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Original Research Article

Allicin-mediated renal protection in mitigating streptozotocin-induced diabetic nephropathy in rats through comprehensive restoration of kidney function and morphology

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Abstract

Purpose: *To evaluate the benefits of allicin, a vital garlic component, against streptozotocin-induced diabetic kidney damage in rats.*
Methods: *A total of thirty male albino Wistar rats were divided into five groups of six rats each, with groups II – IV induced with diabetes using a single intraperitoneal administration of streptozotocin (55 mg/kg). Group I served as normal control while Group II was untreated diabetic control, receiving vehicle injections. Diabetic rats in Groups III and IV were treated with allicin (diallyl thiosulfinate, DATS, 20 mg/kg/day orally for 15 days) and aminoguanidine (AG, 100 mg/kg/day orally for 15 days), respectively, while Group V rats served as normal control rats receiving DATS only. Various biochemical, and kidney marker assessments and histological examinations were conducted at the end of treatment.*
Results: *Streptozotocin-induced diabetic rats exhibited significant ($p < 0.05$) alterations in body weight, kidney metrics, kidney markers and renal histopathology compared to normal control (Group I). Treatment with DATS showed significant improvements in body weight, kidney metrics and biochemical markers ($p < 0.05$) indicating potential nephroprotective effect against diabetic nephropathy by significantly restoring kidney weight, protein levels, albumin, potassium, sodium and other urinary markers ($p < 0.05$). Furthermore, DATS effectively reversed STZ-induced renal damage with outcomes comparable to standard drug (aminoguanidine).*
Conclusion: *Administration of DATS in rats with nephropathy from diabetes may have kidney protective benefits. The study highlights allicin's potential as a therapeutic agent in managing diabetic complications, particularly diabetic nephropathy, prompting further exploration prior to future use.*

Keywords: *Allicin, Nephropathy, Renoprotection, Streptozotocin, Biomarkers, Herbal, Diabetes, Rats*

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INTRODUCTION

Diabetes mellitus is a major global health issue causing microvascular complications like retinopathy, neuropathy and nephropathy as well as macrovascular problems like heart attacks, strokes and peripheral vascular diseases [1]. The International Diabetes Federation (IDF) highlights the growing burden worldwide. According to the 2021 IDF Diabetes Atlas, diabetes affects 10.5 % of adults aged 20 - 79 years with projections suggesting 783 million people will have diabetes

by 2045 [2]. Fourteen percent of diabetic deaths and forty percent of instances of advanced renal disease are related to diabetic nephropathy (DN), which is a primary cause of chronic kidney failure [3].

Despite progress in antidiabetic treatments, diabetes remains incurable. Insulin therapy is still the best way to manage diabetes, but it comes with some serious side effects such as insulin resistance, anorexia, brain atrophy and formation of fatty liver with continued use [4]. Protective effects of various medicinal plants are attributed to the presence of phytoconstituents, including carotenoids, vitamin C and phenolic acids [5]. Allicin (Diallyl thiosulfinate), the primary biologically active compound found in garlic (*Allium sativum* L. Family: Amaryllidaceae), has garnered considerable interest due to its potential to offer a wide range of health benefits, including antihypertensive effects, cardioprotective effects, anti-inflammatory properties and anticancer activities [6,7]. These health benefits provide a strong rationale for investigating if allicin is potentially effective in DN treatment. Thus, the purpose of this work was to assess the effect of allicin on DN in streptozotocin-induced diabetic rats.

EXPERIMENTAL

Animals

This study employed thirty (30) male albino rats of the Wistar strain, with body weights ranging between 200 and 240 grams, procured from Central Animal Facility, King Saud University, Saudi Arabia. Rats were housed within polypropylene cages, set within a precisely controlled environment. Ambient conditions were maintained at a temperature of 22 ± 2 °C and a humidity level of 55 ± 5 %. To ensure a consistent light-dark cycle, rats were exposed to 12 hours of illumination followed by 12 hours of darkness in a specialized experimental chamber. They were given a standard laboratory diet from local Mills in Saudi Arabia. Experimental protocols were approved by the Animal Care and Use Committee, University of Hail, Saudi Arabia (No. 27/5/8530) and were performed according to the guidelines on the care and use of laboratory animals [8].

Induction of diabetes

Streptozotocin (STZ; Sigma Aldrich Chemical Pvt Limited in India) (was dissolved in citrate buffer (pH 4.5) and rats were administered a single intraperitoneal injection (55 mg/kg STZ) to induce diabetes [9]. To mitigate any initial hypoglycemic effects, rats were provided with a 5 % glucose solution in their drinking water for the first 24 hours following injection and on the third day, animals underwent blood glucose level assessments and those with readings exceeding 180 mg/dL were included, following the procedure outlined by Rathod *et al* [9].

Design

The rats were divided into five groups, each consisting of six rats, as illustrated in Figure 1. Group I served as normal control rats and were given vehicle (citrate buffer, IP) injections while rats in Group II were induced with STZ and thereafter received vehicle (citrate buffer, IP) injections. Group III rats, after STZ induction, received an oral daily administration of diallyl thiosulfinate (DATS; Shanghai Harvest Pharmaceutical Co., Ltd., China) at a dose of 20 mg/kg of body weight for 15 days via gastric intubation with a force-feed needle and rats in Group IV, post-STZ induction, were daily treated with the standard drug, aminoguanidine (AG), at a dose of 100 mg/kg of body weight via oral administration for 15 days. Lastly, rats in Group V were normal control rats administered DATS orally daily for 15 days. At the end of the protocol, the rats were fasted overnight and were humanely euthanized via cervical dislocation under light ether anesthesia. After decapitation, blood samples were collected and serum was separated through centrifugation (20 minutes at 2,000 rpm) and stored at - 20 °C for subsequent biochemical assays. Kidney tissues were rapidly dissected and a portion was used to prepare a 10 % (w/v) homogenate in 0.1 M Tris HCl buffer (pH 7.4). The homogenate was centrifuged at 7,000 rpm for 10 minutes at 4 ºC and the resulting supernatant was used for further assays.

Determination of kidney markers

Serum and urine creatinine levels were determined using the alkaline picrate technique. Blood urea content was estimated with the NED Dye technique, while blood urea nitrogen (BUN) and urine urea nitrogen (UUN) were quantified using kits from Parsazmoon Company (Iran) and Span Diagnostics (India), respectively. Serum uric acid levels were determined following the procedure described by Buzanovskii [10]. Total protein in serum was assayed colorimetrically. Serum albumin levels were estimated using the Bromocresol Green (BCG) method while urinary albumin was quantified via competitive ELISA. Serum and urinary sodium and potassium levels were determined using flame photometry [11].

Histological examination

The kidneys were dissected and abnormalities were inspected using standard histological procedures. The kidney tissues underwent a series of steps including fixing in buffered formalin, ethanol dehydration, xylene clearing, paraffin embedding and microtome sectioning. Then the sections were stained with hematoxylin and eosin (H&E) and images were captured using a light microscope.

Statistical evaluation

Data are reported as mean values along with their accompanying standard deviations (mean ± SD). Data was analyzed using SPSS version 12.0 for Windows (SPSS Inc.). One-way analysis of variance (ANOVA) was used to compare experimental groups while Duncan's multiple range test (DMRT) was used to confirm the level of significance between groups. $P < 0.05$ was considered significant.

RESULTS

The impact of allicin on body mass and renal metrics in STZ-induced DN is shown in Figure 2. Rats given STZ had significantly lower body weight than normal controls ($p < 0.05$). However, STZ-induced DN rats in Group III and Group IV showed increased body weight compared to normal rats ($p < 0.05$). Group V animals exhibited no significant body weight changes ($p > 0.05$). Kidney weights in DN rats were significantly higher than normal controls ($p < 0.05$). Diabetic rats administered DATS (Group III) and AG (Group IV) showed improved kidney weight similar to Group I. Animals in Group V treated with DATS alone did not exhibit a statistically significant change in kidney weight as compared to controls ($p > 0.05$). Additionally, in groups treated with DATS, the modified kidney-to-body weight index in Group II animals returned to normal.

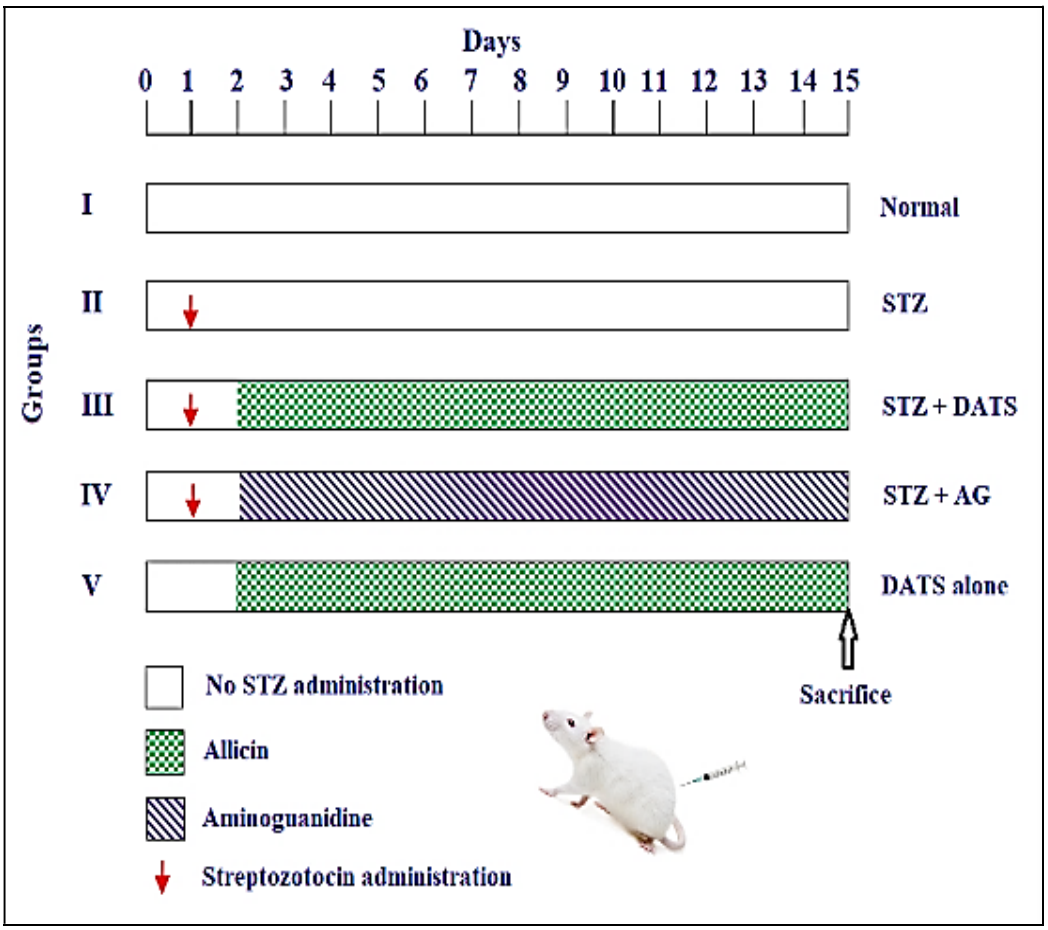

Figure 1: Protocols for experimental nephroprotective investigations

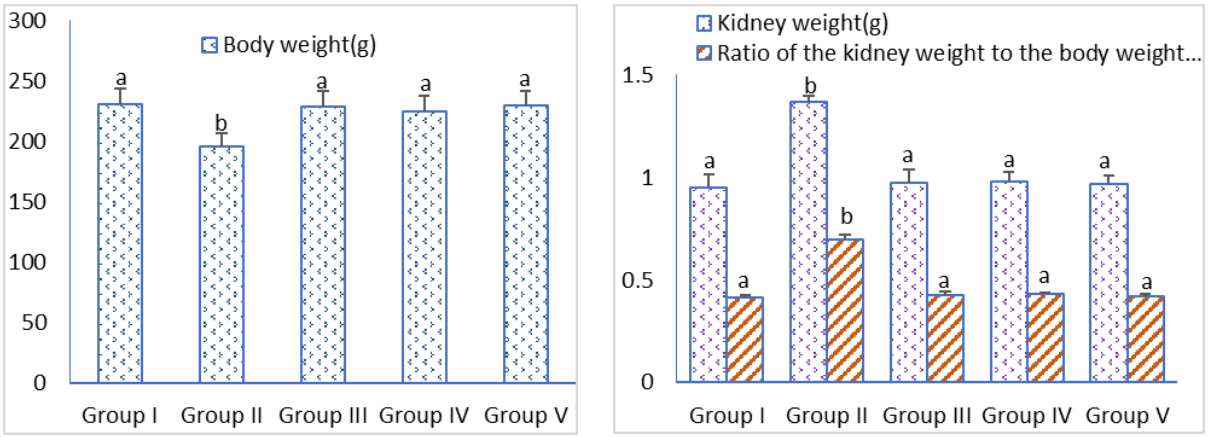

Figure 2: Effect of DATS on physiological parameters (body mass and renal metrics) in STZ-induced diabetic nephropathy. Bars with different letters are significantly different ($p < 0.05$)

Effect of DATS on kidney markers

As shown in Figure 3, animals in Group II had significantly higher serum creatinine, urea, uric acid and blood urea nitrogen levels than animals in Group I. However, there was a significant decrease ($p < 0.05$) in these markers after oral DATS administration. When compared with control, a significant increase ($p < 0.05$) in serum total protein levels alongside a decrease in albumin was observed (Figure 4). However, administering DATS and AG to STZ-induced animals effectively restored total protein and albumin levels, bringing them closer to normal.

Additionally, Figure 5 shows that animals with DN in Group II had higher serum potassium and sodium levels compared to normal group ($p < 0.05$) but, DATS treatment reversed these levels to normal. It's interesting to note that kidney indicators did not significantly change between animals in Group V, which received DATS alone, and those in the control group.

Figures 6 - 8 illustrate variations in kidney function markers between control and experimental animals. Group II animals displayed significantly heightened levels of urinary urea nitrogen (UUN), creatinine, albumin and glucose ($p < 0.05$). However, administration of DATS and AG effectively restored these altered kidney function markers to normal levels. In contrast, DN was associated with elevated potassium and sodium levels. Kidney function analysis of STZ-induced diabetic rats with nephropathy indicated increased glucose, along with elevated levels of potassium, sodium, BUN, creatinine, uric acid and albumin ($p < 0.05$). Treatment with DATS and AG notably alleviated these elevated kidney markers. Normal animals treated solely with DATS did not exhibit significant differences in kidney markers compared to control.

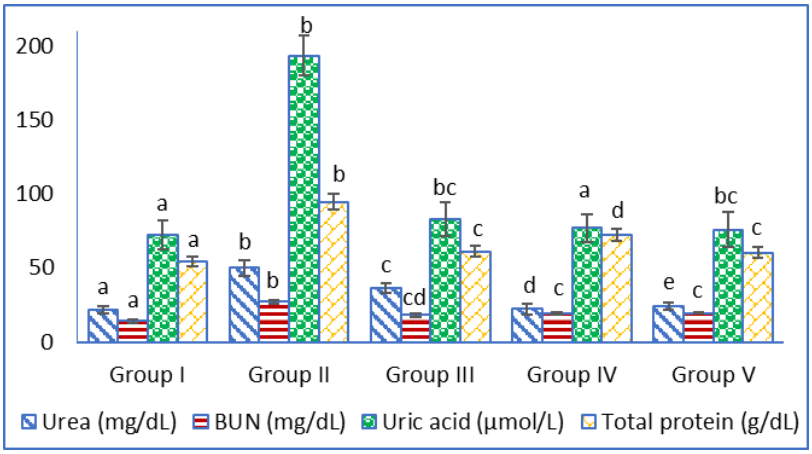

Figure 3: Effect of DATS on serum renal markers (Urea, BUN, Uric acid and Total protein) in STZ-induced diabetic nephropathy. Bars with different letters are significantly different ($p < 0.05$)

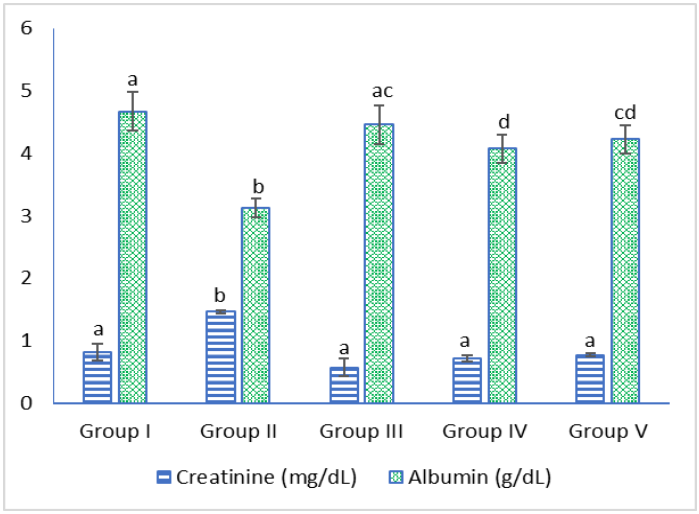

Figure 4: Effect of DATS on serum kidney markers (Creatinine and Albumin) in STZ-induced diabetic nephropathy. Bars with different letters are significantly different ($p < 0.05$)

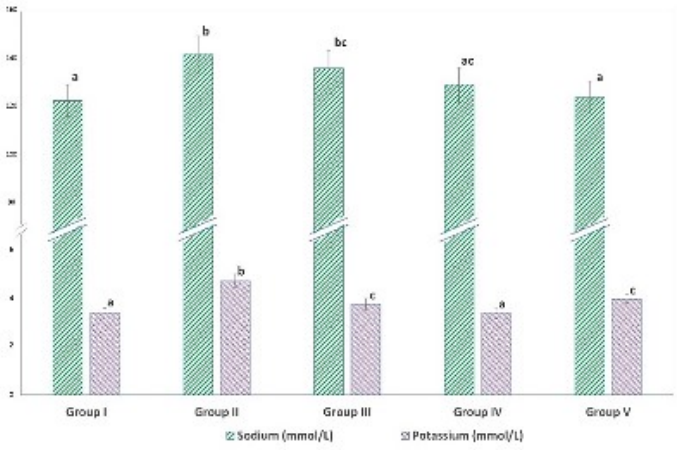

Figure 5: Effect of DATS on serum sodium and potassium concentrations in STZ-induced diabetic nephropathy. Bars with different letters are significantly different ($p < 0.05$)

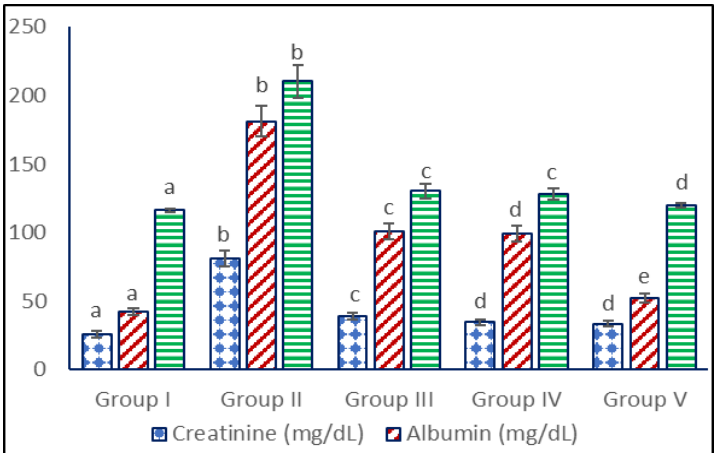

Figure 6: Effect of DATS on urinary kidney markers in STZ-induced diabetic nephropathy. Bars with different letters are significantly different ($p < 0.05$)

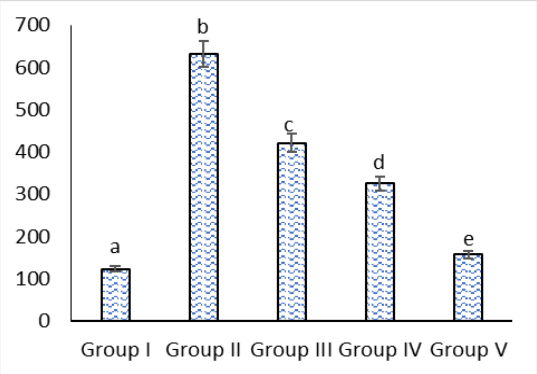

Figure 7: Effect of DATS on urinary urea nitrogen levels in STZ-induced diabetic nephropathy. Bars with different letters are significantly different ($p < 0.05$)

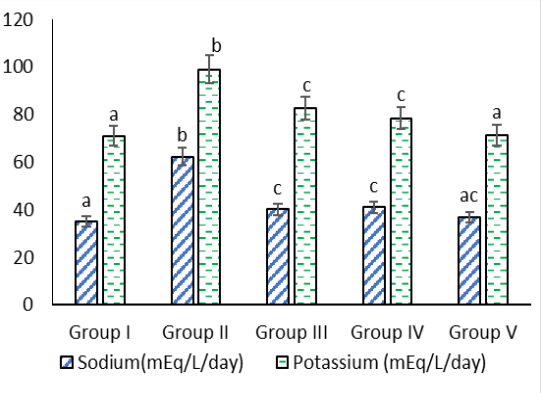

Figure 8: Effect of DATS on urinary sodium and potassium excretion in STZ-induced diabetic nephropathy. Bar with different letters are significantly different ($p < 0.05$)

Histomorphology

Renal histopathological assessments unveiled distinct alterations in kidney architecture between control and study groups (Table 1 and Figure 9). Control (Group I) kidney specimens exhibited typical histological features consistent with a healthy morphology during examination. On the other hand, tubular epithelium of kidneys from animals that were administered STZ showed clear changes, including vacuolization, desquamation, atrophy, necrosis and noticeable interstitial edema and inflammation. These changes collectively disrupt normal renal structure and function indicative of DN. Interestingly, administration of DATS and AG show a reversal of these pathological changes in the renal tissue. This significant amelioration in histopathological features suggests a potential nephroprotective effect conferred by DATS against the progression of DN. Notably, in the group treated solely with DATS, the histological appearance of the tubular and glomerular structures exhibited a striking resemblance to features in normal control.

DISCUSSION

Diabetic nephropathy (DN) affects approximately one-third of individuals with diabetes, estimated to reach 592 million people (8 – 10 % of global population) [12]. It is the leading cause of chronic kidney disease and adult end-stage renal disease with fibrosis playing a key role in its progression in both types of diabetes [13]. Among Group II DN rats, there was a distinct decline in renal function, as evidenced by increased kidney weight/body weight ratios, higher BUN and serum creatinine levels. They also displayed substantial renal damage affecting glomeruli, tubules and tubulointerstitial fibrosis. However, serum urea and creatinine levels may not precisely be dependent on renal function due to factors like diet and body weight [14].

Allicin (DATS) was administered orally at 20 mg/kg/day to assess its potential in reducing hyperglycemia and DN. The drug notably reversed the increase in blood glucose levels in Group III animals, potentially owing to its hypoglycemic properties. In contrast, untreated rats in Group II showed uncontrolled increase in glucose levels and an increased risk of DN [15], suggesting that DATS may offer kidney protection.

Table 1: DATS impact on histopathological alterations in kidney tissues in STZ-induced diabetic nephropathy

Tissue Histomorphology	Group I	Group II	Group III	Group IV	Group V
Tubular necrosis	-	+	-	-	-
Tubular dilatation	-	++	-	-	-
Tubular epithelial desquamation	-	+++	+	+	-
Tubular atrophy	-	++	+	-	-
Interstitial inflammation	-	+	-	-	-
Interstitial edema	-	+	-	+	-
Tubular casts	-	+	-	-	-

Quantification scores: (-) no significant alteration in histopathology; (+) mild degree; (++) moderate degree; (+++) severe degree

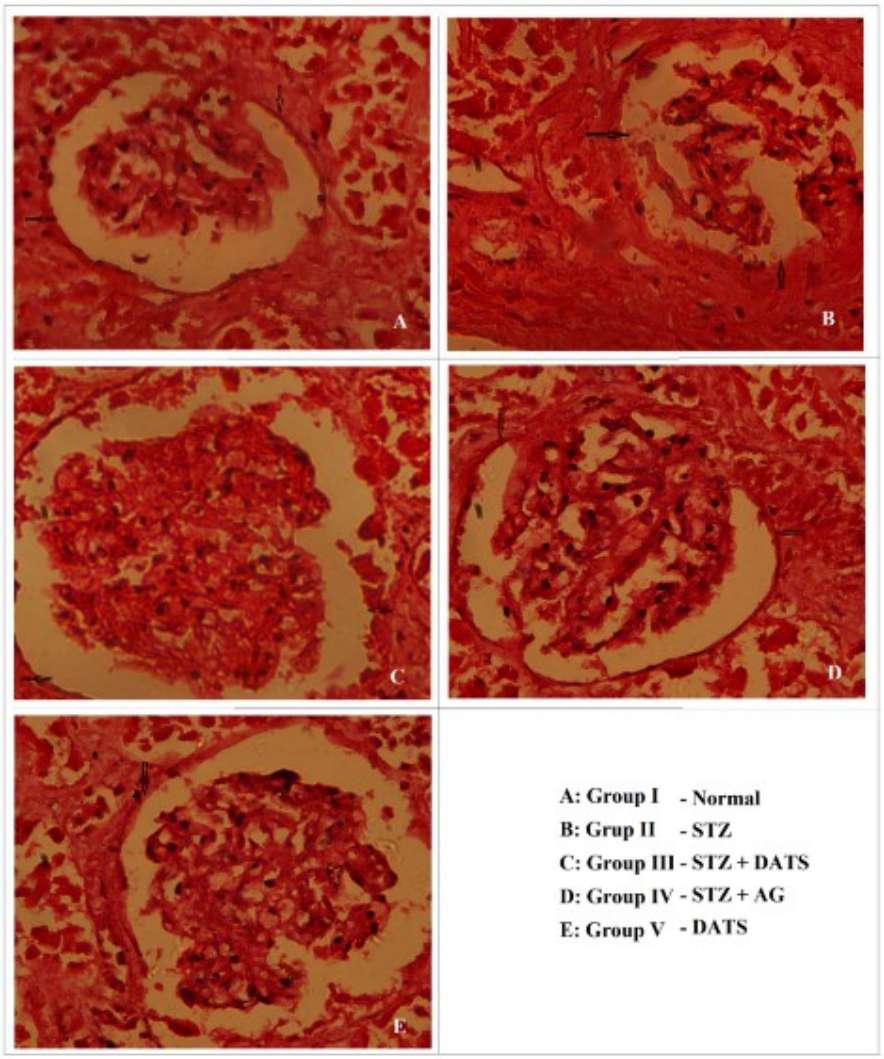

Figure 9: Effect of DATS on kidney histopathology in diabetic nephropathy. STZ - Streptozotocin; DATS -Allicin; AG - Aminoguanidine

Kidney weight index is critical for assessing enlargement. Treating Group III animals with DATS for 15 days reduced kidney enlargement. Group II DN animals showed decreased body weight with hyperglycemia, due to muscle loss from protein breakdown [16]. Treatment with DATS was instrumental in maintaining body weight by controlling blood glucose levels and preventing protein breakdown.

This study showed an increase in urinary albumin linked to hyperglycemia after the onset of diabetes. Furthermore, markers of renal function such as urine creatinine, kidney weight/body weight ratio, serum creatinine, BUN, urea, uric acid and albumin were significantly higher in DN rats [17] compared to normal rats. Hyperglycemia triggers an increase in serum uric acid, BUN and serum creatinine levels, sensitive indicators of kidney malfunction in DN rats [18]. Increased uric acid and creatinine in STZ-induced Group II animals indicate DN with renal hyperfiltration and creatinine elevated levels may indicate muscle loss [19]. In DN rats, DATS restored these markers, hinting at its potential to protect against DN by possibly improving kidney function or reducing protein breakdown.

As diabetes progresses, increased serum BUN and creatinine result from extracellular matrix (ECM) accumulation in glomeruli and interstitium, leading to characteristic renal fibrosis in DN [20]. Despite the extracellular matrix (ECM) buildup and structural renal changes following STZ-induced DN, treatment with DATS in DN rats demonstrated enhancements in glomerular fibrosis and renal structure. Reduction in sodium transport from renal proximal tubules to blood occurs when sodium is extruded into the cytosol by basolateral Na^+K^+ ATPase pump, with

alterations in renal sodium transporters in DN animal models leading to increased urinary sodium excretion [21]. Mesangial hypertrophy and glomerular thickening are early markers of DN. Podocyte damage leads to proteinuria, triggering initial renal dysfunction and tubular damage in diabetes. Diabetic Group II STZ-induced kidneys exhibit hyperfiltration, enlargement, mesangial expansion, fibrosis and increased ECM [22].

Diabetic nephropathy presents distinct structural and functional alterations with tubulointerstitial injury playing a key role in DN progression. Interstitial fibrosis, tubular atrophy, T-lymphocyte and macrophage infiltration, podocyte loss and decreased endothelial cell spacing are among subsequent progressions in DN [23]. Renal fibrosis is a multifaceted process involving diverse cellular responses to injury. Renal analysis in DN (Group II) rats compared to normal rats (Group I) reveals tubular lesions and evidence of glomerulosclerosis [24]. Histopathological modifications in Group II DN rats include interstitial edema and inflammation in addition to tubular epithelial abnormalities such as vacuolization, desquamation, atrophy and necrosis. Glomerular changes include thickening of capillary basement membranes and diffused or nodular glomerulosclerosis [25]. Administration of DATS shows substantial enhancements in renal morphology, indicating nephroprotective effects against DN, comparable to those observed with AG. Tubular and glomerular structures tend toward normalcy with DATS treatment alone. Simultaneous DATS administration effectively prevents DN-associated renal damage, as evidenced by various biochemical and histological examinations. Changes in mean body weight, BUN, creatinine and uric acid related to DN are lessened by DATS therapy. These findings propose Diallyl thiosulfinate (DATS) as a potential nephroprotective agent against DN with outcomes comparable to the standard drug aminoguanidine (AG).

Limitations of the study

This study utilized STZ-induced DN rats, which may not fully represent human diabetes. Treatment duration was short and longer studies are needed to evaluate long-term efficacy and safety. Additionally, the study focused on biochemical and histopathological parameters. Further mechanistic investigations are required. Finally, using a single DATS dose limits the understanding of its dose-response relationship, warranting exploration in future studies.

CONCLUSION

Allicin mitigates the effects of streptozotocin-induced diabetic kidney damage in rats. Allicin administration in STZ-induced diabetic rats shows promising outcomes, maintaining body weight, normalizing kidney parameters and ameliorating renal markers. Histological examinations reveal improved kidney structure, buttressing allicin's nephroprotective effects. These outcomes emphasize the therapeutic potential of allicin for diabetic renal problems and suggest additional studies into the use of allicin in the management of renal damage caused by diabetes.

DECLARATIONS

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Ethical approval

All protocols were approved by the Animal Care and Use Committee, University of Hail, Saudi Arabia (approval no. 27/5/8530).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. *Umar A, Ahmed QU, Muhammad BY, Dogarai BB, Soad SZ. Antihyperglycemic activity of the leaves of Tetracera scandens Linn. Merr. (Dille-niaceae) in alloxan-induced diabetic rats. J Ethnopharmacol 2010; 1: 140-145.*
2. *International Diabetes Federation. IDF Diabetes Atlas. 10th ed. Brussels, Belgium: International Diabetes Federation; 2021.*
3. *Ziyadeh FN, Sharma K. Overview: combating diabetic nephropathy. J American Soc Nephrol 2003; 14: 1355-1357.*
4. *Piédrola G, Novo E, Escobar F, García-Robles R. White blood cell count and insulin resistance in patients with coronary artery disease. Annales d Endocrinologie 2001; 62: 7-10.*
5. *Tan AC, Konczak I, Sze DM, Ramzan I. Molecular pathways for cancer chemoprevention by dietary phytochemicals. Nutr Cancer 2011; 63: 495–505.*
6. *Gonen A, Harats D, Rabinkov A, Miron T, Mirelman D, Wilchek M, Weiner L, Ulman E, Levkovitz H, Ben-Shushan D, et al. The antiatherogenic effect of allicin: possible mode of action. Pathobiol 2005; 72: 325–334.*
7. *Lang A, Lahav M, Sakhnini E, Barshack I, Fidder HH, Avidan B, Bardan E, Hershkoviz R, Bar-Meir S, Chowers Y. Allicin inhibits spontaneous and TNF-alpha induced secretion of proinflammatory cytokines and chemokines from intestinal epithelial cells. Clin Nutr 2004; 23:1199–208.*
8. *National Research Council. Guide for the care and use of laboratory animals, National Academies Press. Washington, DC; 2010.*
9. *Rathod N, Raghuveer I, Chitme HR, Chandra R. Antidiabetic activity of Nyctanthes arbortristis. Pharmacogn Mag 2008; 16: 335-340.*
10. *Buzanovskii VA. Determination of uric acid in blood. J Chem 2015; 5: 281–323.*
11. *Tubino M, de Souza RL, Hoehr NF. Rapid quantitative turbidimetric spot test analysis of potassium in blood serum. J Braz Chem Soc 2004; 15: 635-639.*
12. *International Diabetes Federation. IDF Diabetes Atlas; 6th ed. - Brussels, Belgium: International Diabetes Federation. 2013.*
13. *Zeng L-F, Xiao Y, Sun L. A glimpse of the mechanisms related to renal fibrosis in diabetic nephropathy. Adv Exp Med Biol 2019; 1165: 49-79.*
14. *Sasaki T, Nakagawa K, Hata J, Hirakawa Y, Shibata M, Nakano T, Tsuboi N, Oda Y, Kitazono T, Yokoo T, et al. Pathologic diabetic nephropathy in autopsied diabetic cases with normoalbuminuria from a Japanese community-based study. Kidney Int Rep 2021; 6: 3035-3044.*
15. *Arul B, Kothai R, Christina AJ. Hypoglycemic and antihyperglycemic effect of Semecarpus anacardium Linn in normal and streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 2004; 26: 759-762.*
16. *Andallu B, Varadacharyulu NC. Antioxidant role of mulberry (Morus indica L. cv. Anantha) leaves in streptozotocin-diabetic rats. Clin Chim Acta 2003; 338: 3-10.*
17. *Sefi M, Fetoui H, Soudani N, Chtourou Y, Makni M, Zeghal N. Artemisia campestris leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. Pathol Res Pract 2012; 208: 157-162.*
18. *Hovind P, Rossing P, Johnson RJ, Parving HH. Serum uric acid is a new player in the development of diabetic nephropathy. J Ren Nutr 2011; 21: 124-127.*
19. *Stevens LA, Coresh J, Greene T, Andrew SL. Assessing kidney function-measured and estimated glomerular filtration rate. N Engl J Med 2006; 354: 2473-2483.*
20. *Lee HS, Ku SK. Effect of Picrorrhiza rhizoma extracts on early diabetic nephropathy in streptozotocin-induced diabetic rats. J Med Food 2008; 11: 294-301.*
21. *Fekete A, Rosta K, Wagner L, Prokai A, Degrell P, Ruzicska E, Vegh E, Toth M, Ronai K, Rusai K, et al. Na+K+-ATPase is modulated by angiotensin II in diabetic rat kidney – another reason for diabetic nephropathy? J Physiol 2008; 586: 5337-5348.*
22. *Thomas HY, Ford VAN. Pathophysiology of mesangial expansion in diabetic nephropathy: mesangial structure, glomerular biomechanics, and biochemical signaling and regulation. J Biol Eng 2022; 16: 19.*
23. *Collins KS, Eadon MT, Cheng YH, Barwinska D, Ferreira RM, McCarthy TW, Janosevic D, Syed F, Maier B, El-Achkar TM, et al. Alterations in protein translation and carboxylic acid catabolic processes in diabetic kidney disease. Cells 2022; 11: 1166.*
24. *Distler JHW, Györfi AH, Ramanujam M, Whitfield ML, Königsho M, Lafyatis R. Shared and distinct mechanisms of fibrosis. Nat Rev Rheumatol 2019; 15: 705-730.*
25. *Osterby R, Asplund J, Bangstad HJ, Nyberg G, Rudberg S, Viberti GC, Walker JD. Neovascularization at the vascular pole region in diabetic glomerulopathy. Nephrol Dial Transplant 1999; 14: 348-352.*