RESEARCH PAPER

Caffeic Acid Attenuates Streptozotocin-Nicotinamide-Induced Diabetic Nephropathy by Inhibiting Renal Angiotensin II-Signalling Pathways

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ABSTRACT

Diabetes mellitus (DM), a widespread metabolic disorder in India, poses a growing public health challenge, particularly due to its serious complications like diabetic nephropathy (DN). Among these, DN stands out as a leading cause of end-stage renal disease (ESRD), with no definitive cure or therapy currently available to halt or reverse its progression.

The current study aims to address the treatment of DN, focusing on the improvement of the diseased condition using caffeic acid (CA), a polyphenolic compound, known for its kidney-aiding properties associated with DM. Additionally, we attempted to determine the probable mechanism of action for these activities. DN was induced in Wistar rats using streptozotocin and nicotinamide, and CA was administered orally to the rats. The study included three experimental groups; the treatment lasted continuously up to 63 days, and CA reduced Ang II levels by 56 %, among other improvements. Biochemical and urine parameters, as well as the level of inflammatory markers and HbA1c, were then assessed. Histological changes were evaluated, and the estimation of the peptide Ang II was conducted using the ELISA technique.

CA improved biochemical and urine parameters and HbA1c and also mitigated morphological derangements in the histological structure of the nephron and its associated structures by reducing the levels of Ang II in the kidney.

In conclusion, the study revealed that CA has a preventive effect on chemically induced diabetic nephropathy by downregulating Ang II pathways and also may have the insulin-sparing effect.

Keywords: Caffeic acid, angiotensin II, oxidative stress, inflammation, diabetic nephropathy.

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1. INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic condition in which a persistently elevated blood glucose level develops as a result of inadequate insulin release, impaired insulin activity and effectiveness, or both. A number of long-term systemic complications can arise as a result of the metabolic and vascular abnormalities caused by persistent hyperglycemia. One of the most serious and prevalent microvascular complications among these is diabetic nephropathy (DN), which, if untreated, is a predominant cause underlying end-stage renal disease (ESRD). Nevertheless, diabetes also impacts other organ systems, resulting in cardiovascular problems like cardiomyopathy and atherosclerosis, as well as retinopathy and neuropathy, all of which raise morbidity and mortality.1 According to the International Diabetes Federation (IDF), diabetes mellitus (DM) has reached epidemic proportions globally, with cases rising from 108 million in 1980 to 463 million in 2019, and projections indicating an alarming increase in 2045 to 700 million.2 However, 90-95% of cases of type 2 diabetes are primarily due to insulin resistance, while Type 1 diabetes stems from autoimmune destruction of insulin-producing cells.3,4 Similarly, diabetes is the leading cause of ESRD, primarily due to structural and functional alterations in the kidney with alteration of the thickness of the glomerular basement membrane and mesangial matrix expansion, that's are collectively impair glomerular filtration rate.5 However, these pathological changes are driven by haemodynamic stress and hyperglycaemia,

which activate different intracellular signalling molecules like protein kinase C (PKC), mitogen-activated protein kinase (MAPK), as well as angiotensin (Ang), all of which contribute to the progression of DN.6 Moreover, the activation of angiotensin has been observed in both tubules and glomeruli of diabetic kidneys, contributing to immune cell recruitment and progression of renal damage.7 Studies suggest that Ang II is an important protein of the renin-angiotensin system (RAS), is activated through AT1 and AT2 receptors, further promoting inflammation via upregulation of pro-inflammatory cytokines and oxidative markers.8 So, this Ang-II driven pathway, particularly in the context of proteinuria, is closely associated with tubulointerstitial injury- a major predictor of renal dysfunction in DN.9 Recognizing these systemic impacts adds to the clinical significance of creating treatment plans that address the underlying oxidative and inflammatory processes that cause multi-organ damage in diabetes, in addition to renal damage. To explore these underlying mechanisms and potential treatments for DN, rat models have long served as a valuable experimental tool due to their close resemblance to human disease. A commonly employed approach involves inducing diabetes through intraperitoneal (*i.p.*) administration of streptozotocin (STZ-65 mg/kg), freshly prepared buffer solution (pH 4.5), administered 15 minutes after nicotinamide (NA) at 230 mg/kg.10 However, the STZ model is widely used to induce DN in animal models due to its selective toxicity to pancreatic β cells via GLUT2-mediated uptake, leading to insulin deficiency, hyperglycaemia, and elevated oxidative stress.11 To enhance β-cell specificity and reduce systemic toxicity, NA is often administered prior to creating a more stable and moderate diabetic state.

In recent years, natural compounds, particularly flavonoids and polyphenols (extracted from vegetables, fruits, and medicinal herbs), have received more attention for their antioxidant, anti-inflammatory, and hypoglycemic properties. Among these, caffeic acid (CA), a polyphenolic hydroxycinnamic acid derivatives present in various plant-based foods like apples, berries, carrots, and propolis have emerged as promising therapeutic agents.12 Additionally, CA exerts potential biological effects through modulation of several key signaling molecules and transcription factors, inducing proinflammatory cytokines, COX-2, HIF-1, and iNOS, all of which are implicated in inflammation and oxidative stress in DN. 13,14 However, its strong antioxidant and anti-inflammatory actions help to counteract the cellular damage induced by hyperglycemia.12 Additionally, CA also significantly decreases oxidative markers (MDA) and increases antioxidant markers like SOD and GSH in serum, liver, and kidney in diabetic rats.15 These observed outcomes demonstrate the therapeutic value of CA as a natural, accessible, and cost-effective agent for managing DN. The present work focuses on elucidating the renoprotective effects of CA and its role in the modulation of Ang II-mediated pathways in diabetic nephropathy by evaluating both biochemical parameters (BUN, serum creatinine, albumin) and inflammatory mediators (TNF-α, IL-6). These results could advance knowledge of the pharmacological underpinnings of using natural antioxidants to reduce the renal complications associated with diabetes. However, it is yet to be explored for its anti-nephropathic activity, especially its role in the RAAS system, which is a major contributor to fibrosis of the nephron, leading to its permanent damage and inflicting chronic kidney disease.

2. MATERIALS AND METHODS

2.1 Experimental animals

For this experiment, Wistar rats (180-220g; male: 14-16 weeks old) were acquired from the central animal house, and they were housed under standard laboratory conditions (room temperature: 22 ± 3 ºC; humidity 55 ± 6% and 12:12 hours light/dark cycle) and maintained on a pellet-based diet with food available ad libitum. For this study, male rats were included, as they exhibit greater sensitivity to the diabetogenic effect of STZ compared to females.

2.2. Informed Consent:

Experimental procedures involving animals were reviewed and received approval from the Institutional Animal Ethics Committee (IAEC), with approval number SETGOI/01/A/2024. These procedures also comply with the guidelines issued by the CCSEA (Committee for the Control and Supervision of Experiments on Animals) and the TR Dizin ethical requirements for preclinical research.

2.3. Drugs and Chemicals

STZ (extra pure, 98%, C8H15N3O7, M.W. 265.22) and nicotinamide were purchased from SRL for the induction of diabetes. Caffeic acid was obtained from Sigma-Aldrich, USA. For the determination of plasma and urine parameters, a creatinine, albumin, and urea kit (Accurex Biomedical Pvt. Ltd.) and the antibodies were acquired from Santa Cruz Pharmaceuticals, Mumbai. The ELISA kits were taken from Elabsciences Pvt. Ltd.

2*.4. Experimental protocol and treatment schedule*

Before starting the protocol, the animals were habituated for 10 days in laboratory conditions. Firstly, all 30 approved Wistar rats were divided into 3 groups; among them, 1st one was marked as the normal control group containing 10 rats, while 10 rats were in the 2nd group, namely diabetic group, in which overnight fasted rats were administered with a single dose of STZ at 65 mg/kg, *i.p*., that prepared in 0.1 mol fresh sodium citrate buffer (pH of 4.5), and NA (230 mg/kg) and the 3rd*p.o.),* Group II*:* STZ or disease group, received only STZ, and lastly Group III was the treatment group (received STZ+ CA-40

mg/kg/*p.o.)* for 2 months (Figure 01). At the end of 8 weeks, all animals were sacrificed, and biochemical and histological studies were performed.

3.3 Evaluation parameters

3.3.1 Body weight

Change in body weight was observed using a weighing machine, and the body weight of each animal was measured on the first and last day of the experiments between 9:30 and 10:30 a.m.

3.3.2. Determination of the kidney weight and kidney hypertrophy (%)

At the end of the experimental process, all kidneys were isolated, and individual kidney weights were taken in each group, divided by the respective animal's body weight, and multiplied by 100 to ensure the % change in the KW/BW ratio. Formula: Kidney weight (g)/ Body weight (g) × 100.

3.3.3 Blood glucose level

Fasting blood glucose levels (mg/dL) were measured by a glucometer (Dr Morepen Gluco One, New Delhi). Blood glucose levels were measured between 10:30 a.m. and 12:30 p.m.

3.3.4 Oral glucose tolerance test (OGTT)

For the OGTT, food was discontinued last night before test. On the day of the test, firstly, rats were administered 2 g/kg of glucose orally dissolved in saline. After that, the glucose levels in blood were checked by a glucometer from the tail at 0, 30, 60, and 120-minute intervals. 16

3.3.5 Urine albumin and creatinine

For assessment of the level of urine albumin and creatinine, rats were placed in a metabolic cage for 24 hours, and their urine was collected. The albumin and creatinine concentrations were then determined using the Liquid Gold albumin and creatinine kit, followed by the Bromocresol Green endpoint assay procedure. We combined the diluted urine with albumin reagent and albumin standard reagent, then evaluated the absorbance in a colorimeter.17

3.3.6 Assessment of HbA1c (%)

HbA1c levels were measured by MISPRA-i2 diagnostic kits, which rely on antigen–antibody interactions for direct HbA1c detection. Upon completion, a heart puncture was used to get 0.5 ml of blood into tubes coated with EDTA. An equal volume (0.5 ml) of hemolysis reagent was added, and 10 µl of the lysed blood was mixed thoroughly and incubated for 5 minutes. The samples were then analyzed using a protein analyzer, following the instructions provided in the kit.16

3.3.7. Serum albumin and creatinine

For assessment of serum albumin and creatinine levels, we used the Liquid Gold albumin and creatinine kit, followed by modified Jaffe's reaction, the initial rate assay method. Following the extraction of serum from animal tail veins, samples were combined with sodium hydroxide and picrate reagents and subjected to absorbance testing. 18

3.3.8 Blood urea nitrogen (BUN)

For measuring BUN levels in blood samples, the urea kit is followed by the Urease Berthelot endpoint assay method. Data was expressed as mg/dL.17

3.3.9. Assessment of insulin level

Insulin levels from the animals in each group were measured using a solid-phase enzyme-linked immunosorbent assay (ELISA) with a commercially available kit from Monobind Inc., USA. Calibrators, controls, and samples were incubated with enzyme-labeled monoclonal anti-insulin antibodies in streptavidin-coated microplate wells. After washing, tetramethylbenzidine (TMB) substrate was added to develop color, and the reaction was stopped with 1N HCl. The absorbance was measured at 450 nm using a microplate reader, and insulin concentrations were expressed in µU/mL based on the standard calibration curve.

3.3.10. Animal dissection and tissue preparation

The kidneys were separated and cleansed with ice-cold water after the animals were killed by cervical dislocation on day 63 of the regimen. After weighing each sample, the tissue was separated for histopathology and to estimate the levels of different molecular markers. Kidney tissue samples were homogenized in ice-cold phosphate-buffered saline (50 mM, pH around 7.4) and subsequently centrifuged at 10,000 rpm (15 mins). The resulting supernatant was collected for biochemical assays.

3.3.11. Determination of malonaldehyde (MDA)

MDA is used as a marker of lipid peroxidation (LPO), as determined by the method of Wills (1966). The absorbance (532 nm) of the supernatant was measured.19

3.3.12 Determination of superoxide dismutase (SOD)

SOD enzyme's activity is measured by using nitro blue tetrazolium (NBT), followed by the Del-Maestro technique. The ability of SOD to scavenge superoxide anion radicals (O2) reduces the pyrogallol autooxidation. For the SOD assay, 2 mL of NBT solution was combined with 0.5 mL of hydroxylamine hydrochloride, followed by the addition of 0.1 mL of PMS. The reaction mixture was then illuminated with white light (15 mins) at room temperature to initiate the enzymatic reaction, after which the absorbance was measured at 560 nm.20

3.3.13. Determination of reduced glutathione (GSH)

GSH levels were estimated using the Ellman (1959) method. Briefly, 1 mL of the sample was mixed with 1 mL of 4% sulfosalicylic acid and kept at 4 °C for 1 hour. The mixture was then centrifuged at 1200 g (15 min, 4

°C), after which 0.1 mL of the supernatant was collected and diluted with 2.7 mL of PBS (0.1 M) at pH 8.0 and 0.2 mL of DTNB reagent (5,5-dithiobis (2-nitrobenzoic acid) (0.01 M). The resulting yellow color developed in the solution, and absorbance was recorded spectrophotometrically (412 nm).21

3.3.14. Estimation of inflammatory markers

Inflammatory markers (TNF-α and IL-1β) levels in the kidney tissue sample were measured by using ELISA kits (Elabsciences, Texas, USA). Inflammatory biomarkers were quantified using a solid-phase sandwich ELISA (BIO-RAD, iMARK model). Absorbance was read at 450 nm on a microplate reader, and concentrations were calculated from standard calibration curves.22

3.3.15. Estimation of Serum Angiotensin II Level

For determining serum Ang-II level, we used an ELISA kit (Elabsciences Pvt. Ltd, CAS no: E-EL-R1430), in which tissue homogenization of the coded tissue (blinding process) from each of the 3 groups was used in ELISA plate readers for the estimation of serum. The Ang-II biomarkers were detected using a solid-phase sandwich ELISA, in which the plates were pre-coated with antibodies specific for rat Ang II, and these samples were used to analyze protein expression. Biotinylated antibodies specific to rat Ang II and an avidin-horseradish peroxidase (HRP) conjugate were sequentially added to the microplate wells and incubated at 37°C. Following a wash step, the substrate solution was introduced, and the optical density was recorded (450 nm). 21,23

3.4 Histopathological assessment

Following animal sacrifice, kidney samples were meticulously separated and preserved in 10% neutral-buffered formalin. Following tissue fixation, they proceeded to tissue processing using various ethanol concentrations. Tissues were prepared and embedded in paraffin before being stained with hematoxylin and eosin. A microtome was used to cut paraffin sections to a thickness of 3–4 micrometers. Hematoxylin and eosin-stained slices were examined microscopically at 40X magnification to assess morphology and structural changes and then examined using a microscope. Throughout the process, histopathologists remained blinded to the samples, which were coded to eliminate any psychological bias. Once everything was finished, ImageJ was used to analyze the photos under a microscope.17

3.5. Statistical Analysis

All data were presented as means ± standard error of the mean (SEMs), in which statistical analyses were performed using GraphPad Prism 9.0 software (GraphPad Software, Inc., USA). Statistical comparisons among groups were performed using one-way ANOVA for single-time-point comparisons among multiple groups (e.g., body weight, insulin, and biochemical parameters), followed by Tukey's multiple comparison post-hoc test for pairwise group comparisons. Two-way ANOVA was applied for parameters measured across time and treatment (e.g., OGTT and blood glucose levels), with time and treatment as independent factors, followed by Bonferroni's post-hoc test to determine intergroup differences at specific time points. A probability value of $P < 0.05$ was regarded as statistically significant.

4. RESULTS

4.1 Effect of the CA on body weight

Following the conclusion of the experiment, the body weight of the diabetic rats was dramatically reduced by the administration of STZ-NA compared to the normal group ($P<0.05$), and it was greatly recovered by the administration of CA (40 mg/kg) compared to the diabetic-induced group ($P<0.001$). (Figure 02).

4.2 Effect of the CA on Kidney weight and percentage of kidney hypertrophy

When compared to the normal group, the administration of STZ-NA significantly reduced the kidney weight and kidney hypertrophy (%) of diabetic rats ($P<0.05$), while the administration of CA (40 mg/kg) significantly restored these parameters when compared to the diabetic-induced group ($P<0.001$). (Table 01).

4.3 Effect of CA on blood glucose level

After inducing T2DM in animals with STZ and NA, the fasting blood glucose levels (FBG) of the rats were measured at the end of the study. Compared with the normal group, the FBG level was increased significantly in the diabetic group of rats ($P<0.05$). CA treatment group animals significantly restored the FBG level as compared to the diabetic-induced group ($P < 0.001$) (Figure 03 (a)).

4.4 Effect of CA on OGTT

At the end of the experiment, the OGTT of diabetic rats was significantly lower ($P< 0.001$) after receiving CA (40 mg/kg) than in the diabetic-induced group, and it was significantly higher after administering STZ-NA than in the normal group ($P<0.05$). (Figure 03 (b)).

4.5 Effect of CA on urine albumin and creatinine

Following the conclusion of the experiment, CA (40 mg/kg) treatment significantly reduced urine albumin but increased urine creatinine levels in comparison to the diabetic-induced group ($P<0.001$), and STZ-NA administration significantly increased urine albumin but decreased urine creatinine levels in diabetic rats compared to the normal group ($P<0.05$). (Figure 04 (a and b)). Also, figure 04 (c) described the urine albumin/creatinine ratio, which was increased in the diabetic group as compared to the normal control group ($P<0.05$), and decreased in the CA-treated group as compared to the diabetic group.

4.6 Effect of CA on HbA1c (%), serum albumin, creatinine, and BUN

When compared to the normal group, the administration of STZ-NA significantly raised HbA1c (%), serum creatinine, and BUN but significantly decreased serum albumin levels in diabetic rats (P<0.05). Similarly, the administration of CA (40 mg/kg) significantly decreased HbA1c (%), serum creatinine, and BUN but significantly increased serum albumin levels compared to the diabetic-induced group (P<0.001). (Table 02).

4.7 Effect of CA on SOD, GSH, and MDA

At the end of the experiment, the diabetic rats given STZ-NA had significantly higher MDA levels and lower SOD and GSH levels compared to the normal group (P<0.05), whereas the diabetic-induced group received significantly lower MDA levels and higher SOD and GSH levels compared to the CA (40 mg/kg) group (P<0.001). (Figure 05).

4.8. Effect of CA on inflammatory markers (TNF-α and IL-1β)

Administration of STZ-NA significantly increased TNF-α and IL-1β levels of diabetic rats as compared to the normal group (P < 0.05), and CA (40 mg/kg) treatment significantly decreased TNF-α (70%) and IL-1β levels (67%) as compared to the diabetic-induced group (P< 0.001) (Figure 06, (a)).

4.9. Effect of CA on serum angiotensin II levels

The injection of STZ-NA considerably raised the Ang II levels of diabetic rats compared to the normal group (P<0.05) after the experimental procedure was finished, while the administration of CA (40 mg/kg) dramatically lowered the Ang II levels compared to the diabetic-induced group (P<0.001). (Figure 06, (b)).

4.10 Effect of CA on kidney morphology

Hematoxylin and eosin were used to stain the kidney tissue of rats in each group. After completion of the experimental protocol, administration of STZ-NA in the induced group, the glomerular diameter was significantly less and had higher tubular collapse and thyroidization than in the control group. Rats treated with CA had fewer globally sclerosed glomeruli, a smaller decrease in glomerular diameter, and less tubular collapse and thyroidization in their kidneys compared to rats in the induced group (Figure 07).

Table 01: Effect of the CA on Kidney weight and percentage of kidney hypertrophy

Groups	Normal control group	Diseased group (STZ+NA)	Treatment group (CA 40 mg/kg/*p.o.*)
Kidney weight (g) **Mean ± S.E.M.**	1.24±0.03	1.51±0.07*	1.31±0.05#
Kidney hypertrophy (kidney weight/Body weight) %	0.52±0.01	0.96±0.04*	0.70±0.03#

Effects of CA on the Kidney weight and percentage of kidney hypertrophy of the diabetic induced animals. Data are shown as mean ±SEM: *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased group. One-way ANOVA followed by Tukey's post hoc test.

Table 02: Effect of CA on HbA1c (%), serum albumin, creatinine, and BUN

Groups	Normal control group	Diseased group (STZ+NA)	T Treatment group (CA 40 mg/kg p.o.)
HbA1c (%)	4.73±0.87	12.24±1.14*	8.15±0.96#
Serum albumin (mg/dL)	4.42±0.13	3.15±0.17*	4.02±0.14#
Serum creatinine (mg/dL)	0.51±0.07	1.82±0.14*	0.96±0.21#
BUN (mg/dL)	15.61±2.81	34.61±3.14*	19.25±3.05#
Creatinine Clearance (mL/min)	3.15 ± 0.46	0.44 ± 0.05*	1.41 ± 0.32#

Effects of CA on the serum: (a) HbA1c%, (b) serum albumin, (c) serum creatinine, and (d) BUN of the animals induced with diabetes. Data are shown as mean ± SEM. *p<0.05, significantly different from the normal control; #p < 0.001, significantly different from the diseased group. One-way ANOVA followed by Tukey's post hoc test.

4.11 Effect of CA on insulin level

After inducing T2DM in animals with STZ and NA, the insulin levels of the rats were measured at the end of the study. Compared with the normal group, the insulin level was decreased significantly in the diabetic group of rats (P<0.05). CA treatment group animals significantly restored the insulin level as compared to the diabetic induced group (P< 0.001) (Figure 08).

5. DISCUSSION

Naturally derived compounds possess diverse and unique structures, frequently engage with various biological targets, and have historically played a crucial role in the development of new drugs. In our current study, we evaluated the nephroprotective effect of the CA in STZ-NA-induced diabetic nephropathy rats. Diabetes associated with kidney dysfunction involves multifactorial pathophysiological mechanisms, including chronic hyperglycemia, oxidative stress, inflammation, and Ang-II.24 It is well evident from the results that the CA has increased body weight and improved glucose tolerance in the treatment group of rats compared to the induced group. Additionally, the drug also improved the antioxidant markers (SOD, GSH, and MDA) as well as decreased biochemical markers related to diabetic nephropathy, viz, urine and serum albumin, and creatinine and BUN. CA also improved the histopathological parameters, such as globally sclerosed glomeruli, glomerular collapse, and thyroidization of the renal tubules. Lastly, the extract was also found to reduce the serum angiotensin II levels in kidney tissues compared to the diabetic-induced group.

Angiotensin plays a pivotal role in the pathogenesis of DN by activating the RAAS signaling pathway, which in turn upregulates pro-inflammatory cytokines like IL-6, TNF-α, and MCP-1.25 This cascade contributes to glomerular inflammation, fibrosis, and tubular damage. In DN models, CA, a naturally occurring hydroxycinnamic acid derivative present in various plants, fruits, and drinks, including wine and coffee, has shown encouraging renoprotective properties. Modification of the RAS system, namely the inhibition of Ang II production and signaling, seems to be its mechanism. A strong vasoconstrictor, Ang II, is essential for glomerular hypertension, oxidative stress, inflammation, and kidney fibrosis. CA successfully lowers renal inflammation and fibrotic remodelling by probably reducing Ang II levels by 56 %, which enhances kidney function and histological integrity in general. This mechanism is somewhat conceptually similar to that of traditional RAS inhibitors, including Ang II receptor blockers (ARBs) like losartan and valsartan, and Angiotensin-converting enzyme inhibitors (including captopril and enalapril), however further studies are required to study the impact of the drug's ability to alter the levels of the downstream effector molecules of the RAS pathway. These well-established medications, like captopril and enalapril, lower glomerular pressure and lessen renal fibrosis by either inhibiting Ang- I's conversion to Ang- II or preventing it from binding to AT_1 receptors. Summarizing, CA has additional antioxidant and anti-inflammatory properties in addition to being mechanistically aligned with conventional RAS inhibitors due to its capacity to modulate Ang II levels and signaling. To identify its exact molecular targets within the RAS and assess its possible synergism with ACE inhibitors or ARBs in combination therapies, more mechanistic and clinical research is necessary.

6. CONCLUSION

CA reduced circulating Ang II levels and was associated with improved renal parameters, suggesting possible partial involvement of RAS modulation in diabetic nephropathy. In conclusion, our results suggest that CA treatment may improve diabetic nephropathy in rats induced by STZ-NA, potentially by altering Ang II-mediated pathways. Although these findings are promising, more thorough research is necessary to validate the underlying mechanisms and determine CA's therapeutic potential in diabetic renal complications. Treatment also attenuated inflammation and oxidative stress, in addition to an anti-diabetic effect. However, further studies are still needed to identify the Ang II-coupled pathological pathways, which could provide more insightful outcomes related to the pathological prevention of CA in diabetic nephropathy.

Abbreviation

CA: Caffeic acid; CCSEA: Committee for Control and Supervision of Experiments on Animals; DM: Diabetes mellitus; DN: Diabetic nephropathy; ESRD: End-stage renal disease; GSH: Reduced glutathione; IAEC: Institutional Animal Ethics Committee; IDF: International diabetes federation; IL: Interleukin; MDA: Malonaldehyde; NA: Nicotinamide; OGTT: Oral glucose tolerance test; SOD: Superoxide dismutase; STZ: Streptozotocin; TNF: Tumor necrosis factor.

Figure legends:

Figure 01:

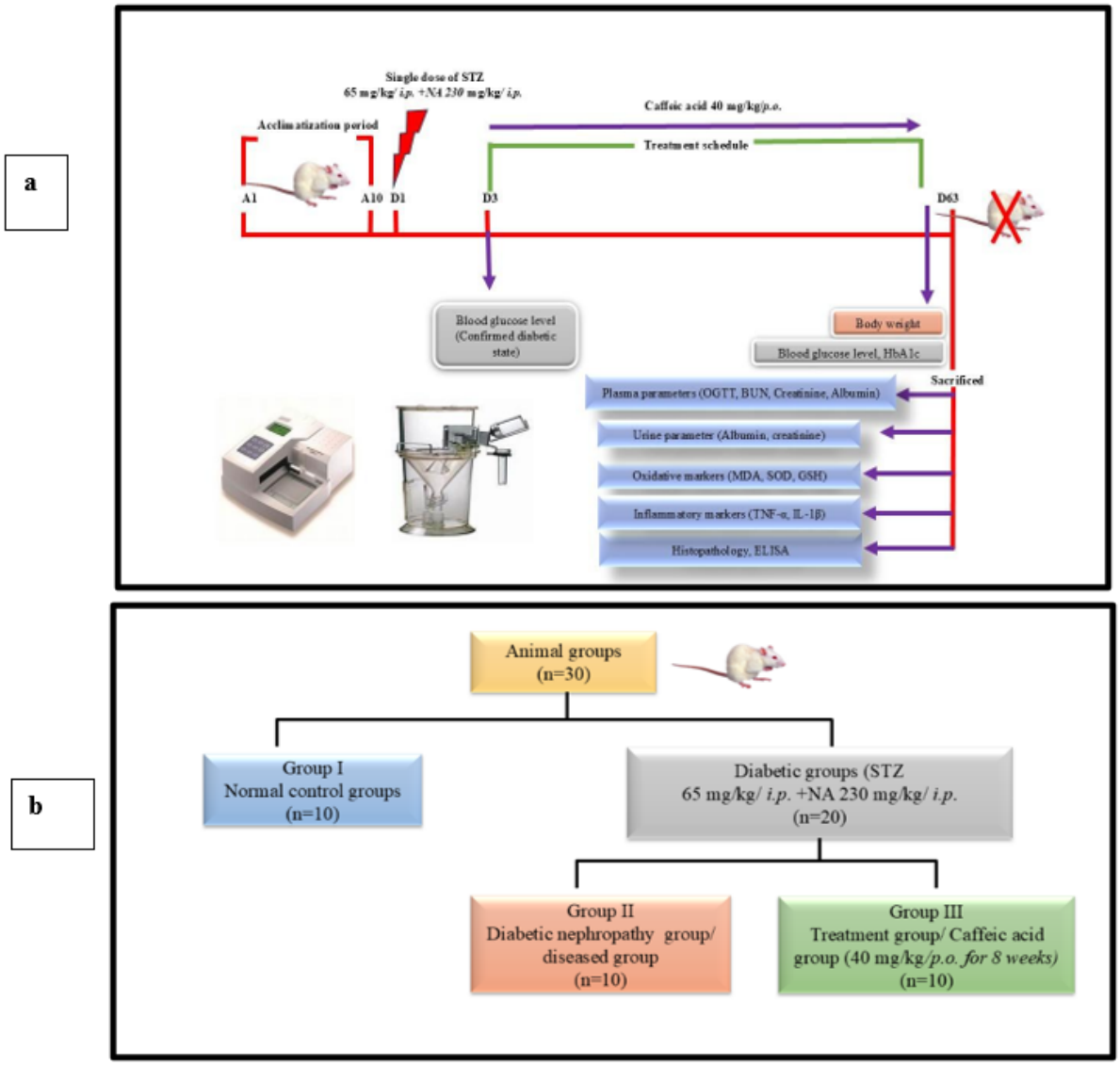


Figure 01: (a) Experimental design, & (b) grouping of the study.

Figure 02:

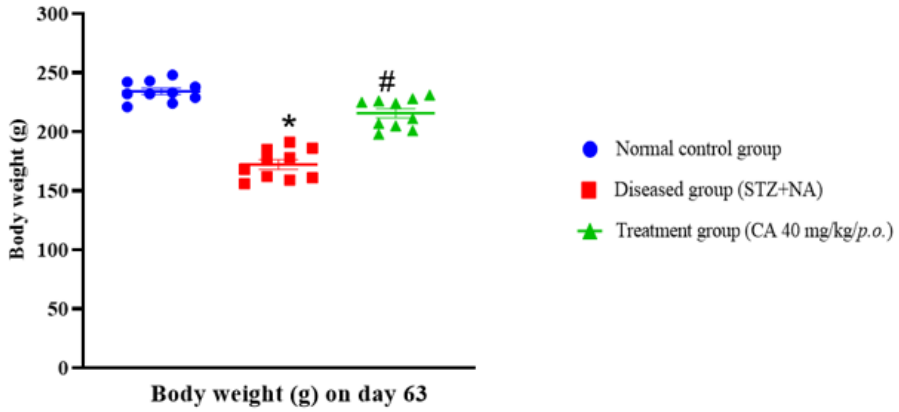
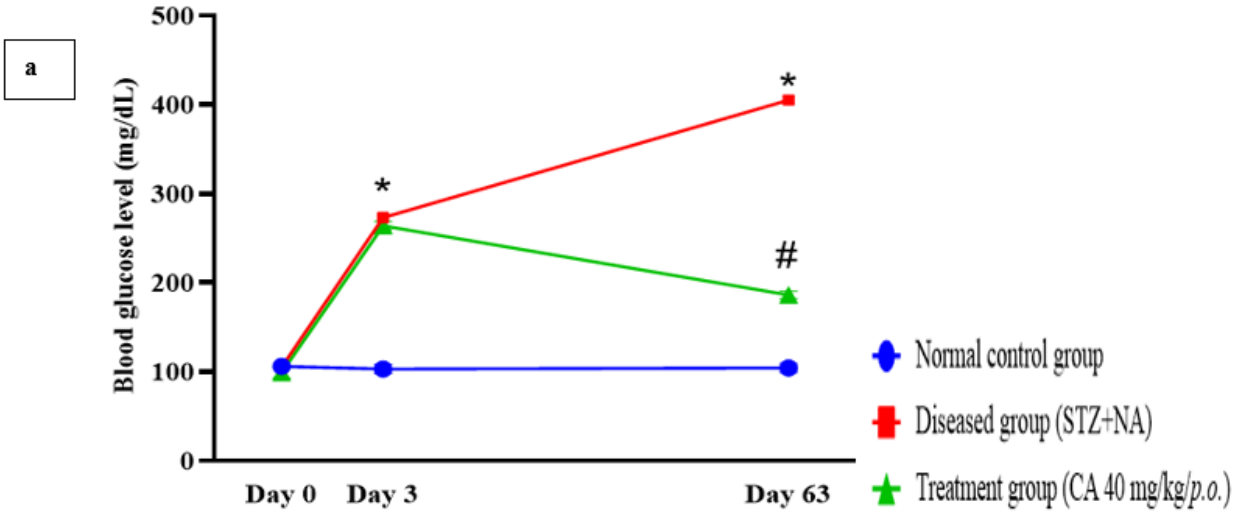


Figure 02: Effects of CA on the body weight (g) of the diabetic induced animals. Data were expressed as mean ± standard error of mean (SEM). *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased (STZ+NA) group: One-way ANOVA followed by Tukey's post hoc test.

Figure 03:



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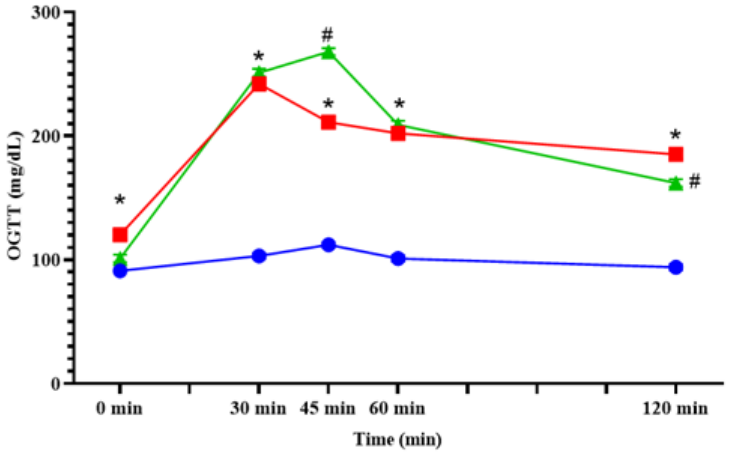
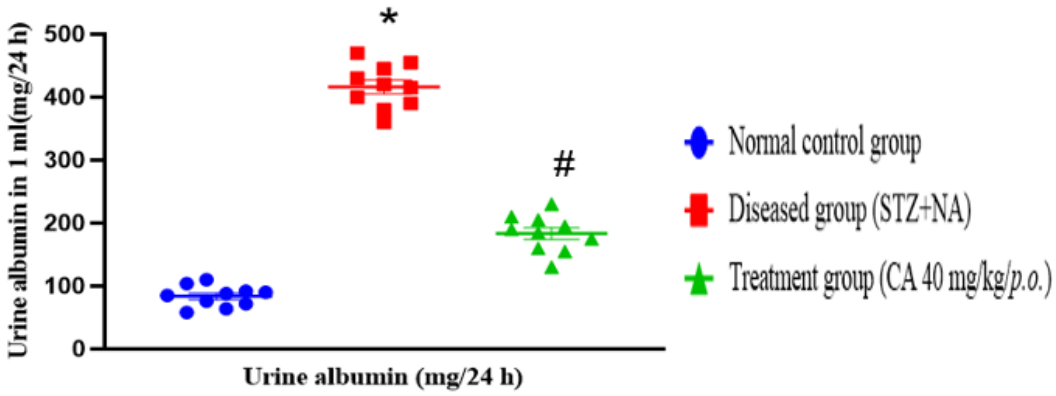


Figure 03: Effects of CA on the (a) fasting blood glucose level (mg/dL), (b) OGTT of the diabetic induced animals. Data were expressed as mean ± standard error of mean (SEM). *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased (STZ+NA) group: Two-way ANOVA followed by Bonferroni test.

Figure 04:

a



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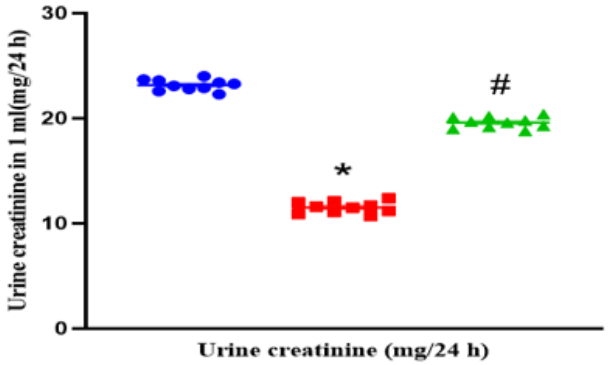

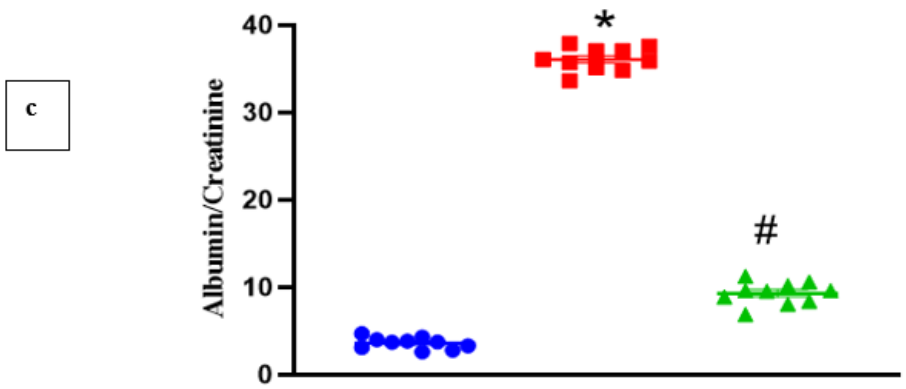

Figure 04: Effects of CA on the (a) urine albumin, (b) urine creatinine (c) urine albumin/creatinine ratio of the diabetic induced animals. Data were expressed as mean ± standard error of mean (SEM). *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased (STZ+NA) group: One-way ANOVA followed by Tukey's post hoc test.

Figure 05:

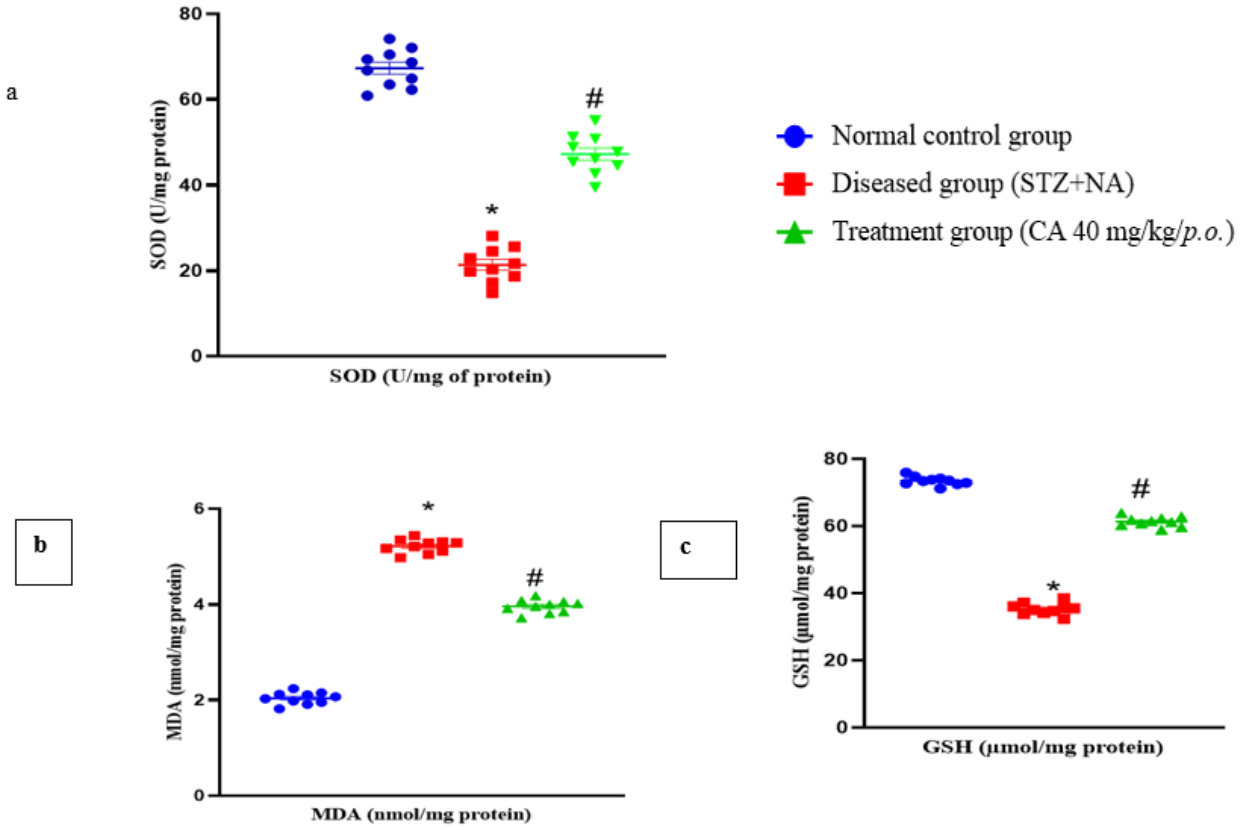

Figure 05: Effects of CA on the tissue (a) SOD, (b) MDA, (c) GSH of the diabetic induced animals. Data were expressed as mean ± standard error of mean (SEM). *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased (STZ+NA) group: One-way ANOVA followed by Tukey's post hoc test.

Figure 06:

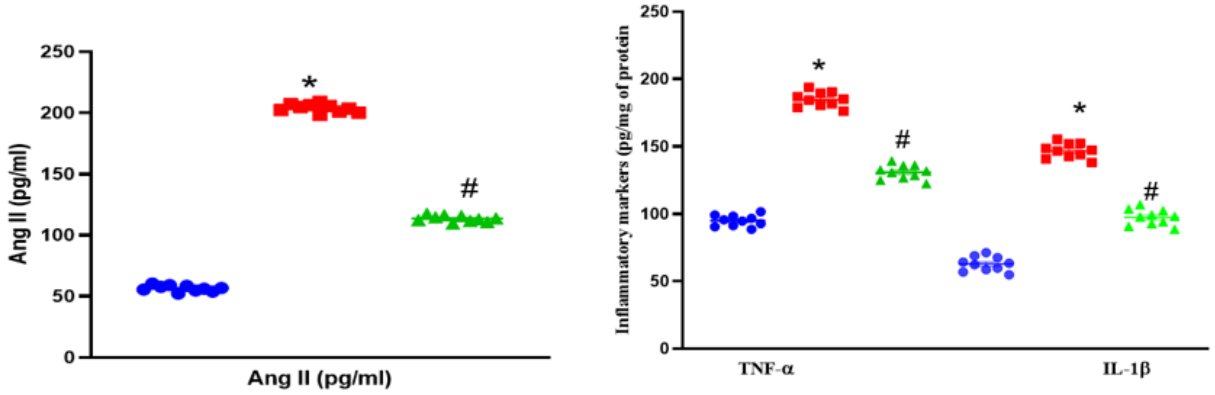

Figure 06: Effects of CA on the (a) inflammatory markers (TNF-α and IL-1β), (b) serum Ang II levels of the diabetic induced animals. Data were expressed as mean ± standard error of mean (SEM). *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased (STZ+NA) group: One-way ANOVA followed by Tukey's post hoc test.

Figure 07:

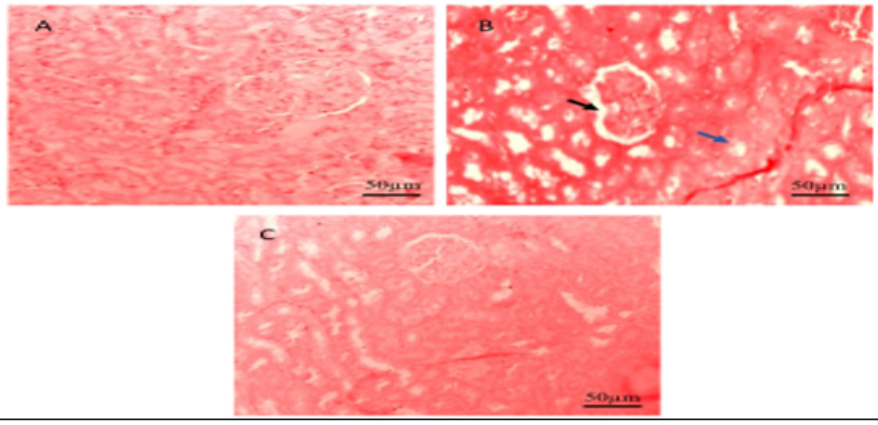

Figure 07: Effects of CA on the histopathological changes of the kidney (A) normal control group, (B) Diabetes group, (C) CA-treated group, in the diabetic induced animals, in which the black arrow denotes glomerular sclerosis and the blue arrow is marked as tubular collapse.

Figure 08:

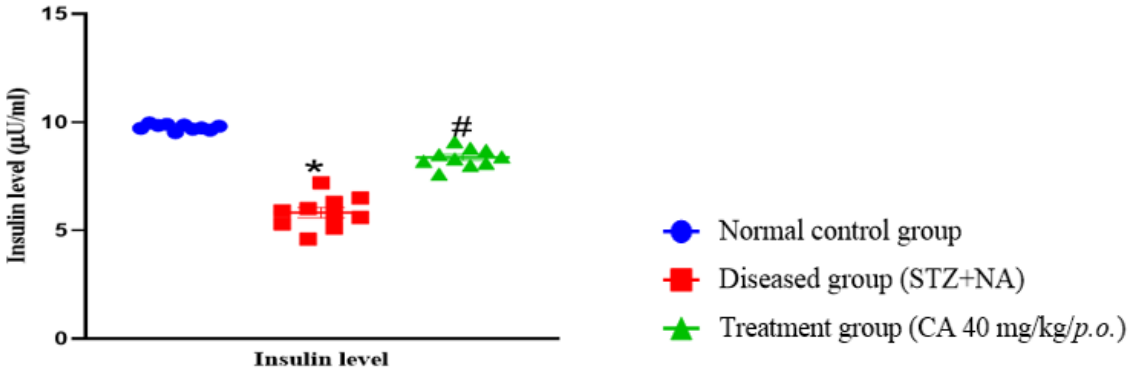

Figure 08: Effects of CA on the insulin level (µU/ml) of the diabetic induced animals. Data were expressed as mean ± standard error of mean (SEM). *p<0.05, significantly different from the normal control group, #p <0.001, significantly different from the diseased (STZ+NA) group: One-way ANOVA followed by Tukey's post hoc test.

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