pedneo.2013.09.005

Irwin, M. R., Cho, J. H., Kim, C. H., and Bhutani, S. (2021). A comprehensive review and meta-analysis of clinical studies on the anti-inflammatory benefits of melatonin. Brain Behav. Immun. 93, 245–253. doi:10.1016/j.bbi.2021.01.034

Wapnir, R. A., and C. N. Codipilly (2004). Gum Arabic's proabsorptive effect in isotonic solutions given orally to rats. II. Impact on solutes in both secretory and normal circumstances. 473–1478 in Dig. Dis. Sci. doi:10.1023/B:DDAS.0000042249. 85357.65

Lopes, R. H., Lamy-Freund, M. T., and Costa, E. J. X. (1995). Melatonin permeability of pure lipid bilayers. tb00180.x J. Pineal Res. 19, 123–126. doi:10.1111/j.1600-079X.1995.

Chamekh-Coelho, I., Vaysse, C., Baury, A., Joseph, C., Abrous, H., Couëdelo, L., et al. (2022). Rats' intestinal bioavailability of n-3 polyunsaturated fatty acids is impacted by the gum Acacia. Biomolecules doi:10.3390/biom12070975 12, 975

Blewett, H., Aliani, M., Shariati, S., and Defries, D. (2021). Flaxseed enterolignans make cytochrome P450 enzymes more active. 5, 312. doi:10.1093/cdn/nzab037_022; Curr. Dev. Nutr.

Figueiredo, L. M., Nunes, B. P., and Delpino, F. M. (2021). Melatonin supplementation's effects on diabetes: a meta-analysis and comprehensive review of randomized clinical trials. 40, 4595–4605. Clin. Nutr. doi:10.1016/j.clnu.2021.06.007

Pastor, K., Dobrijević, D., Özogul, F., Krulj, J., Kokić, B., Nastić, N., et al. (2023). The metabolism, health-promoting qualities, dietary sources, uses, and analytical techniques of betaine as a functional ingredient. doi:10.3390/molecules28124824 Molecules 28, 4824

Rodella, L. F., Veronese, F. V., Stacchiotti, A., dos Santos, M., Favero, G., Bonomini, F., et al. (2018). In a lupus mouse model produced by pristane, oral

melatonin administration prevents kidney damage from lupus nephritis. 1. doi:10.1016/j.lfs. 2017.10.038; Life Sci. 193, 242–251.
Ballantyne, C. M., Duncan, B. B., Couper, D., Vigo, A., Pankow, J. S., Schmidt, M. I., et al. (2003). Atherosclerosis risk in communities: a study of low-grade systemic inflammation and the onset of type 2 diabetes. doi:10.2337/diabetes.52.7.1799 Diabetes 52, 1799–1805.
Hossein-Nezhad, A., Emamgholipour, S., and Ansari, M. (2016). In a model of oxidative stress caused by hydrogen peroxide in human peripheral blood mononuclear cells, may melatonin function as an antioxidant? doi:10.1155/2016/5857940 Biochem. Res. Int. 2016, 5857940
Cooper, M. E., and J. M. Forbes (2013). diabetes problems' mechanisms.
93, 137–188. doi:10.1152/physrev.00045.2011. Physiol. Rev.
Ong, A. C. M., Francis, A., Harhay, M. N., Ortiz, A., Fogo, A. B., Tummalapalli, S. L., et al. (2024). An worldwide agreement on chronic kidney disease and the global public health agenda. 20 473–485. doi:10.1038/s41581-024-00820-6 In Nat. Rev. Nephrol.
Eid, F. A., Mashaal, A., EL-Shamy, S. A. E.-M., Ibrahim, R. M., and Abdelhafez, H. M. (2023). In rats with diabetes induced by alloxan monohydrate, Arabic gum improves systemic regulation. doi:10.1038/s41598-023-31897-x Sci. Rep. 13, 5005
Stojanovska, L., Feehan, J., Bataineh, M. F., Ismail, L. C., Apostolopoulos, V., Jarrar, A. H., et al. (2021). A randomized clinical research examined the impact of Arabic gum (Acacia Senegal) on gastrointestinal symptoms and cardiovascular risk factors in persons at risk for metabolic syndrome. The nutrients doi:10.3390/nu13010194 13, 194.
Modi, G. K., and V. Jha (2018). Learning more about the enemy—the prevalence of chronic renal disease worldwide. 94, 462–464. doi:10.1016/j.kint. Kidney Int. May 09, 2018
Pourfarziani, P., Kavyani, Z., Mollaghasemi, N., Sedgh Ahrabi, S., Hossein Moridpour, A., Mohamad Jafari Kakhki, A., et al. (2023). An updated comprehensive review and meta-analysis of randomized controlled trials examined how flaxseed supplements affected adults' glycemic management. 10.1016/j.jff. J. Funct. Foods 110, 105816. 2023.105816
Kanzaki, M., Teshigawara, S., Wada, J., Kurose, Y., Nakatsuka, A., Murakami, K., et al. (2013). Patients with chronic renal disease and type 2 diabetes had higher serum levels of galectin-9. 14. 23. doi:10.1186/1471-2369-14-23 BMC Nephrol. Slow, S., and Lever, M. (2010). The therapeutic importance of betaine, an osmolyte that plays a crucial part in the metabolism of methyl groups. doi:10.1016/j.clinbiochem.2010.03.009; Clin. Biochem. 43, 732–744.
Fan, C.-Y., Zhu, Q., Li, J.-M., Pan, Y., Wang, X., Liu, Y.-L., et al. (2013). In hyperuricemic mice, betaine lowers blood uric acid levels and enhances renal function. 39–47 in Planta Medica. doi:10.1055/s-0033-1360127
Tavares, A., and W. Martorina (2023). Melatonin's effects on type 2 diabetes mellitus glycemic variability: a crossover, double-blind, placebo-controlled study procedure. doi:10.2196/47887 JMIR Res. Protoc. 12, e47887
M. P. McRae (2013). A meta-analysis found that betaine supplementation lowers plasma homocysteine in healthy adult subjects. 10.1016/j. jcm. J. Chiropr. Med. 12, 20–25. doi. 11.001, 2012.
Mueed, A., Esatbeyoglu, T., Madjirebaye, P., Korma, S. A., Shibli, S., and Deng, Z. (2022). The chemical makeup, functional characteristics, culinary uses, and gut bacteria associated with flaxseed bioactive substances and their health effects. doi:10.3390/foods11203307; Foods 11, 3307.
Kavyani, Z., Vajdi, M., Askari, G., Musazadeh, V., and Morovatshoar, R. (2024). A GRADE-accredited systematic review and meta-analysis of randomized controlled trials examined the impact of flaxseed supplementation on inflammatory biomarkers. doi:10.1016/j.prostaglandins.2024. 106868 Prostagl. & Other Lipid Mediat. 174, 106868
Somay, A., Simsek, A., Cekmen, M., Ozbek, E., Ozbek, M., and Ilbey, Y. O. (2009). Melatonin reduces oxidative stress, iNOS, MAPK, and NF-kB expression to lessen kidney damage brought on by unilateral ureteral obstruction. 23 1165–1173, J. Endourology, doi:10.1089/end.2009.0035
Vardi, N., Batcioglu, K., Ozturk, F., Ucar, M., and Ozturk, I. C. (2003). Nephrotoxicity caused by carbon tetrachloride and the protective role of betaine in Sprague-Dawley rats. doi:10.1016/s0090-4295(03)00255-3 Urology 62, 353–356
Garcia-Ocaña, A., Palit, S. P., Li, Y., Patel, R., Parmar, N., Rathwa, N., et al. (2022). In mouse and human islets, a new therapeutic combination of melatonin and sitagliptin regenerates pancreatic β-cells. Mol. Cell Res. 1869, 119263, Biochimica Biophysica Acta (BBA) doi:10.1016/j.bbamcr.2022.119263
Patschan, D., Wessels, J. T., Patschan, S., Becker, J. U., Hildebrandt, A., Rinneburger, J., et al. (2012). In acute ischemic kidney damage, the hormone melatonin promotes the renoprotective properties of "early outgrowth" endothelial progenitor cells. 302, F1305–F1312; doi:10.1152/ajprenal.00445.2011; Am. J. Physiol. Ren. Physiol.
Qian, Q. (2017). One of the main factors in the development and advancement of chronic renal disease is inflammation. 191, 72–83. doi:10.1159/000479257. Contrib. Nephrol.
Lubega, M., Katureebe, S. K., Oundo, A., Kiweewa, F., Mukanga, D., and Rutebemberwa, E. (2013). A

qualitative investigation of the factors influencing the use of traditional medicine to treat diabetes in Eastern Uganda. Human Rights in BMC Int. Health 13, 1 doi:10.1186/1472-698X-13-1
S. Samanta (2022). Melatonin from a physiological and pharmacological standpoint. 128, 1346–1367; Archives Physiology Biochem. doi:10.1080/13813455.2020.1770799
El-Rayes, S. M., Ahmed, N., Abdelkader, A., Aborayah, N. H., Shanab, O., Khalil, W. F., et al. (2023). Acacia Senegal has nephroprotective properties that prevent aflatoxicosis by focusing on inflammatory and apoptotic signaling pathways. doi:10.1016/j.ecoenv.2023.115194 Ecotoxicol. Environ. Saf. 262, 115194
Singh, H., Ahmad, N., Mishra, P., Tiwari, A., and Sharma, S. (2015). Melatonin's function in diabetes: potential treatment applications. 59, 391–399; doi:10.1590/2359-3997000000098; Arch. Endocrinol. Metab.
Y.-L. Shih, C.-C. Shih, and J.-Y. Chen (2022). A community-based cross-sectional research found that middle-aged and older people in Taiwan who live in the community have elevated homocysteine levels, which are a sign of chronic kidney disease. doi:10.3389/fmed.2022.964101 Front. Med. 9, 964101.
Nicholas, S. B., and S. K. Sinha (2023). diabetic renal disease pathogenesis.
doi:10.3390/jcm12237349 J. Clin. Med. 12, 7349
Rezzani, R., Bianchi, R., Lavazza, A., and Stacchiotti, A. (2002). An electron microscopy study found that melatonin limits the kidney changes caused by cyclosporine A in rats. Ultrastruct. Pathol. 26, 81–87. doi:10.1080/01913120252959254
Tsigalou, C., Konstantinidis, T., Romanidou, G., Voidarou, C., Stavropoulou, E., Kantartzi, K., et al. (2021). Pay attention to the kidney-gut axis in both health and illness. doi:10.3389/fmed.2020.620102 Front. Med. 7, 620102
Murase, T., Tanaka, G., Sueyoshi, M., Fukunaga, M., Mei, M., Nakajima, A., et al. (2019). Lactulose's effects on gut microbiota and renal function in rats with chronic kidney disease caused by adenine. 23 908–919. Clin. Exp. Nephrol. doi:10.1007/s10157-019-01727-4
Sun, H., Ogurtsova, K., Duncan, B. B., Pinkepank, M., Karuranga, S., Saeedi, P., et al. (2022). IDF Diabetes Atlas: 2021 diabetes prevalence estimates and 2045 predictions at the national, regional, and worldwide levels. doi:10.1016/j.diabres.2021.109119 Diabetes Res. Clin. Pract. 183, 109119
Esteban-Zubero, E., Zhou, Z., Manchester, L. C., Tan, D.-X., and Reiter, R. J. (2015). The production and metabolism of melatonin, a strong and inducible endogenous antioxidant. doi:10.3390/molecules201018886; Molecules 20, 18886–18906.
Papamikroulis, G.-A., Vogiatzi, G., Papaioannou, S., Tsalamandris, S., Antonopoulos, A. S., Oikonomou, E., et al. (2019). Current understandings and prospective viewpoints on the function of inflammation in diabetes. 14. 50–59. doi:10.15420/ecr. 2018.33.1 Eur. Cardiol.
Hsieh, H.-Y., Chu, P., Lin, S.-H., Wu, C.-C., Lu, K.-C., Lin, G.-J., et al. (2012).
Melatonin improves experimental murine membranous nephropathy by suppressing immunological responses and increasing endogenous heme oxygenase-1. 460–469 in J. Pineal Res. doi:10.1111/j.1600-079X.2011.00960.x
Chen, G., Liu, H., and Xu, N. (2017). Twenty amino acids are categorized as antioxidants based on experimental examination. doi:10.3390/molecules22122066; Molecules 22, 2066