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Original Article

Conessine Attenuates Diabetic Nephropathy in Rats through Inhibition of Hyperglycemia-Induced Oxidative Stress, Inflammation and Apoptosis

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ABSTRACT

Background: One type of chronic kidney disease is diabetic nephropathy. One of the main causes of end-stage renal disease and chronic kidney disease is diabetic nephropathy. The objective of this study was to assess the effects of treating diabetic nephropathy with conessine extract on male wistar rats with diabetes that had been triggered by streptozotocin. **Materials and Methods:** Twenty-four rats were split up into four groups. The regular diet was fed to the negative control animals in the first group. After receiving a single intravenous injection of streptozotocin to cause diabetes, the remaining 18 rats were split equally into three groups: the diabetic control group was placed in group 2, the third group received oral treatment with 20 mg/kg of conessine, and the fourth group received oral treatment with 5 mg/kg of gliclazide. **Results:** In comparison to the negative control, the rats in the second group had higher glucose and lipid peroxide levels and lower SOD, CAT, GR, GPx, and GSH activity. Diabetes also led to an increase in immunoglobulins, interleukin-6, and carboxymethyl lysine. Potassium and sodium levels were lowered, while kidney function metrics were also raised. Renal tissues also displayed significant histological alterations. **Conclusion:** Conessine treatments, administered to the diabetic rats in the third improved all altered biochemical and pathological tests that were getting closer to the negative control.

Keywords: Conessine, Diabetic nephropathy, Streptozotocin, Antioxidants.

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INTRODUCTION

The most prevalent endocrine condition, Diabetes Mellitus (DM), affects about 100 million individuals globally (6% of the population). It is brought on by the pancreas' inability or lack of ability to produce enough insulin, which causes variations in blood glucose levels. Numerous bodily systems are shown to be harmed by it, specifically the blood vessels, eyes, kidneys, heart, and nerves.[1] Type I insulin-dependent diabetes and Type II non-insulin-dependent diabetes are the two distinct forms of diabetes mellitus.[1] Due to changes in lifestyle and socioeconomic growth, the global prevalence of diabetes has increased dramatically in recent decades.[2]

Diabetic Nephropathy (DN) is a prevalent cause of End-Stage Renal Disease (ESRD) and one of the main significant microvascular consequences of Diabetes Mellitus (DM). According to data, around 40% of DM patients have variable stages of DN. At this point, many metabolic problems are regarded to be the root of DN, which is a chronic inflammatory illness. As a result, inflammations is essential to the onset and course of DN.[3] Numerous consequences of diabetes, including DN, are linked to oxidative stress generated by hyperglycemia. A substantial amount of research demonstrated the interaction between oxidative stress and the inflammatory response in the development of DN. Prolonged hyperglycemia induces oxidative stress and generates significant Reactive Oxygen Species (ROS) in renal tissues. This, in turn, activates the nuclear transcription factor NF-κB, resulting in renal inflammation.[4]

Experimental investigations relying on the pathogenic factors of DN have led to the development of many unique treatment possibilities, such as rigorous glycaemic control, accurate blood pressure control, optimal RAAS blockade with ACEI/ARB, dietary restrictions and exercise, and a host of novel agents. However, despite the prevalent use of these therapeutic methods that target the management of the aforementioned factors, the

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proportion of ESRD caused by DN remains high. Medications that could successfully stop the progression of DN are therefore desperately needed.[5]

Before the development of modern medicine, people relied on herbs all throughout the world to treat a variety of illnesses. Many plants have been utilized without any understanding of their correct ingredients or activities as dietary adjuvants for a variety of disorders.[6] Herbal medications and their active compounds have been shown in multiple research studies to have anti-diabetic activities with lower toxicity and side effects. Alkaloids possess antibacterial, antihyperglycemic, antimalarial, and anticancer properties. Many of these have been used in drug discovery processes for both conventional and contemporary medicine.[7]

Conessine is a steroidal alkaloid compound isolated from Holarrhena antidysenterica. As an ethnobotanical plant, Holarrhena antidysenterica, a member of the Apocynaceae family, has been used to cure bacterial infections, fever, diarrhoea, and dysentery. There is evidence of the plant's biological actions, which include anti-urolithic, anti-malarial, anti-diabetic, CNS-stimulating, acetylcholinesterase inhibitory, anti-mutagenic, and anti-oxidant properties.[8]

The primary goal of the current study is to evaluate Conessine's effectiveness against STZ-provoked diabetes in Wistar rats by examining the biochemical and histological markers. The impact of conessine on levels of fasting blood glucose, body weight, insulin, antioxidants and pro-inflammatory markers, kidney functional markers, electrolytes, immunoglobulins, carboxymethyl lysine, and apoptosis levels in untreated and treated rats was examined.

MATERIALS AND METHODS

Materials

Conessine, gliclazide, and Streptozotocin (STZ) were purchased from Sigma-Aldrich in the United States. All of the other necessary chemicals and reagents that were acquired were of a high analytical category.

Animal housing and dietary treatment

After obtaining approval from the Institutional Animal Ethical Committee, we purchased mature, healthy Male Wistar rats weighing 220±50 g from the Animal Facility. Within a meticulously maintained laboratory environment, the rats were acclimated by regulating the temperature at 25°C, maintaining a relative humidity of 55% for duration of one week, and ensuring a 12-hr light-dark cycle. After acclimation, the rats were provided with standard rat food and access to fresh water at all times. The bedding was replaced daily, while the rat cages underwent replacement every three days. All experimental methodologies employed in the present study received approval from the ethics committee, and the rats were handled with the utmost diligence and care.

Diabetes induction

Streptozotocin (STZ) was used to induce diabetes in the experimental animals. They received an intraperitoneal injection of 45 mg/kg of STZ in a 0.1M citrate buffer with pH 4.4. After five days of STZ treatment, the blood glucose level was measured to confirm the start of diabetes.[9]

Experimental design and sampling

Four groups of six rats each were used in the study. Groups were randomly split. The first group of rats was the control group, which received an intraperitoneal injection of about 0.5% of DMSO. The diabetic-triggered group followed in second, with the rats receiving an intraperitoneal injection of the medication STZ at a dose of 45 mg/kg. The third group received Conessine orally (20 mg/kg b.wt) after being given a diabetic induction drug for duration of 8 weeks. The fourth group received gliclazide orally (5 mg/kg b.wt) after being given a diabetic induction drug for duration of 8 weeks. Following the conclusion of the course of therapy, the rats were sacrificed, their blood was obtained for biochemical analysis, and their kidney tissues were obtained for histological research.

Estimation of fasting blood glucose, body weight and HbA_{1c} of the animals

On the first and last day of the experiment, The Body Weight (BW) of the experimental animals was measured and compared across all experimental groups. After the drug was administered, blood samples were taken while fasting. The rats fasted for the whole night before the Fasting Blood Glucose (FBG) was measured. Using the glucose measurement kit, precise orbital sinus blood samples were utilized to quantify glucose levels. Using an insulin ELISA kit purchased from Thermo Fisher Scientific in the USA, the levels of serum insulin hormone and glycosylated hemoglobin were measured.

Estimation of kidney function markers of the animals

An enzymatic colorimetric kit was used to estimate the levels of urea, creatinine, and uric acid in the serum using the methods outlined by[10-12] respectively. Using a commercial kit and the technique of[10] the concentration of creatinine in urine samples was measured. Albumin was quantified in urine by ELISA method, as per,[13] using a commercially available kit.

Estimation of electrolyte in the serum of animals

Using commercially available enzymatic kits, the amounts of Sodium (Na+) and potassium (K+) in the serum were determined in accordance with[14,15] respectively.

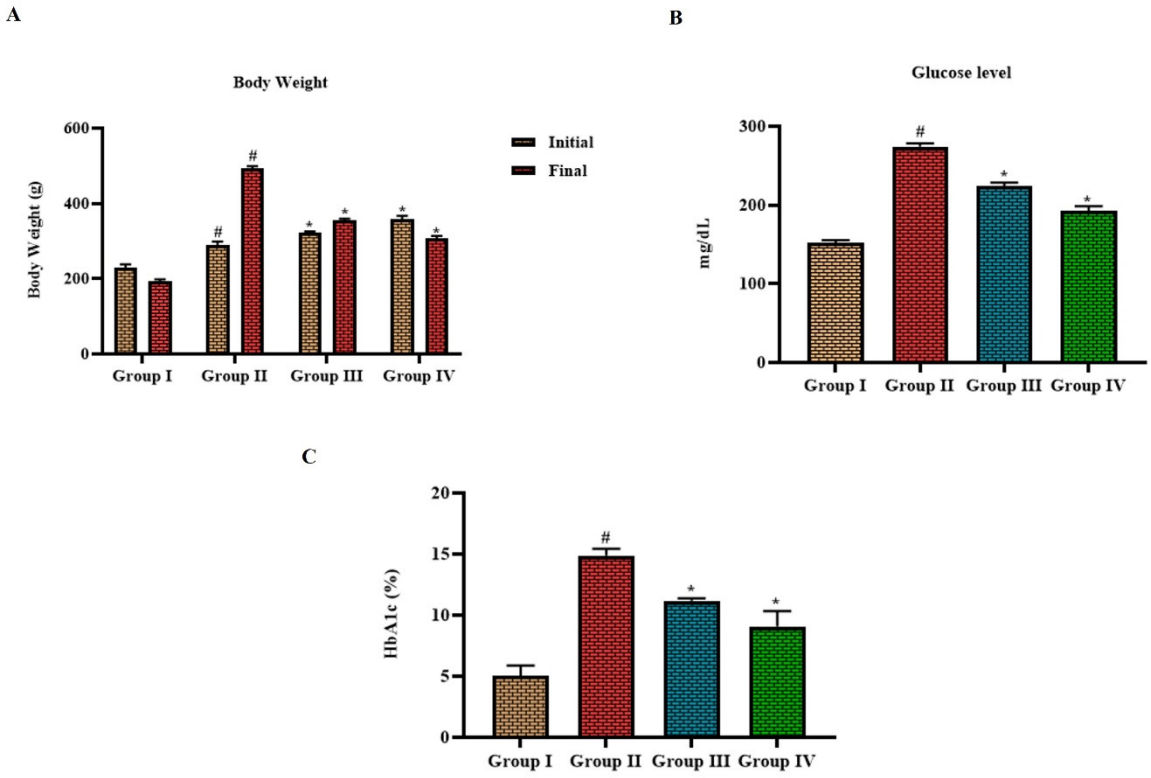

Figure 1: Effect of Conessine on body weight, blood glucose, and Glycosylated haemoglobin levels in diabetic rats.

Data are depicted as mean±SD of triplicate assays. Utilizing Graphpad Prism 3.0, statistical analysis was performed. Dunnett's t-test was used to compare data groups after an analysis of one-way ANOVA. '#' denotes that values are significant at $p<0.01$; '*' denotes that values are significant at $p<0.05$.

Estimation of immunoglobins of the animals

A commercial kit was utilized to measure immunoglobulins (IgA, IgM, and IgG) in the serum in accordance with.[16,17]

Estimation of carboxymethyl lysine level of the animals

Employing a commercial accessible kit, the amount of Carboxymethyl Lysine (CML) in the serum was determined using the protocol outlined by.[18] This kit incorporates the use of the Double Antibody Sandwich Method.

Estimation of Antioxidant enzymes

The amount of the antioxidant biomarkers, such as SOD, CAT, and GPx, were measured calorimetrically using kits that were marketed commercially and by implementing the manufacturer's instructions.

Estimation of MDA

Using the technique outlined by,[19] the amount of Malondialdehyde (MDA) in the serum and kidney tissue homogenate was used to quantify the amount of lipid peroxide utilizing kits.

Estimation of Glutathione Reductase (GR)

Employing a commercial accessible kit, the amount of Glutathione Reductase (GR) was determined using the protocol outlined previously.[20]

Estimation of GSH and GSSG

The amounts of reduced Glutathione (GSH) and oxidized Glutathione (GSSG) were determined fluorometrically using o-phthalaldehyde, according to method outlined by.[21]

Estimation of inflammatory markers

The levels of pro-inflammatory markers including Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), and Interleukin-1 (IL-1β) in each group were measured using a commercial ELISA kit that was acquired from market. The experiment followed the instructions given by the manufacturer. The experiment was done in triplicates, and the absorbance at 450 nm was determined. The standard curve with established standard concentrations was used to compute the final results.

Estimation of cas-3, cyt c, and VGEF

Cytochrome c and caspase-3 were estimated in the kidney homogenate using ELISA kits following the manufacturer's

instructions. The estimation of VEGF in the serum tissues of untreated and treated animals was carried out by assay kits by following the manufacturer's instructions.

Histopathological architecture

Following animal sacrifice, kidney tissues were preserved in saline, fixed in 10% formalin, processed regularly, and then embedded in paraffin. Hematoxylin and Eosin (H&E) dye was used to generate 5 µm thick slices for microscopic investigation. The stained sections were seen and captured on camera using a light microscope that was outfitted with a digital camera.

Statistical analysis

Utilizing Graphpad Prism 3.0, statistical analysis was performed. The expression of all the data was mean±SD. Dunnett's t test was used to compare data groups after an Analysis of Variance (ANOVA). When $p<0.01$, values were deemed statistically significant.

RESULTS

Effect of Conessine on body weight, blood glucose, and Glycosylated haemoglobin levels in diabetic rats

Figure 1 depicts the effect of conessine on body weight, blood glucose, and glycosylated haemoglobin level in diabetic rats. The current study's findings show that, as compared to the negative control rats (Group I), the diabetic control rats (Group II) had considerably lower body weight, and higher blood glucose and glycosylated haemoglobin levels, respectively. Results also indicate that the rats treated with conessine (group III) increased body weight, and reduced level of blood glucose and glycosylated haemoglobin in comparison to the obese diabetic control rats (group II). The results obtained in group III were comparable with animals treated with gliclazide (group IV).

Effect of Conessine on kidney function markers and electrolytes in diabetic rats

As a result of the induced diabetes illustrated in Figure 2a, the mean values of urea, creatinine, and uric acid in the blood of the diabetic control rats (Group II) were considerably higher than those of the negative control group (Group I). In comparison to group II, the conessine-treated rats (group III) had lower

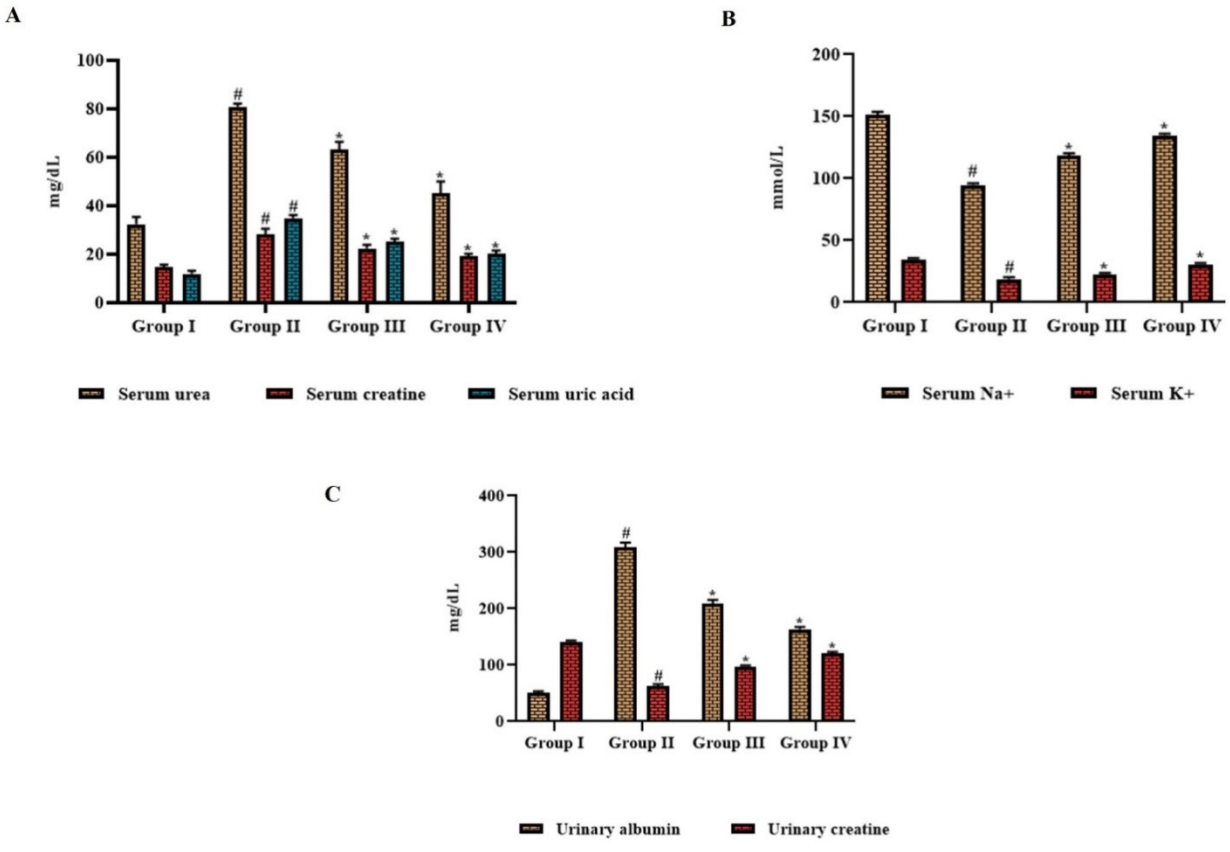

Figure 2: Effect of Conessine on kidney function markers and electrolytes in diabetic rats.

Data are depicted as mean±SD of triplicate assays. Utilizing Graphpad Prism 3.0, statistical analysis was performed. Dunnett's t-test was used to compare data groups after an analysis of one-way ANOVA. '#' denotes that values are significant at $p<0.01$; '*' denotes that values are significant at $p<0.05$.

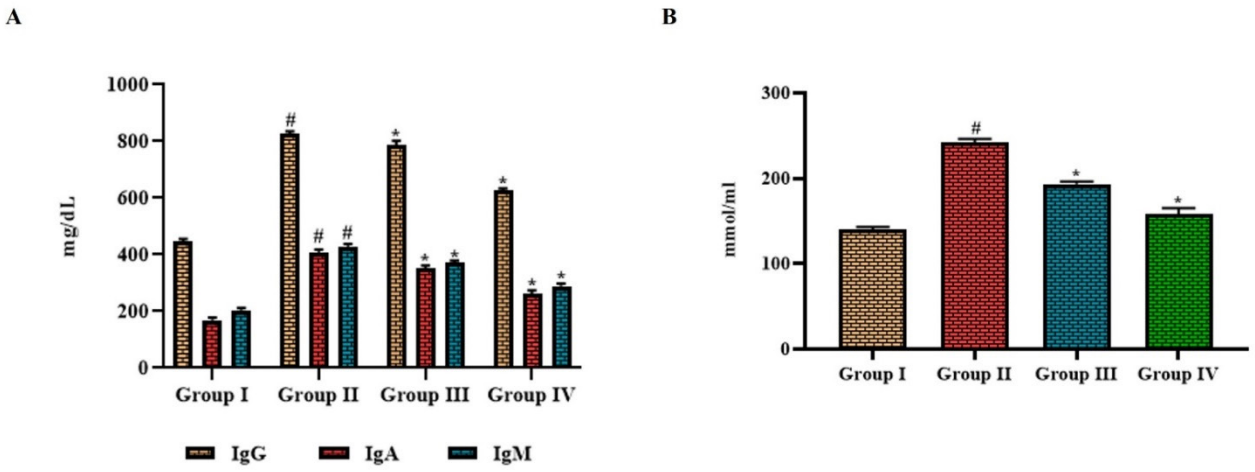

Figure 3: Effect of Conessine on Immunoglobins and carboxymethyl lysine in diabetic rats.

Data are depicted as mean±SD of triplicate assays. Utilizing Graphpad Prism 3.0, statistical analysis was performed. Dunnett's t-test was used to compare data groups after an analysis of one-way ANOVA. '#' denotes that values are significant at $p<0.01$; '*' denotes that values are significant at $p<0.05$.

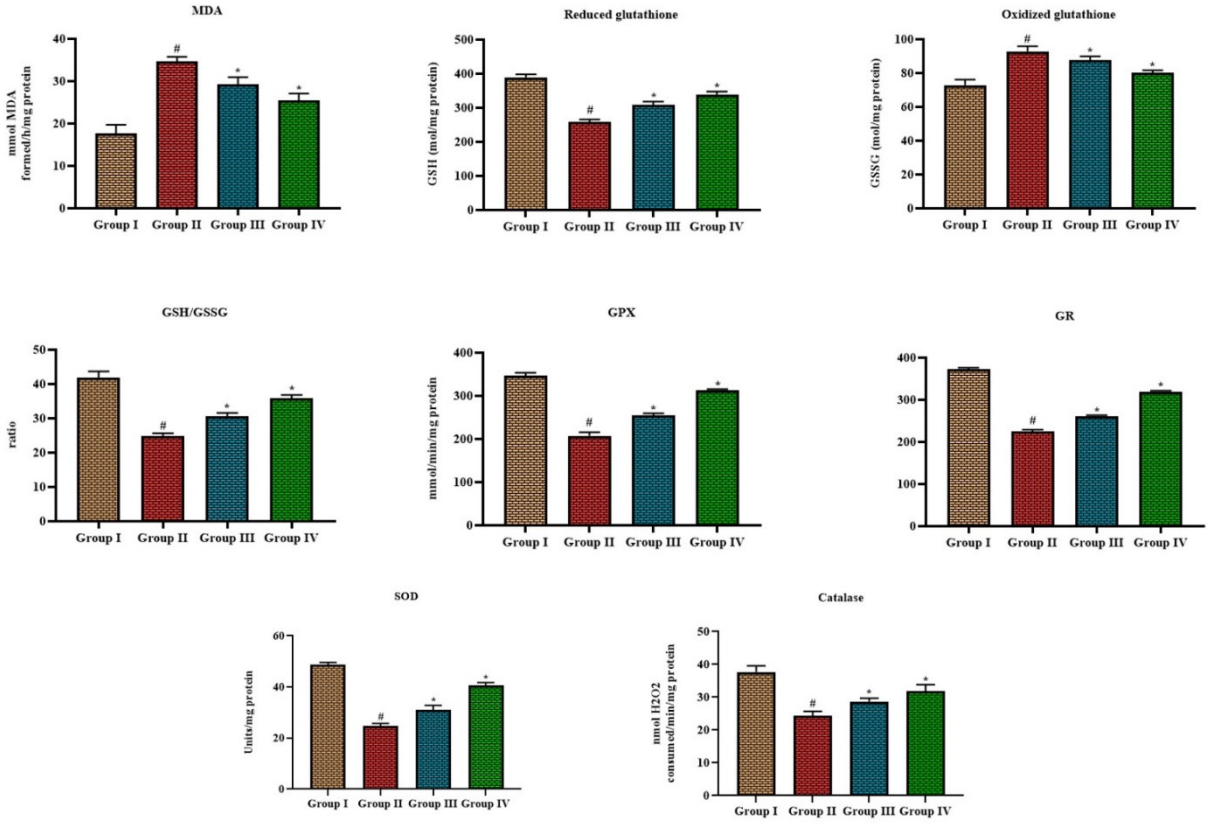

Figure 4: Effect of Conessine on antioxidant enzyme level in diabetic rats.

Data are depicted as mean±SD of triplicate assays. Utilizing Graphpad Prism 3.0, statistical analysis was performed. Dunnett's t-test was used to compare data groups after an analysis of one-way ANOVA. '#' denotes that values are significant at $p<0.01$; '*' denotes that values are significant at $p<0.05$.

levels of urea, creatinine, and uric acid. The impact of conessine treatment on serum electrolytes in diabetic rats is depicted in Figure 2b. When comparing the diabetic control rats (Group II) blood sodium and potassium ion mean values to those of the negative control animals (group II), a substantial drop was observed. When compared to the diabetic control rats (Group I), the conessine-treated diabetic rats (group III) had considerably higher blood electrolyte levels (Na+ and K+). Additionally, Figure

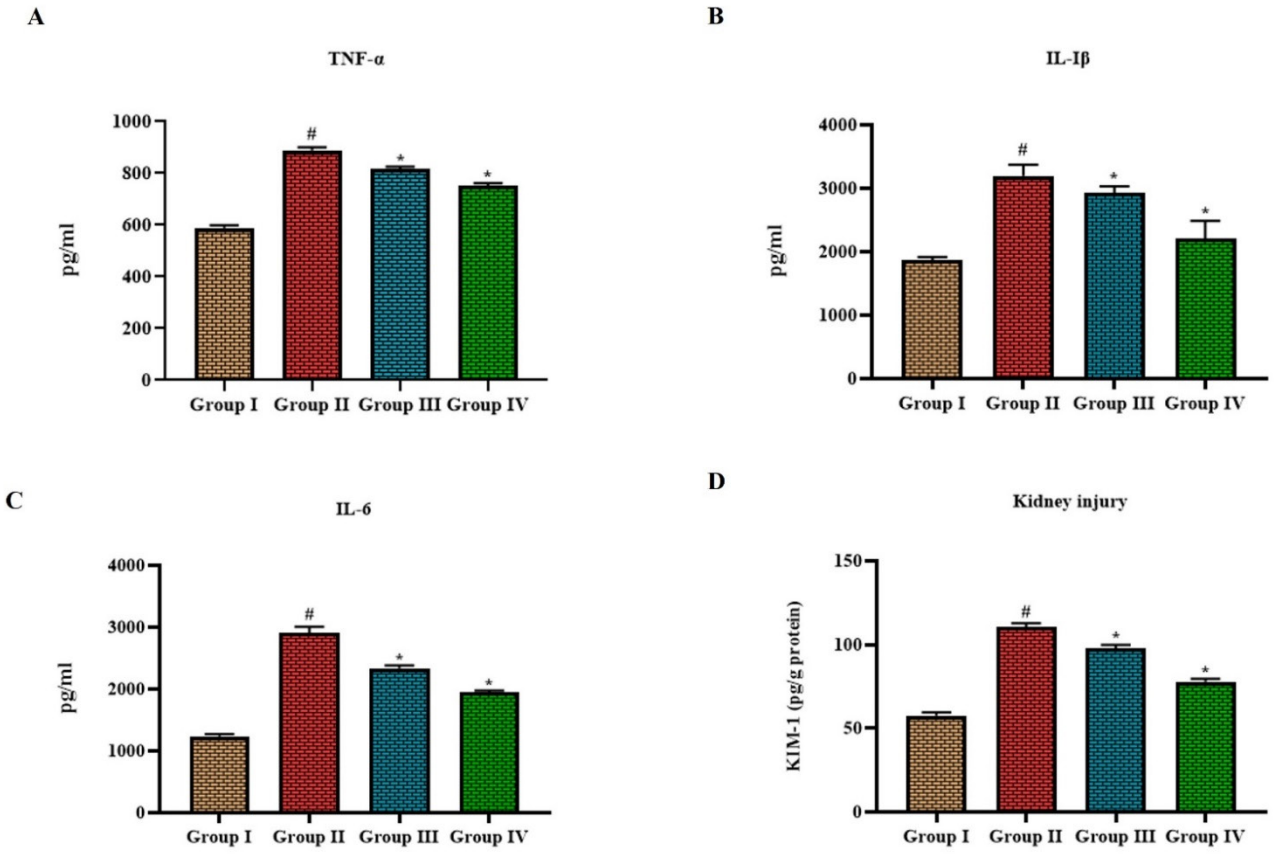

Figure 5: Effect of Conessine on inflammatory markers and kidney injury in diabetic rats.

Data are depicted as mean±SD of triplicate assays. Utilizing Graphpad Prism 3.0, statistical analysis was performed. Dunnett's t-test was used to compare data groups after an analysis of one-way ANOVA. '#' denotes that values are significant at $p<0.01$; '*' denotes that values are significant at $p<0.05$.

2c demonstrates that the diabetic control group's (group II) urine albumin mean values were substantially higher than those of the negative control animals (group I). In the meanwhile, Group II's mean urine creatinine readings were considerably lower than those of Group I, the negative control group. When compared to group II, the diabetic rats treated with conessine (group III) and gliclazide (group IV) saw a substantial drop in urinary albumin and a rise in urinary creatinine.

Effect of Conessine on Immunoglobins and carboxymethyl lysine in diabetic rats

The impact of conessine on immunoglobulin levels in diabetic rats is seen in Figure 3a. When comparing the levels of IgG, IgA, and IgM immunoglobulins between the diabetic control rats (group II) and the negative control animals (group I), a substantial increase was seen. In diabetic animals treated with conessine (group III), resulted in a considerable drop in the mean levels of IgG, IgA, and IgM immunoglobulins. Figure 3b shows the effect of conessine on carboxymethyl lysine in diabetic rats. When compared to the negative control group (group I), the diabetic control group (group II) had a much higher proportion of CML. When compared to the diabetic control animals (group II), the proportion of CML was much lower in the diabetic animals treated with conessine (group III) and gliclazide (group IV).

Effect of Conessine on antioxidant enzyme level in diabetic rats

The effect of conessine on kidney antioxidant enzyme expression is seen in Figure 4. Compared to the negative control rats (group I), the diabetic control rats (group II) had higher levels of MDA and GSSG. The expression of SOD, CAT, GR, and GPx activity in the diabetic control rats (group-II) was significantly lower than in the control group. Diabetic rats treated with conessine (group III) and gliclazide (group IV) resulted in a considerable increase in these antioxidant enzymes and a decrease in MDA levels as compared to the diabetic control rats (group II). Compared to control rats (group I), diabetic rats (group II) had significantly higher levels of GSSG and concurrently lower levels of GSH/GSSG. Treatment with conessine (group III) and gliclazide (group IV) tended to return GSSG and the GSH/GSSG ratio to almost normal levels.

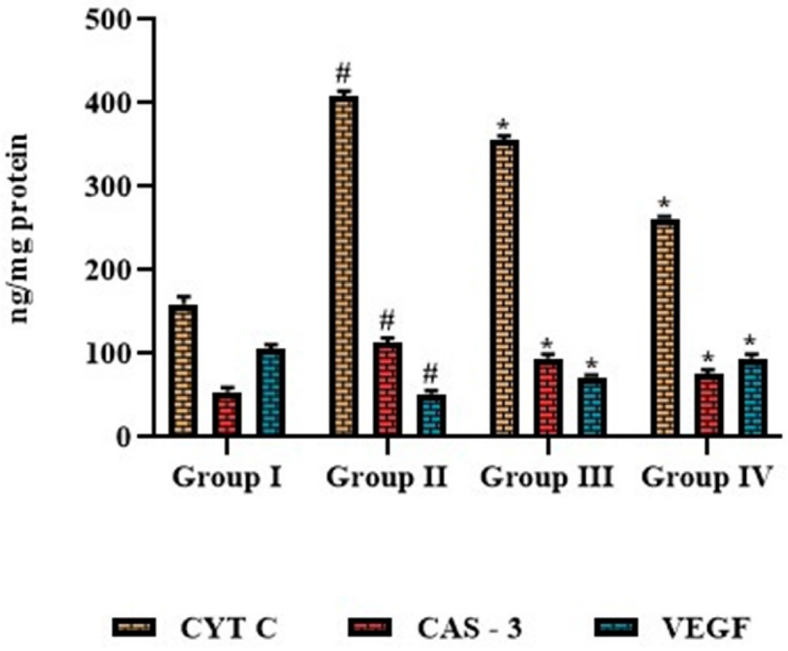

Figure 6: Effect of conessine on apoptosis in the kidney of diabetic rats.

Data are depicted as mean±SD of triplicate assays. Utilizing Graphpad Prism 3.0, statistical analysis was performed. Dunnett's t-test was used to compare data groups after an analysis of one-way ANOVA. '#' denotes that values are significant at $p<0.01$; '*' denotes that values are significant at $p<0.05$.

Effect of Conessine on inflammatory markers and kidney injury in diabetic rats

The amounts of IL-6, TNF-α, IL-1β, and kidney injury were measured in the retinal tissues of untreated and treated rats and are shown in Figure 5. The levels IL-6, TNF-α, and IL-1β in the retinal tissues were significantly increased in the diabetic control rats (group II) as compared to the control animals (group I). Similarly, the level of kidney injury was high in the diabetic control rats (group II) as compared to the control animals (group I). In diabetic rats treated with conessine (group III) and gliclazide (group IV), the IL-6, TNF-α, and IL-1β expression was significantly decreased. In addition, the level of kidney injury was reduced significantly in the group III and group IV animals in comparison to diabetic control animals.

Effect of Conessine on apoptosis in the kidney of diabetic rats

The effect of conessine on apoptosis in the kidney diabetic rats is depicted in Figure 6. The expression of cyt-c, VEG-F, and CAS-3 were significantly increased in the diabetic control rats (group II) as compared to the control animals (group I). In diabetic rats treated with conessine (group III) and gliclazide (group IV), the expression of cyt-c, VEG-F, and CAS-3 was downregulated.

Impact of Conessine on the renal histopathology of the experimental rats

The renal tissues from animals treated with conessine and gliclazide as well as control animals underwent histological examination (Figure 7 (a-d)). The kidney of the negative control animals (group I) underwent histopathological analysis, which revealed normal kidney tissues and blood vessels with no histological alterations (Figure 7(a)). As the renal tissues of the rats in the diabetic control group (group II) were examined, it was discovered that the kidney structure of these animals had pathological abnormalities in comparison to the negative control group (group I). Figure 7(b) depicted a collapsed glomerular tuft with prominent tubular atrophy linked to interstitial bleeding and inflammation. Conversely, the kidney sections of diabetic rats receiving conessine treatment (group III) appeared to be recovering interstitial haemorrhage-induced glomeruli and regenerating tubules in their typical morphology (Figure 7(c)). However, following gliclazide therapy (group IV), the kidney almost fully recovered its normal cortical tissue (Figure 7(d)).

DISCUSSION

A growing proportion of people globally are suffering from Type 2 Diabetes Mellitus (T2DM), which is strongly associated with the obesity trend. Due to hyperglycemia and specific elements of the insulin resistance (metabolic) syndrome, people with

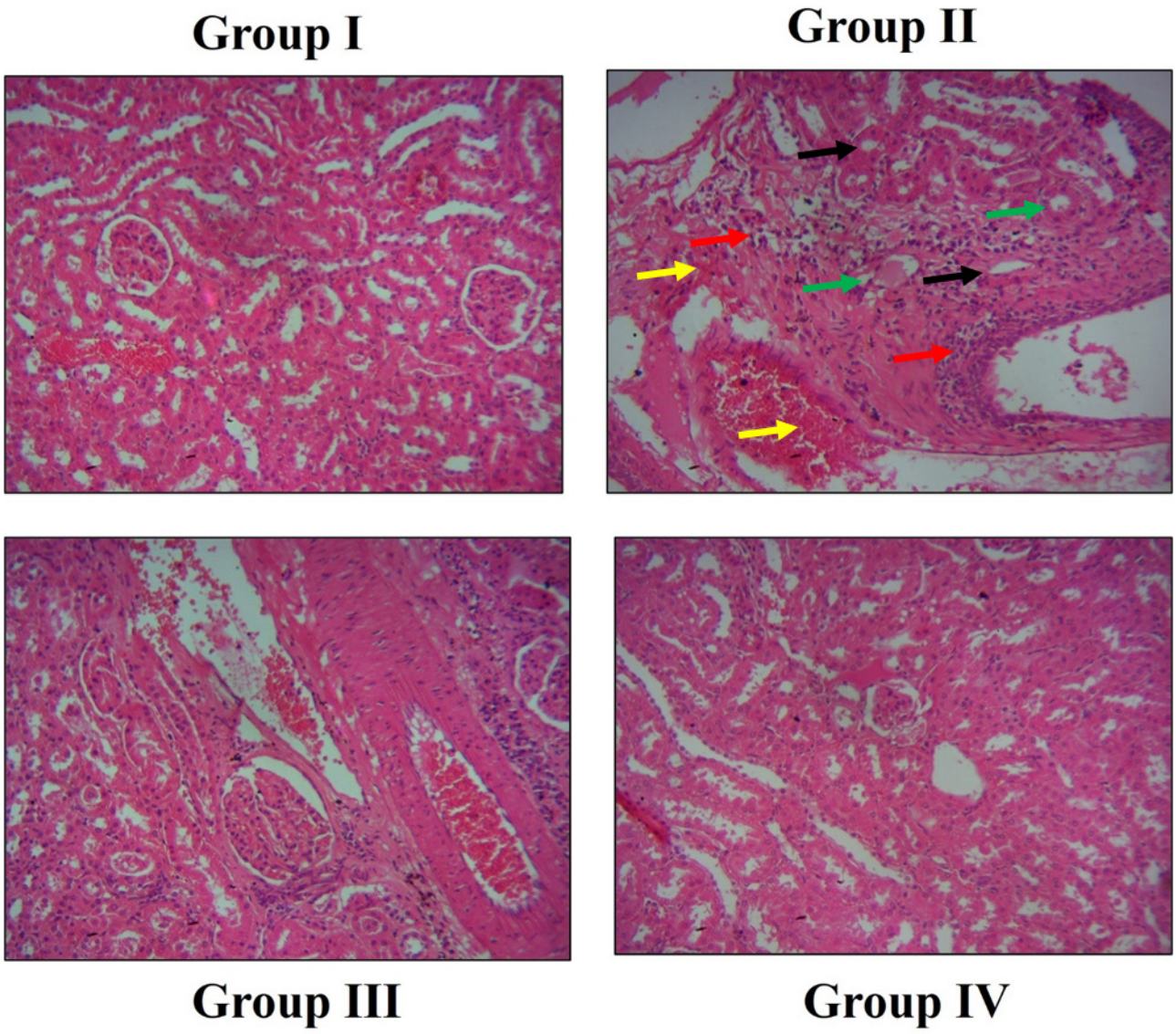

Figure 7: Impact Of Conessine On The Renal Histopathology Of The Experimental Rats.

Group I: Normal control group; Group II: STZ-induced Diabetic Nephropathy (DN) group; STZ-induced DN+Conessine (20 mg/kg b.wt)-treated group; STZ-induced DN+Gliclazide (5 mg/kg b.wt)-treated group. Black arrows: Collapsed glomerular tuft; Green arrows: tubular atrophy; Yellow arrows: interstitial bleeding; Red arrows: inflammation.

Type 2 Diabetes Mellitus (T2DM) are at a higher probability of developing microvascular complications, such as nephropathy, retinopathy, and neuropathy, as well as macrovascular complications, such as cardiovascular comorbidities. T2DM is marked by irregularities of lipid, protein, and carbohydrate metabolism and is brought on by either reduced secretion of insulin, a resistance to insulin, or a combo of the two. T2DM is the most prevalent kind of diabetes among the three main varieties.[22] Numerous investigations on diabetes and its consequences frequently employ the STZ-induced mouse model of the disease.[23]

The emphasis of the current study was to investigate the way a naturally occurring substance called conessine affected the progression of problems related to diabetes in STZ-stimulated rats. To compare conessine's anti-diabetic activity, gliclazide, a common medication, was used.

An antibiotic called Streptozotocin (STZ) destroys pancreatic islet β-cells and is commonly used in experiments to create a model of diabetes mellitus. As a decrease in β-cell function, which eventually leads to hyperglycemia and an insulin shortfall.[24] In the current investigation, rats in the diabetic control group were given 45 mg/kg of Streptozotocin (STZ) to induce diabetes, and this resulted in a significantly higher blood glucose level than in the control group. Following conessine medication, the rats that were triggered with STZ had a substantial reduction in their fasting glucose level. The anti-diabetic effect of conessine is demonstrated by this observation.

Animals administered with STZ exhibit diabetes, which is linked to excessive weight loss from hypoinsulinemia, enhanced muscle atrophy from hyperglycemia, and tissue protein loss.[25] The body weight of the STZ-diabetic rats significantly increased after they were administered with conessine, indicating that the degradation of muscle tissue caused by hyperglycemia had been avoided.

A frequently observed marker for long-term glycemic management is glycated Haemoglobin (HbA_{1c}). Glycation of hemoglobin causes elevated HbA_{1c} levels, which are a sign of sustained hyperglycemia in diabetes. Patients with diabetes were observed to have elevated HbA_{1c} levels as high as 16%. Increased levels of HbA_{1c} are positively correlated with comorbidities such diabetic retinopathy, nephropathy, and neuropathy.[26] In the present study, the level of HbA_{1c} was elevated in diabetic control animals in comparison to control group indicating diabetic condition in the animals. On treatment with conessine, the level of HbA_{1c} was downregulated in STZ induced animals indicating the antidiabetic property of conessine.

The fundamental indicators for an investigation of renal function are elevated concentrations of urea, uric acid, and creatinine, which suggest a clearly reduced functional ability of the kidneys concerning the filtration of waste products from the blood and their excretion in the urine.[27] In the current investigation, the level of urea, uric acid, and creatinine in serum was reduced after the administration of conessine in STZ-induced rats.

Reduced elimination of creatinine and a substantial increase in urine albumin levels are signs of chronic uncontrolled hyperglycemia-induced renal dysfunction. Nephropathy is characterized by microproteinuria or microalbuminuria, which may indicate that diabetes, is the cause of the nephropathy.[28] In the current study, urine examination of the STZ-induced diabetic rats revealed a substantial drop in urine creatinine levels and a surge in albumin levels in the diabetic control group. Treatment with conessine, reduced the level of albumin and increased the level of creatinine in the urine sample of the animals.

The pathophysiology of neuropathy, nephropathy, and vascular problems in diabetes patients may be related to a combo of extracellular and intracellular electrolyte abnormalities.[29] In the untreated diabetic group, our investigation revealed a considerable drop in blood potassium and salt levels. Conessine treatment allowed the serum electrolyte (Na+ and K+) levels in diabetic rats to return to normal.

Advanced Glycation End products (AGEs) are markedly elevated in diabetes because of the non-enzymatic mechanism of glycation, which forms covalent adducts among glucose and plasma proteins. Within this framework, Nε-Carboxymethyl Lysine (CML), one of the most well studied AGEs, becomes an important participant in the pathophysiology of diabetes. In pancreatic β cells and vascular smooth muscle cells, CML destroys mitochondrial DNA, promotes the generation of Reactive Oxygen Species (ROS), decreases the synthesis of Adenosine Triphosphate (ATP), and induces mitochondrial depolarization.[30] In the current study, when compared to the negative control group in our study, the diabetic control group had a significantly higher rate of CML. However, our findings demonstrated a substantial reduction in Carboxymethyl Lysine (CML) in the diabetic group receiving conessine when compared to the control diabetic group.

The elevation in IL-6 is correlated with a spike in immunoglobulins (IgA, IgG, and IgM) spurred on by diabetes, and additional research showed a favourable relationship between these parameters.[31] In the current investigation, on treatment with conessine significantly reduced the level of immunoglobulins in comparison to the diabetic control group.

Advanced Glycation End products (AGEs) and the oxidation process of unsaturated lipids in plasma and membrane proteins produce free radicals, which in turn induce oxidative stress. Free radicals can be scavenged or detoxified by antioxidant defense mechanisms, which lessen their detrimental effects. Enzymes like Catalase (CAT) and Superoxide Dismutase (SOD) are the initial part of the defense against free radicals. The most widely used marker of lipid peroxidation is Malondialdehyde (MDA). Based on many research, it seems that STZ-induced diabetic rats have higher levels of oxidative stress and lipid peroxidation.[32] Reducing glutathione disulfide to Glutathione Sulfhydryl (GSH), which is necessary to prevent oxidative stress, is catalyzed by glutathione reductase.[33] One important endogenous antioxidant that regulates damage caused by free radicals is GSH. The previous work shown that, in comparison to control rats, the STZ-induced diabetic rats' plasma and tissue GSH concentrations were considerably reduced. Reduction of reduced GSH is the mechanism by which Glutathione Peroxidase (GPx), an enzyme that contains selenium and is found in large quantities, converts $H2O_2$ to H_2O.[34] In the present investigation, the level of antioxidant enzymes including SOD, GSH, CAT, GR and GPx elevated on treatment with conessine. In addition, the level of GSSG and MDA reduced on treatment with conessine. This demonstrates the antioxidant property of conessine. This indicates that conessine can be a potent therapeutic for treating DN.

Elevated blood glucose level causes inflammation, which is shown by increased levels in many inflammatory markers, including Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6). Furthermore, diabetes may make free fatty acids more readily available as a result of the lipolysis mechanism. An elevation in free fatty acids will trigger the immune system to release IL-6, TNF-α, and IL-1β.[35] The levels of these inflammatory mediators significantly decreased after conessine treatment in diabetic rats, indicating the drug may have anti-inflammatory properties.

A pro-angiogenic protein that functions in endothelial tissue is called VEGF. VEGF causes a rise in vascular permeability during acute inflammation. Numerous secondary problems

associated with diabetic pathology arise from modifications to both macro and micro vessels, resulting from dysfunction of endothelial cells and enhanced vascular permeability.[36] The degree of oxidative stress and mitochondrial performance are tightly correlated. The area between the mitochondria's outer membrane and endometrium is where Cytochrome C (Cyto) is found. It is a vital part of the mitochondrial oxidative respiratory chain and is essential to the process of apoptosis. Cyto C can be released from the mitochondria into the cytoplasm by an apoptosis inducer. One characteristic of apoptosis is the release of Cyto C, which happens before to the activation of caspases and DNA breaks.[37] Apart from the impact of oxidative stress on renopathy induction, the current study indicates that inflammation and apoptosis may also play a significant role in kidney dysfunction and histological deteriorations. This is supported by the significant upregulation of caspase-3, an apoptotic marker, in the kidney dysfunction-ridden STZ-induced diabetic rats. Apoptosis may contribute to the development of diabetic nephropathy.[38] Herein, the level of VEG-F, cyto-c, and cas-3 was increased in the diabetic control rats in comparison to the control rats. On treatment with conessine significantly reduced the level of VEG-F, cyto-c, and cas-3 levels indicating the anti-oxidative and anti-inflammatory property of conessine.

Our histological results, which were in line with the biochemical results and those from previous studies, revealed damage to renal tubular epithelial cells, glomerular shrinkage and necrosis, edema, and neutrophil infiltration in rats given STZ treatment. Conessine treatment was seen to have a reduction in edema, neutrophil penetrations in the renal tissues, and glomerular and tubular damage. Furthermore, the limitations of the present work are that it lacks in-depth molecular studies, which will be addressed in our future studies.

CONCLUSION

Our research showed that conessine treatment considerably decreased hyperglycemic and oxidative stress brought on by hyperglycemia in STZ-induced diabetic rats. They also corrected every negative histological and biochemical alteration brought on by diabetes. Because of their strong antioxidant properties, these natural resources demonstrated safe and effective antidiabetic activity. Most of the cells not only recovered the normal circumstances, but also overcame the majority of the histopathological alterations in the renal tissues. As a result, conessine is advised to be great adjuvant assistance in the treatment of diabetes mellitus and the avoidance of its consequences.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

STZ: Streptozotocin; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutases; **CAT**- Catalase; **GSH:** Glutathione; **GPx:** Glutathione peroxidase; **ELISA:** Enzyme Linked Immunosorbent Assay; **DN:** Diabetic nephropathy; **DM:** Diabetes Mellitus; **ROS:** Reactive oxygen species; **RAAS:** Renin-angiotensin-aldosterone system; **FBG:** Fasting blood glucose; **B.W:** Body weight; **GR:** Glutathione reductase; **GSSG:** Oxidized glutathione; **H&E:** Hematoxylin and eosin; **Ig:** Immunoglobulin; **CML:** Carboxy methyl lysine; **HbA_{1c}:** Glycated haemoglobin; **ATP:** Adenosine triphosphate.

SUMMARY

The ameliorative effects of conessine against STZ-induced nephrotoxicity were thoroughly examined in this work. In the course of the study, the Body Weight (B.W) and glucose level of the conessine treated animals was unaffected. Moreover, it decreases the activity of MDA and pro-inflammatory cytokines, indicating the anti-inflammatory properties of conessine. Additionally, there was a substantial increase in the level of antioxidant enzymes demonstrating conessine's antioxidant properties. Thus, it is anticipated that conessine will be used as a therapeutic drug to reduce the nephrotoxicity.

ETHICS APPROVAL

This work was approved by the institutional ethical committee Hebei University, Baoding, 071000, China.

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