Ind. J. Pharm. Edu. Res., 2025; 60: 10.5530/ijper.20251228.
https://www.ijper.org

Original Article

Anti-Diabetic and Renoprotective Effects of Menthone in Streptozotocin-Induced Diabetic Nephropathy in Rats

Tihua Zhang[1], Danni Wang[2], Xin He[3,*]

[1]Department of Clinical Medical, Shangqiu Medical College, Shangqiu City, Henan Province, CHINA.
[2]Department of Pharmacy, Shangqiu Medical College, Shangqiu City, Henan Province, CHINA.
[3]Department of Medical, Luohe Medical College, Luohe City, Henan Province, CHINA.

ABSTRACT

Aim: This study aimed to investigate the renoprotective effects of menthone on Streptozotocin (STZ)-induced Diabetic Nephropathy (DN) in rats. **Materials and Methods:** The animals were divided into five groups. Each group contains six animals. Groul I: control; Group II: Menthone (50 mg/kg BW); Group III: Diabetes; Group IV: Diabetic+Menthone (50 mg/kg) An intraperitoneal injection of 50 mg/kg bt.; Group V: Diabetic+Gliclazide (10 mg/kg BW). STZ was used to induce diabetes in rats, creating a diabetic rat model. **Results:** Following induction, rats were orally administered menthone (50 mg/kg BW) for eight weeks. Diabetic rats exhibited elevated levels of glucose, glycosylated hemoglobin, reduced insulin level, and increased oxidative stress marker such as malondialdehyde and decreased activities of antioxidants status in kidney tissue. Significant increases in blood creatinine, Blood Urea Nitrogen (BUN), and urine albumin were also observed in diabetic rats. Furthermore, diabetic rats displayed elevated levels of pro-inflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1) and Interleukin-6 (IL-6) in renal tissues. However, administration of menthone in diabetic rats led to reductions in hyperglycemia, oxidative stress, and inflammation while enhancing antioxidant levels in the kidneys. This suggests that menthone possesses renoprotective properties by improving renal function, reducing oxidative stress, and mitigating inflammation in diabetic rats. **Conclusion:** In conclusion, menthone shows promise as a potential therapeutic agent for the treatment of diabetic nephropathy, warranting further investigation in future clinical trials.

Keywords: Diabetic Nephropathy, Hyperglycemia, Inflammation, Menthone, Oxidative stress, Streptozotocin.

Correspondence:
Dr. Xin He
Department of Medical, Luohe Medical College, Luohe City, Henan Province, CHINA.
Email: lhyzhhexin@sina.com

Received: 16-05-2025;
Revised: 24-07-2025;
Accepted: 02-09-2025.

SIGNIFICANCE OF THE STUDY

Menthone plays a crucial role in protecting against DN by reducing oxidative stress, inflammation, and hyperglycemia while improving renal function. These findings highlight menthone as a promising candidate for future therapeutic interventions in diabetic nephropathy. With limited treatment options for DN, menthone emerges as a natural compound with therapeutic potential. This study supports its possible clinical application in managing diabetic nephropathy.

INTRODUCTION

Diabetic Nephropathy (DN) emerges as a persistent and incapacitating kidney ailment, evolving as a complication of diabetes mellitus.[1] It assumes a significant role in the escalation of end-stage renal disease globally, impacting individuals with both type 1 and type 2 diabetes. The onset of diabetic nephropathy is intricately linked with prolonged elevation in blood glucose levels, triggering a cascade of interlinked physiological processes within the kidneys.[2] Streptozotocin (STZ) is a widely used chemical to induce Diabetes Mellitus (DM) in experimental animal models. It primarily targets pancreatic β-cells, leading to hyperglycemia, which in turn contributes to DN over time. The mechanism of STZ-induced DN involves several key pathological pathways like β-cell toxicity, hyperglycemia and glucose toxicity, oxidative stress and mitochondrial dysfunction, inflammatory pathways, renal fibrosis and glomerulosclerosis etc.[3] Elevated blood sugar levels lead to glomerular hyperfiltration, renal hypertension, and subsequent thickening of the glomerular basement membrane. Consequently, renal permeability increases, resulting in renal hypoperfusion and proteinuria, notably marked by albumin excretion. With time, chronic inflammation and oxidative stress foster renal fibrosis, compromising kidney function.[4,5] Clinical manifestations include microalbuminuria progressing to proteinuria, hypertension, edema, and a decline in glomerular filtration rate. Diagnosis involves urinalysis, serum creatinine

DOI: 10.5530/ijper.20251228

Copyright Information :
Copyright Author (s) 2025 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

assessment, and monitoring of glomerular filtration rate.[6] Treatment strategies prioritize stringent glycemic control, blood pressure regulation using medications like ACE inhibitors or ARBs, lifestyle adjustments, and regular monitoring.[7] The early identification through routine screenings and proactive management of risk factors plays pivotal roles in forestalling or postponing the onset of diabetic nephropathy, emphasizing the significance of holistic care in diabetic individuals. Present therapeutic approaches for diabetic nephropathy focus on managing the progression of renal impairment and alleviating associated complications.[8] Tight glycemic control serves as a cornerstone, with optimal blood glucose levels pivotal in retarding nephropathy progression.[9] These medications not only aid in hypertension control but also contribute to reducing proteinuria, thereby preserving renal function. Lifestyle modifications, encompassing a kidney-friendly diet, weight management, and consistent physical activity, complement pharmacological interventions. In instances of advanced diabetic nephropathy, renal replacement therapies like dialysis or kidney transplantation may be contemplated. Regular assessment of kidney function, blood pressure, and glycemic levels is crucial for ongoing diabetic nephropathy management.[10,11] Ongoing research endeavours seek novel therapeutic avenues, emphasizing the necessity of a tailored and comprehensive treatment regimen to address the multifaceted nature of this condition. Early detection, timely intervention, and a holistic care approach remain pivotal in enhancing outcomes and augmenting the quality of life for individuals afflicted with diabetic nephropathy.[12]

Menthone, a naturally occurring cyclic monoterpene ketone found in essential oils of various mint species, has gained attention for its potential therapeutic effects in diabetes and diabetic nephropathy.[13] While previous reports on the direct efficacy of menthone specifically are limited, several studies have investigated the pharmacological properties of mint extracts containing menthone and its derivatives, suggesting promising benefits in managing diabetes and its complications.[14] Menthone has been associated with anti-diabetic properties through its effects on glucose metabolism and insulin sensitivity. One of the studies indicates that mint extracts rich in menthone may enhance insulin secretion from pancreatic beta cells and improve glucose uptake in peripheral tissues, thereby helping to regulate blood glucose levels.[15,16] Moreover, menthone-containing mint extracts have shown potential renoprotective effects in experimental models of diabetic nephropathy. By mitigating oxidative stress, inflammation, and fibrosis in the kidneys, menthone may help attenuate the progression of renal damage and preserve renal function.[17] Zhao *et al.*, (2023) reported that Menthone had potent antibacterial effect on Methicillin Resistant *Staphylococcus aureus* by affecting cell membrane properties and lipid profile.[18] Most of the studies suggest that mint extracts may reduce proteinuria, inhibit the accumulation of extracellular matrix proteins, and improve renal hemodynamics, thereby delaying the onset and progression of diabetic nephropathy.[19,20] Despite the promising findings from preclinical studies, further research is warranted to elucidate the specific mechanisms of action of menthone and its potential therapeutic utility in clinical settings. This research delves into the multifaceted role of menthone, a naturally occurring compound found in mint extracts, in mitigating diabetes and its renal complications. By exploring different concentrations of menthone, the study seeks to elucidate its dose-dependent effects on various parameters related to diabetes and diabetic nephropathy, including blood glucose levels, insulin sensitivity, biochemical analysis, renal function markers and anti-inflammation activity. Moreover, this study underscores the importance of further investigation into menthone's efficacy, safety, and mechanisms of action, paving the way for its translation into *in vivo* practice as a promising adjunctive therapy for diabetes and diabetic nephropathy.

MATERIALS AND METHODS

Chemicals

Menthone and streptozotocin were purchased from Sigma Aldrich, USA. TNF-α, IL-1, IL-6, and NF-κB were used in this study by an ELISA kit and procured from Sunred Biological Technology Company, Shanghai. The TRIzol reagent, RNA isolation kit, cDNA synthesis kit, and the SYBR green RT-qPCR kit were sourced from Takara Bio, Japan.

Animal maintenance

A total of 30 male albino Wistar rats (weighing 160-180 g) were used in this proposed study. All the rats were kept in a clean, air-conditioned environment with a temperature of 25°C, 50% humidity, and a 12:12 hr dark/light cycle. Animals were fed normal diets and given access to drinking water *ad libitum*. The experimental animal procedure was approved by Institutional Animal Ethics Committee (IAEC).

Animal grouping and treatment

The experiments involving animals were conducted with approval from the appropriate regulatory body. The experimental rats were randomly divided into five groups based on the body weight. Diabetes was induced by intraperitoneal injection of STZ (50 mg/kg BW in 0.1 mmol/l citrate buffer) following 12 hr of fasting. Blood glucose level was monitored three days after STZ administration and then twice a week. The groups were organized as follows:

Group I: Control,

Group II: Menthone (50 mg/kg BW) was administered orally for eight weeks,[21]

Group III: Diabetic control STZ injected single dose (50 mg/kg BW in 0.1 mmol/l citrate buffer),[15]

IN PRESS

Group IV: Diabetic+Menthone (50 mg/kg BW) was administered orally for eight weeks,

Group V: Diabetic+Gliclazide (10 mg/kg BW) was administered orally for eight weeks.

The body weight of each animal was determined at the end of the study. After eight weeks, the animals fasted for 12 hr, were anesthetized, blood was withdrawn via cardiac puncture and finally sacrificed by decapitation. Blood was centrifuged at 1300 g for separation of serum and plasma separated by respective parameters and stored at 20°C until assay. Then the urine was collected for urine related parameters.

Kidney function markers test

Serum creatinine (mg/dl), BUN (mg/dl) and urine albumin (mg/dl) levels were determined following established protocols using autoanalyzer (Hitachi 7600, Japan).[22]

Antioxidant activity

The oxidative stress markers such as thiobarbituric acid reactive substances (TBARS),[23] Superoxide Dismutase (SOD),[24] Catalase (CAT),[25] Glutathione Peroxidase (GPx)[26] and reduced glutathione (GSH)[27] were evaluated using the standard protocols. The evaluation of lipid peroxidation, specifically the quantification of Thio Barbituric Acid Reactive Substances (TBARS) was performed using known procedures. The sample was diluted with 0.5 mL of double distilled water and thoroughly mixed. Subsequently, 2.0 mL of TBA-TCA-HCL reagent was added. The combination was incubated in a water bath at boiling temperature for 15 min. After cooling, the tubes were subjected to centrifugation at a force of 1000 rpm at 10 min and the liquid above the sediment was measured. The chromophore's absorbance was measured at a wavelength of 535 nm relative to the reagent blank.

0.5 mL of tissue homogenate was diluted to 1.0 mL with water. Added 2.5 mL of ethanol and 1.5 mL of chloroform (chilled), shaken for 90 sec at 4°C and centrifuged. The assay mixture (1.2 mL sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate, 0.3 mL of nitroblue tetrazolium) was combined with the enzyme preparation. The reaction was started with 0.2 mL NADH, incubated at 30°C from 90 sec, and stopped with 1 mL glacial acetic acid. The mixture was shaken with 4 mL n-butanol, allowed to stand for 10 min, centrifuged, and the n-butanol layer's density was measured at 510 nm. 0.1 mL of tissue homogenate was mixed with 0.4 mL of hydrogen peroxide. The reaction was stopped at 15, 30, 45, and 60 s by adding 2.0 mL of dichromate-acetic acid mixture boiling for 10 min, cooled, and the color developed was read at 620 nm.

0.2 mL of tris and 0.2 mL of GSH were mixed, followed by 0.1 mL of H_2O_2. The mixture were incubated at 37°C for 10 min, with a control containing all reagents except homogenate. The reaction was stopped with 0.5 mL 10% TCA, centrifuged, and the supernatant was assayed for GSH using the Ellman method.[27] The measurement of reduced Glutathione (GSH) was conducted using the Ellman method. 0.5 mL of tissue homogenate was precipitated with 2.0 mL of 5% TCA. After centrifugation, 2.0 mL of supernatant was mixed, with 1.0 mL of Ellman's reagent and 4.0 mL of 0.3M disodium hydrogen phosphate. The yellow color developed was read at 412 nm.

Anti-Inflammation activity

The inflammatory regulation markers such as TNF-α, IL-1, IL-6, and NF-κB were used in this study by an ELISA kit and procured from Sunred Biological Technology Company, Shanghai, China, following standard procedures.[22] In the protocol, 50 µL of the sample and 50 µL of an antibody cocktail were added to the experimental wells and incubated at 37°C for 1 hr. The wells were then washed with 100 µL of 3,3′,5,5′-Tetramethylbenzidine substrates and incubated for 10 min. Finally, 100 µL of stop solution was added, and the color development was read at OD 450 nm using an ELISA.

Histopathological analysis

Tissue sections of kidney (3-4 µm thick) from all experimental groups were prepared using paraffin embedding. These sections were then stained with Hematoxylin and Eosin (H&E) according to standard procedures to observe cellular alterations.[28]

Real-time PCR

Total RNA was isolated from the homogenized cardiac material using Ribozol RNA extraction reagent according to the manufacturer's instructions. The reaction mixture had a total volume of 25 µL, which contained 1 µL of total RNA, 1 µL of forward primer and 1 µL of reverse primer, 1 µL of Reverse Transcriptase (M-MLV RT), and 10 µL of single-step SYBR Green PCR Master Mix, plus 20 µL of nuclease-free water. TNF-α, IL-6 and IL-1 genes were compared to Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), a reference gene. The primer sequences were shown in Table 1. The relative expression level of each gene was determined using the formula $2^{(-\Delta\Delta Ct)}$.

Statistical analysis

The study reported all data as mean±standard deviation derived from six rats in each group. Statistical analysis was conducted using One-Way Analysis of Variance (ANOVA) with GraphPad Prism 8 software. Pairwise comparisons between groups were carried out utilizing the least significant difference test, with statistical significance defined as $p<0.05$.

IN PRESS

Table 1: Primer sequences for inflammatory markers.

Gene Name	Primer Sequences
TNF-α	Forward primer: 5′- GTACTAACTCCCAGAAAAGCAAGC-3′
	Reverse primer: 5′- CAGTAGACAGAAGAGCGTGGTG-3′
IL-1	Forward primer: 5′- TAAATGAAATTGACATTC-3′
	Reverse primer: 5′-TGATAAATGTTATTACTC-3′
IL-6	Forward primer: 5′- CAATGCTCTCCTAATGGAGAAGTTA-3′
	Reverse primer: 5′ -ATGTCCACAAACTGATATGCTTAGG-3′

RESULTS

Menthone effect on glycemic index and renal function of diabetic rats

Four weeks post-STZ injection, diabetic rats demonstrated elevated body weight compared to the control group. However, administration of menthone resulted in decreased body weight and glucose levels. Additionally, diabetic-induced rats exhibited reduced insulin levels, which were increased upon menthone treatment. Furthermore, the HbA_{1c} level was elevated in diabetic animals but was reduced in menthone-treated diabetic rats, as outlined in Figure 1.

Diabetic-induced rats displayed elevated levels of serum creatinine, BUN and urine albumin. However, administration of menthone at a dose of 50 mg/kg BW to diabetic rats significantly decreased (improved in) the levels of serum creatinine, BUN and urine albumin compared to the administration of the conventional medication, gliclazide in Table 2.

Effect of menthone on antioxidant enzyme activity in the kidney of diabetic rats

Renal TBARS levels, reflecting the extent of lipid peroxidation, were observed to be elevated in diabetic-induced animals. However, the menthone-treated group exhibited reduced TBARS levels. Additionally, oxidative enzymes such as SOD and catalase activity were measured, revealing decreased levels in the diabetic group compared to the control. Conversely, the menthone-treated group displayed increased levels of these enzymes compared to the control group, as illustrated in Table 3. Moreover, levels of GPx and GSH were found to be decreased in diabetic rats but increased upon treatment with menthone, surpassing levels observed in the control group. Overall, treatment with menthone in diabetic-induced rats resulted in reduced MDA levels and increased levels of SOD, CAT, GPx, and GSH compared to the control group.

Effect of menthone on inflammatory markers in serum

Levels of TNF-α was found to be elevated in diabetic-induced rats compared to those treated with menthone, where a reduction was observed. Similarly, other inflammatory regulatory markers such as IL-1 and IL-6 showed increased levels in the diabetic group, whereas the menthone-treated groups exhibited reduced levels of these inflammatory regulators. Furthermore, the menthone-treated group demonstrated a significant decline in NF-κB level. In contrast, the diabetic group indicated elevated levels, as depicted in Figure 2. These findings collectively indicate that menthone possesses antioxidant and anti-inflammatory activity in the renal system under diabetic conditions.

Menthone on inflammatory gene expression in kidney tissue

Gene expression analysis revealed significantly upregulated levels of inflammatory targets, including TNF-α, IL-1, and IL-6 in DN-induced rats compared to the control group, as depicted in Figure 3. Conversely, the menthone-treated group exhibited reduced expression levels of TNF-α, IL-1, and IL-6 compared to the DN control group ($p<0.05$).

Histopathology of diabetic-induced kidney tissues

The study conducted a histopathological analysis of normal kidney tissues, revealing the morphological architecture depicted in Figure 4. Notably, diabetic-induced kidney tissues exhibited ruptured epithelial lining of cells and evident damage primarily in the proximal convoluted tubules. Treatment with menthone resulted in a higher degree of kidney morphology atrophy compared to the diabetic-induced group. Additionally, there was an observable enhancement of normal kidney shapes in the treated group.

DISCUSSION

Diabetic nephropathy, a severe complication arising from diabetes mellitus, remains a predominant cause of End-Stage Renal Disease (ESRD) globally.[2] It primarily affects individuals diagnosed with either type 1 or type 2 diabetes, characterized by progressive kidney impairment due to sustained hyperglycemia. Prolonged exposure to elevated blood glucose levels leads to damage in the small blood vessels within the kidneys, particularly the glomeruli. This results in increased deposition of extracellular matrix within mesangial cells, leading to mesangial expansion and glomerulosclerosis.[5,6] As the disease advances, the glomerular filtration barrier becomes increasingly permeable, resulting in the leakage of albumin and other proteins into the urine, a condition known as albuminuria or proteinuria. Chronic inflammation, oxidative stress, and activation of profibrotic signaling pathways further contribute to renal fibrosis, ultimately culminating in irreversible kidney damage. The clinical presentation of diabetic

IN PRESS

nephropathy varies depending on the stage of the disease,[7] initially manifesting with minimal symptoms that may exacerbate as kidney function deteriorates.

The diagnosis of diabetic nephropathy requires a comprehensive approach involving clinical evaluation, laboratory analyses, and imaging techniques. Measurement of Urinary Albumin-to-Creatinine Ratio (UACR) or Protein-to-Creatinine Ratio (PCR) is employed to assess albuminuria/proteinuria,[8] while serum creatinine levels and estimated Glomerular Filtration Rate (eGFR) calculations aid in evaluating renal function. Renal ultrasound or other imaging modalities are utilized to assess kidney size, structure, and the presence of renal artery stenosis.[12-13] Over time, extensive research efforts have been dedicated to exploring potential therapeutic interventions for managing diabetic nephropathy, encompassing both pharmacological and non-pharmacological strategies.[29] An emerging area of interest in diabetic nephropathy management lies in phytotherapy, which harnesses plant-derived compounds for therapeutic purposes. Phytotherapy has garnered significant attention due to its perceived efficacy, safety, and relatively low cost compared to conventional medications.[30] Several plant extracts have been studied for their potential renoprotective effects in diabetic nephropathy.

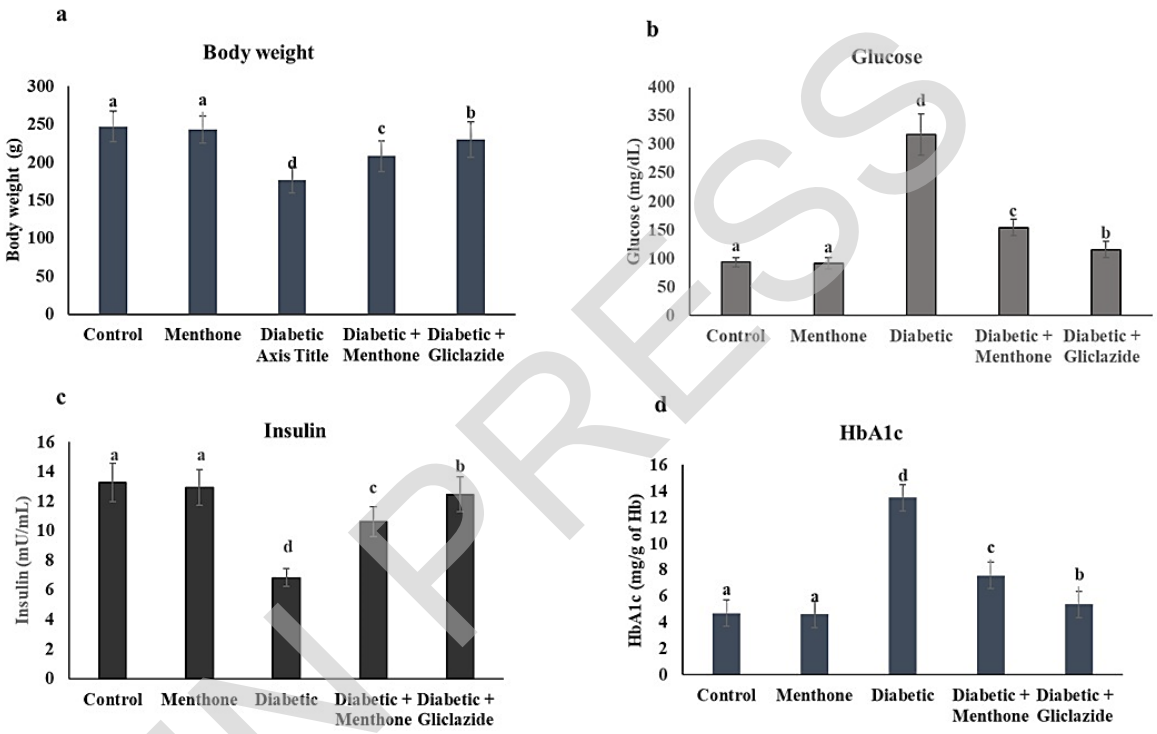


Figure 1: Effect of menthone on body weight and glycemic index in streptozotocin-induced diabetic nephropathy rats. Values are expressed as means±S.D for six rats in each group. Values not sharing a common marking superscript (a, b, c) are different significantly at *p*-value <0.05 (DMRT).

Table 2: Effect of Menthone on kidney function markers in streptozotocin-induced diabetic nephropathy rats.

Groups	Control	Menthone (50 mg/kg BW)	Diabetic control	Diabetic+Menthone (50 mg/kg BW)	Diabetic+Gliclazide (10 mg/kg BW)
Serum creatinine (mg/dl)	0.78±0.06[a]	0.75±0.05[a]	4.72±0.32[b]	1.35±0.96[c]	0.82±0.06[a]
BUN (mg/dl)	33.58±4.61[a]	31.95±3.58[a]	75.49±8.42[b]	48.27±5.59[c]	35.23±4.31[a]
Urine albumin (mg/dl)	0.49±0.03[a]	0.51±0.04[a]	4.57±0.62[b]	2.39±0.61[c]	1.09±0.15[d]

Values are expressed as means±S.D for six rats in each group.Values not sharing a common marking superscript (a, b, c) are different significantly at *p*-value < 0.05 (DMRT).

IN PRESS

Table 3: Effect of Menthone on TBARS and antioxidant status in streptozotocin-induced diabetic nephropathy rats.

Groups	Control	Menthone (50 mg/kg)	Diabetic	Diabetic+Menthone (50 mg/kg)	Diabetic+Gliclazide (10 mg/kg BW)
TBARS (nmol/mg protein)	7.38±0.64[a]	7.29±0.52[a]	18.36±2.69[b]	10.58±2.73[c]	8.47±0.84[d]
SOD (U^1/mg protein)	37.42±2.68[a]	36.29±2.52[a]	15.46±1.73[b]	26.47±2.94[c]	35.94±3.14[a]
CAT (U^2/mg protein)	24.49±1.53[a]	25.37±3.26[a]	12.68±1.52[b]	18.48±1.43[c]	21.62±2.47[d]
GPx (U^3/mg protein)	6.55±0.87[a]	6.89±0.94[a]	2.15±0.37[b]	5.74±0.55[c]	6.22±2.63[a]
GSH (µg/mg protein)	5.09±0.51[a]	5.36±0.83[a]	1.23±0.50[b]	3.81±0.39[c]	4.86±0.48[a]

Values are expressed as means±S.D for six rats in each group.Values not sharing a common marking superscript (a, b, c) are different significantly at *p*-value <0.05 (DMRT).U^1- Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in 1 min; U^2-µmol of hydrogen peroxide consumed per minute; U^3-µg of glutatione consumed per minute.

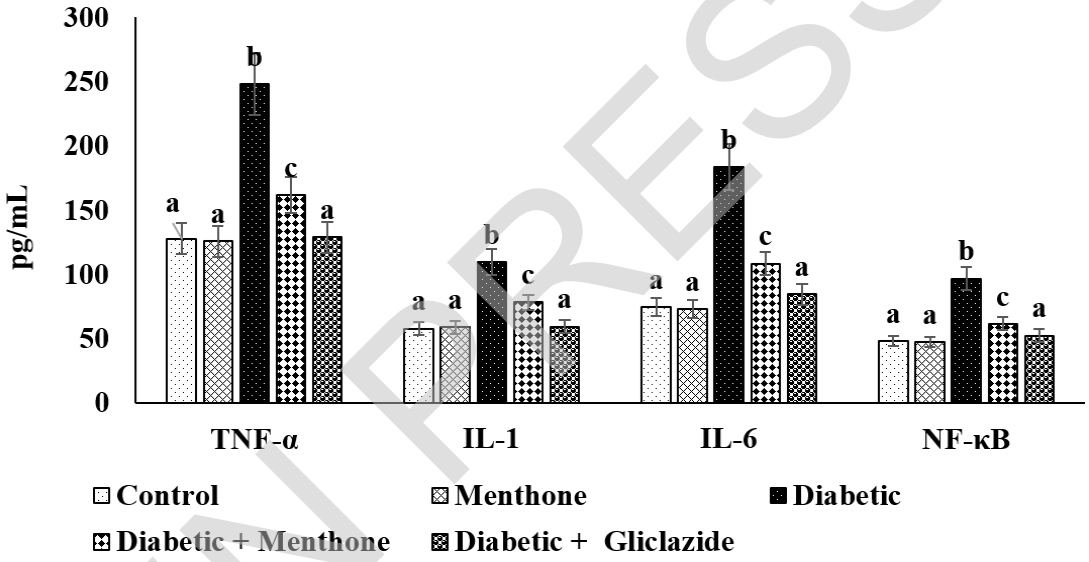


Figure 2: Effect of menthone on serum inflammatory markers in streptozotocin-induced diabetic nephropathy rats. Values are expressed as means±S.D for six rats in each group. Values not sharing a common marking superscript (a, b, c) are different significantly at *p*-value <0.05 (DMRT).

Examples include extracts from plants such as *Salvia miltiorrhiza* (Danshen), *Curcuma longa* (Turmeric), *Camellia sinensis* (Green tea), and *Morus alba* (Mulberry). These extracts have been shown to possess antioxidant, anti-inflammatory, and antifibrotic properties, which may help mitigate kidney damage associated with diabetes.[31,32] Phytochemicals are bioactive compounds found in plants that exhibit therapeutic potential. Examples include polyphenols, flavonoids, alkaloids, and terpenoids. Studies have investigated the effects of individual phytochemicals, such as resveratrol, quercetin, Epigallocatechin Gallate (EGCG), and berberine, in animal models of diabetic nephropathy.[33] These compounds have been shown to attenuate renal oxidative stress, inflammation, and fibrosis, leading to improvements in kidney function. Menthone, a naturally occurring compound found in various essential oils such as peppermint and pennyroyal, has garnered interest for its potential therapeutic effects in diabetes and its complications, including diabetic nephropathy.[34] Menthone exhibits antioxidant properties, which can help combat oxidative stress, a key contributor to the development and progression of diabetic complications, including nephropathy. By scavenging free radicals and reducing oxidative damage, menthone may protect against renal injury in diabetes. Menthone has been reported to modulate various signaling pathways implicated in the pathogenesis of diabetic nephropathy, including pathways involved in oxidative stress, inflammation, apoptosis, and fibrosis.[29] By targeting these pathways, menthone may help prevent or mitigate renal injury in diabetes. Some studies suggest that menthone may have hypoglycemic effects, helping to lower blood glucose levels in diabetes. By improving glucose homeostasis, menthone could potentially reduce the

IN PRESS

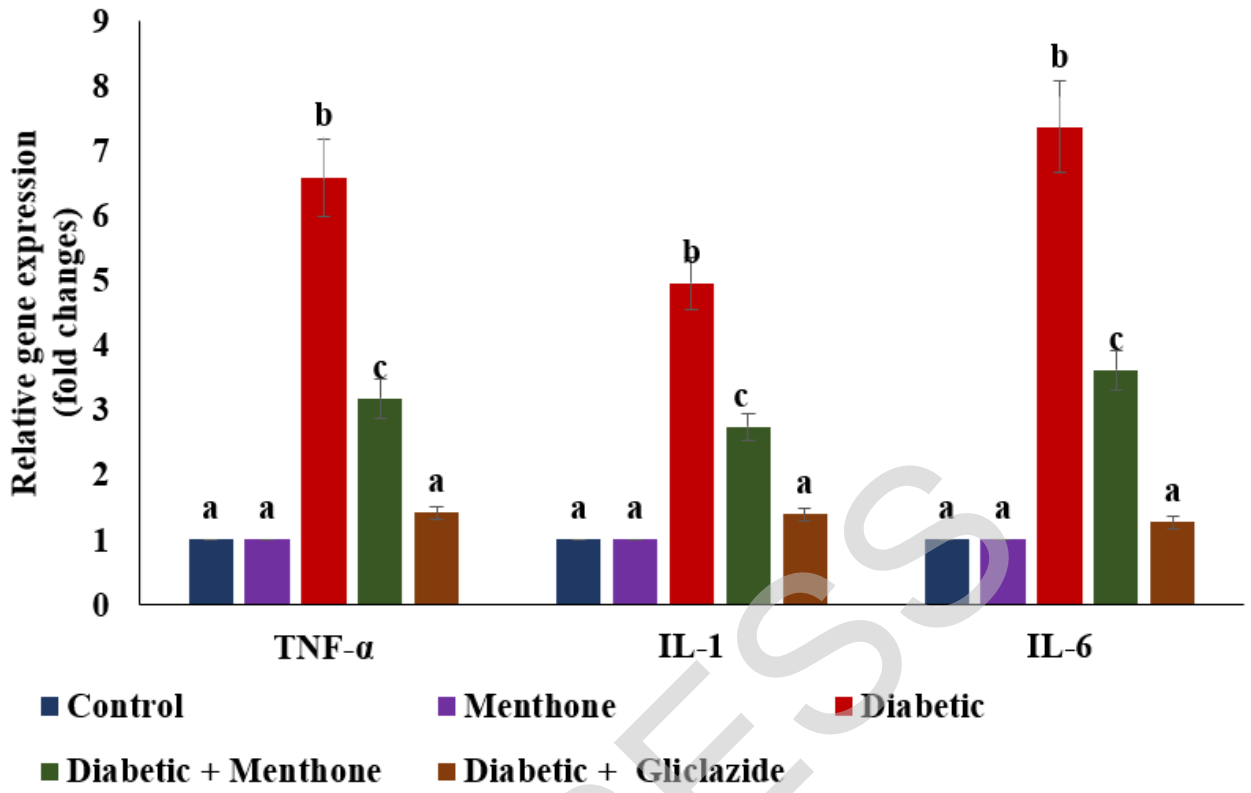


Figure 3: Effect of menthone on inflammatory marker gene expression in kidney tissue of streptozotocin-induced diabetic nephropathy rats. The histogram depicts the quantitation of three independent experiments (means±SD). The TNF-α, IL-6 and IL-1 genes expression levels were normalized with the expression level of the GAPDH mRNA in each sample. Values are expressed as means±SD for three rats in each group. Values not sharing a common marking superscript (a, b, c) are different significantly at *p*-value <0.05 (DMRT).

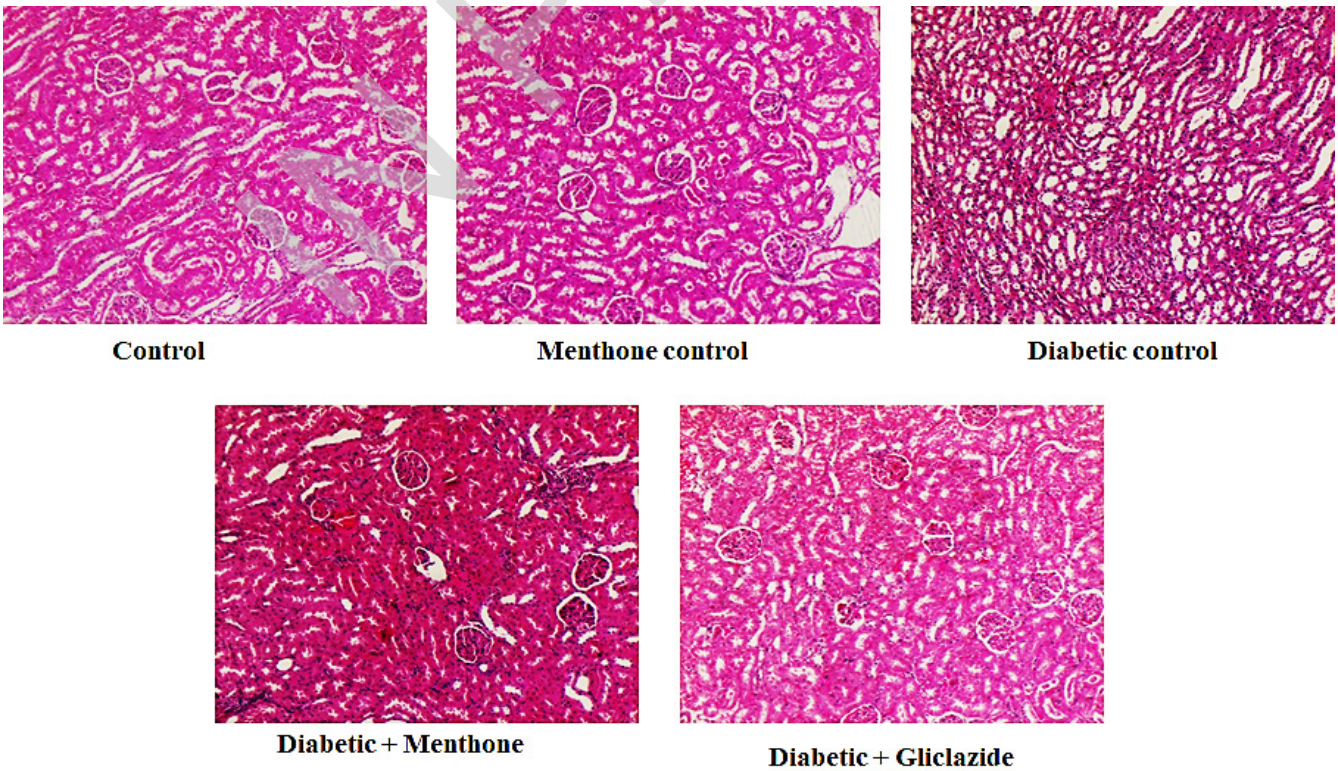


Figure 4: Effect of menthone on histopathological examination in kidney tissue of streptozotocin-induced diabetic nephropathy rats.

risk of diabetic nephropathy and other complications related to hyperglycemia. In this study aimed to understand the role of menthone may exert direct renoprotective effects in diabetic nephropathy. Studies in animal models have demonstrated that menthone treatment can mitigate kidney damage, preserve renal function, and attenuate structural alterations associated with diabetic nephropathy, such as glomerular hypertrophy and tubulointerstitial fibrosis.

Previous studies have reported that the treatment of various drugs resulted in a reduction in glycemic indexes and renal markers.[22] Following four weeks post-STZ injection, diabetic rats displayed notable alterations in various parameters compared to the control group. These alterations encompassed elevated body weight and glucose levels, along with reduced insulin levels and heightened HbA_{1c} levels, indicative of impaired glycemic control. Moreover, diabetic-induced rats exhiited elevated levels of markers of renal dysfunction, including serum creatinine, BUN, and urine albumin. However, administration of menthone at a dose of 50 mg/kg body weight to diabetic rats yielded significant improvements in these parameters. Notably, menthone treatment led to decreased body weight and glucose levels, along with increased insulin levels and reduced HbA_{1c} levels, suggesting enhanced glycemic regulation. Additionally, menthone administration resulted in notable reductions in markers of renal dysfunction, surpassing the effects observed with the standard drug, gliclazide.

Furthermore, menthone treatment exerted robust antioxidant effects on the renal system. Elevated renal TBARS levels, indicative of lipid peroxidation, observed in diabetic-induced animals were mitigated by menthone treatment. Chronic hyperglycemia in diabetes triggers immune responses, leading to the activation of inflammatory pathways in the kidneys. This includes the recruitment of immune cells such as macrophages, T cells, and dendritic cells to the renal tissues.[35] Moreover, menthone treatment led to increased activity of antioxidant enzymes such as SOD, catalase, GPx, and GSH, surpassing levels observed in the control group. In addition to its antioxidative effects, menthone exhibited potent anti-inflammatory properties. An increased level of TNF-α is a key inflammatory marker that contributes to kidney damage and fibrosis. IL-1 promotes inflammatory responses and worsens renal injury. The pro-inflammatory cytokine IL-6 is associated with diabetic complications and oxidative stress. However, menthone administration significantly reduced these elevated cytokines, demonstrating its anti-inflammatory effects and potential to mitigate renal inflammation in diabetic nephropathy.

Furthermore, menthone adsministration resulted in a significant decline in NF-κB level, indicating its ability to modulate the inflammatory response in diabetic nephropathy. Histopathological examination of kidney tissues from DN rats reveals a spectrum of structural abnormalities, including glomerular hypertrophy, tubulointerstitial fibrosis, vascular changes, inflammatory infiltrates, and other pathological features.[36,37] Histopathological examination further corroborated the beneficial effects of menthone treatment on renal morphology. Diabetic-induced kidney tissues exhibited significant structural abnormalities, including ruptured epithelial lining of cells and proximal convoluted tubular damage. Conversely, menthone-treated groups demonstrated improved kidney morphology, characterized by reduced atrophy and enhanced preservation of normal tissue architecture.

Previous study stated that the effect of ursolic acid regulates oxidative stress and involved in inflammatory responses which are strongly corelated with diabetic conditions.[38] Western blot analysis revealed significantly upregulated levels of inflammatory targets, including TNF-α, IL-1, and IL-6, in DN rats compared to the control group. Conversely, the menthone-treated group exhibited reduced expression levels of these inflammatory markers compared to the standard drug group. Overall, these findings collectively underscore the therapeutic potential of menthone in the management of diabetic nephropathy. Its multifaceted effects, encompassing antioxidative, anti-inflammatory, and renoprotective properties, position menthone as a promising therapeutic agent for battling the renal complications associated with diabetes.

CONCLUSION

This study demonstrates that menthone exhibits renoprotective effects in STZ-induced DN rats. The diabetic rats exhibited characteristic signs of DN, including elevated glucose and glycosylated hemoglobin levels, reduced insulin levels, increased oxidative stress markers, decreased antioxidant levels, elevated levels of blood creatinine, BUN, urine albumin, and heightened pro-inflammatory cytokines in renal tissues. However, oral administration of menthone at doses of 50 mg/kg for eight weeks resulted in significant reductions in hyperglycemia, oxidative stress, and inflammation, along with enhancements in antioxidant levels in the kidneys. These findings suggest that menthone possesses potent renoprotective properties by improving renal function, reducing oxidative stress, and mitigating inflammation in diabetic rats. Consequently, menthone emerges as a promising therapeutic agent for the treatment of diabetic nephropathy. Further investigation through clinical trials is warranted to validate the efficacy and safety of menthone as a potential treatment option for diabetic nephropathy. Research should assess the long-term safety and efficacy of menthone treatment in chronic diabetic conditions. Comparing menthone's efficacy with standard anti-diabetic and renoprotective drugs to evaluate its potential as a complementary or alternative therapy.

ACKNOWLEDGEMENTS

NIL.

IN PRESS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The author received no financial support for the research, authorship and/or publication of this article.

DATA AVAILABILITY

The data can be obtained from the corresponding author upon reasonable request.

STATEMENT OF ETHICAL APPROVAL

The experimental methodology was permitted by the Institute Animal Ethics Committee of Luohe Medical College Luohe City, Henan Province, China.

ABBREVIATIONS

DN: Diabetic Nephropathy; **STZ:** Streptozotocin; **SOD:** Superoxide Dismutase; **GPx:** Glutathione Peroxidase; **GSH:** Reduced Glutathione; **TNF-α:** Tumor Necrosis Factor Alpha; **IL-6:** Interleukin-6; **IL-1:** Interleukin-1; **NF-κB:** Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells; **TBARS:** Thiobarbituric Acid Reactive Substances; **BUN:** Blood Urea Nitrogen.

SUMMARY

This study highlights the renoprotective effects of menthone in STZ-induced DN in rats. Diabetic rats displayed typical DN symptoms, including increased glucose, glycosylated hemoglobin, creatinine, blood urea nitrogen, urine albumin, oxidative stress markers, and pro-inflammatory cytokines, along with reduced insulin and antioxidant levels. Oral administration of menthone for eight weeks significantly improved renal function by reducing hyperglycemia, while increasing kidney antioxidant capacity, reduces oxidative stress and protecting kidney cells. These findings suggest menthone's potential as a therapeutic agent for diabetic nephropathy, warranting further validation through clinical trials.

REFERENCES

1. Kaul K, Apostolopoulou M, Roden M. Insulin resistance in type 1 diabetes mellitus. Metabolism. 2015; 64(12): 1629-39. doi: 10.1016/j.metabol.2015.09.002, PMID 26455399.
2. Markell MS, Friedman EA. Diabetic nephropathy: management of the end-stage patient. Diabetes Care. 1992; 15(9): 1226-38. doi: 10.2337/diacare.15.9.1226, PMID 1396019.
3. Guo L, Jiang B, Li D, Xiao X. Nephroprotective effect of Adropinin against streptozotocin-induced diabetic nephropathy in rats: inflammatory mechanism and YAP/TAZ factor. Drug Des Dev Ther. 2021; 15: 589-600. doi: 10.2147/DDDT.S294009, PMID 33623368.
4. Samsu N. Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. BioMed Res Int. 2021; 2021(1): 1497449. doi: 10.1155/2021/1497449, PMID 34307650.
5. Metcalfe W. How does early chronic kidney disease progress? A background paper prepared for the UK Consensus Conference on early chronic kidney disease. Nephrol Dial Transplant. A background paper prepared for the UK Consensus Conference on early chronic kidney disease. 2007; 22 Suppl 9:ix26-30. doi: 10.1093/ndt/gfm446, PMID 17998229.
6. Tong LL, Adler SG. Diabetic kidney disease treatment: new perspectives. Kidney Res Clin Pract. 2022; 41 Suppl 2:S63-73. doi: 10.23876/j.krcp.21.288, PMID 36239062.
7. Boucek P. Diabetic nephropathy/diabetic kidney disease. Vnitr Lek. 2013; 59(3): 201-3. PMID 23713188.
8. Chaurasia S, Thool AR, Ansari KK, Saifi AI. Advancement in understanding diabetic retinopathy: a comprehensive review. Cureus. 2023; 15(11): e49211. doi: 10.7759/cureus.49211, PMID 38143687.
9. Davidson J. Strategies for improving glycemic control: effective use of glucose monitoring. Am J Med. 2005; 118(9) Suppl 9A: 27S-32S. doi: 10.1016/j.amjmed.2005.07.054, PMID 16224940.
10. Borse SP, Chhipa AS, Sharma V, Singh DP, Nivsarkar M. Management of type 2 diabetes: current strategies, unfocussed aspects, challenges, and alternatives. Med Princ Pract. 2021; 30(2): 109-21. doi: 10.1159/000511002, PMID 32818934.
11. Saxena A. Nutritional problems in adult patients with chronic kidney disease. Clin Queries Nephrol. 2012; 1(3): 222-35. doi: 10.1016/j.cqn.2012.06.007.
12. Warren AM, Knudsen ST, Cooper ME. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. Expert Opin Ther Targets. 2019; 23(7): 579-91. doi: 10.1080/14728222.2019.1624721, PMID 31154867.
13. Bungau SG, Vesa CM, Bustea C, Purza AL, Tit DM, Brisc MC, *et al.* Antioxidant and hypoglycemic potential of essential oils in diabetes mellitus and its complications. Int J Mol Sci. 2023; 24(22): 16501. doi: 10.3390/ijms242216501, PMID 38003691.
14. Zhao H, Ren S, Yang H, Tang S, Guo C, Liu M, *et al.* Peppermint essential oil: its phytochemistry, biological activity, pharmacological effect and application. Biomed Pharmacother. 2022; 154: 113559. doi: 10.1016/j.biopha.2022.113559, PMID 35994817.
15. Muruganathan U, Srinivasan S, Vinothkumar V. Antidiabetogenic efficiency of menthol, improves glucose homeostasis and attenuates pancreatic β-cell apoptosis in streptozotocin-nicotinamide induced experimental rats through ameliorating glucose metabolic enzymes. Biomed Pharmacother. 2017; 92: 229-39. doi: 10.1016/j.biopha.2017.05.068, PMID 28549291.
16. Bouyahya A, Mechchate H, Benali T, Ghchime R, Charfi S, Balahbib A, *et al.* Health benefits and pharmacological properties of carvone. Biomolecules. 2021; 11(12): 1803. doi: 10.3390/biom11121803, PMID 34944447.
17. Ueda N. A rheostat of ceramide and sphingosine-1-phosphate as a determinant of oxidative stress-mediated kidney injury. Int J Mol Sci. 2022; 23(7): 4010. doi: 10.3390/ijms23074010, PMID 35409370.
18. Zhao W, Yang C, Zhang N, Peng Y, Ma Y, Gu K, *et al.* Menthone Exerts its antimicrobial Activity against methicillin resistant Staphylococcus aureus by Affecting Cell Membrane Properties and Lipid Profile. Drug Des Dev Ther. 2023; 17: 219-36. doi: 10.2147/DDDT.S384716, PMID 36721663.
19. Shahin D H H, Sultana R, Farooq J, Taj T, Khaiser UF, Alanazi NS, *et al.* Insights into the uses of traditional plants for diabetes nephropathy: a review. Curr Issues Mol Biol. 2022; 44(7): 2887-902. doi: 10.3390/cimb44070199, PMID 35877423.
20. Yadav D, Pandey V, Srivastava S, Tripathi YB. New herbal approaches for the treatment of diabetic kidney diseases and its therapeutic implications. In: Management Association IR, editor. InFood science and nutrition: breakthroughs in research and practice. IGI Global; 2018. p. 321-60. doi: 10.4018/978-1-5225-5207-9.ch015.
21. Feitosa KA, Zaia MG, Rodrigues V, Castro CA, Correia RO, Pinto FG, *et al.* Menthol and menthone Associated with Acetylsalicylic Acid and Their Relation to the Hepatic Fibrosis in Schistosoma mansoni Infected Mice. Front Pharmacol. 2017; 8: 1000. doi: 10.3389/fphar.2017.01000, PMID 29403382.
22. Motawi TK, Ahmed SA, Hamed MA, El-Maraghy SA, Aziz WM. Combination of melatonin and certain drugs for treatment of diabetic nephropathy in streptozotocin-induced diabetes in rats. Diabetol Int. 2016; 7(4): 413-24. doi: 10.1007/s13340-016-0268-9, PMID 30603294.
23. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-10. doi: 10.1016/s0076-6879(78)52032-6, PMID 672633.
24. Nishikimi M, Rae NA, Yagi K. The occurrence of superoxide anion in the action of reduced phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun. 1972; 46: 849-54.
25. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47(2): 389-94. doi: 10.1016/0003-2697(72)90132-7, PMID 4556490.
26. Moron MS, Depierre JW, Mannervik B. Level of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979; 582(1): 67-78. doi: 10.1016/0304-4165(79)90289-7, PMID 760819.
27. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82(1): 70-7. doi: 10.1016/0003-9861(59)90090-6, PMID 13650640.
28. Suzuki H, Suzuki K. Rat hypoplastic kidney (hpk/hpk) induces renal anemia, hyperparathyroidism, and osteodystrophy at the end stage of renal failure. J Vet Med Sci. 1998; 60(10): 1051-8. doi: 10.1292/jvms.60.1051, PMID 9819756.
29. Wang X, Kang J, Liu Q, Tong T, Quan H. Fighting diabetes mellitus: pharmacological and non-pharmacological approaches. Curr Pharm Des. 2020; 26(39): 4992-5001. doi: 10.2174/1381612826666200728144200, PMID 32723251.
30. Pandey M, Debnath M, Gupta S, Chikara SK. Phytomedicine: an ancient approach turning into future potential source of therapeutics. J Pharmacogn Phytother. 2011; 3(3): 27-37.

IN PRESS

31. Kirichenko TV, Sukhorukov VN, Markin AM, Nikiforov NG, Liu PY, Sobenin IA, *et al.* Medicinal plants as a potential and successful treatment option in the context of atherosclerosis. Front Pharmacol. 2020; 11: 403. doi: 10.3389/fphar.2020.00403, PMID 32322201.
32. Shabab S, Gholamnezhad Z, Mahmoudabady M. Protective effects of medicinal plant against diabetes induced cardiac disorder: a review. J Ethnopharmacol. 2021; 265: 113328. doi: 10.1016/j.jep.2020.113328, PMID 32871233.
33. Velu G, Palanichamy V, Rajan AP. Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. Bioorganic phase in natural food: an overview; 2018. p. 135-56.
34. Malekmohammad K, Rafieian-Kopaei M, Sardari S, Sewell RD. Toxicological effects of *Mentha x piperita* (peppermint): a review. Toxin Rev. 2021; 40(4): 445-59. doi: 10.1080/15569543.2019.1647545.
35. Yeshi K, Wangchuk P. Essential oils and their bioactive molecules in healthcare. In: Inherbal biomolecules in healthcare applications. Academic Press; 2022: 215-37. doi: 10.1016/B978-0-323-85852-6.00006-8.
36. Mullins LJ, Conway BR, Menzies RI, Denby L, Mullins JJ. Renal disease pathophysiology and treatment: contributions from the rat. Dis Model Mech. 2016; 9(12): 1419-33. doi: 10.1242/dmm.027276, PMID 27935823.
37. Darenskaya M, Kolesnikov S, Semenova N, Kolesnikova L. Diabetic nephropathy: significance of determining oxidative stress and opportunities for antioxidant therapies. Int J Mol Sci. 2023; 24(15): 12378. doi: 10.3390/ijms241512378, PMID 37569752.
38. Ma X, Zhang Y, Wang Z, Shen Y, Zhang M, Nie Q, *et al.* Ursolic acid, a natural nutraceutical agent, targets caspase3 and alleviates inflammation-associated downstream signal transduction. Mol Nutr Food Res. 2017; 61(12): 1700332. doi: 10.1002/mnfr.201700332, PMID 28801966.

Cite this article: Zhang T, Wang D, He X. Anti-Diabetic and Renoprotective Effects of Menthone in Streptozotocin-Induced Diabetic Nephropathy in Rats. Indian J of Pharmaceutical Education and Research. 10.5530/ijper.20251228.

IN PRESS