

The Attenuative Effects of Zinc Oxide Nanoparticles and S-Methylmethionine Sulfonium Chloride Against D-Galactose Induced Liver Oxidative Stress and Apoptosis in Albino Rats

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ABSTRACT

D-galactose has been documented to induce a sign of aging and is linked to liver injury. Our study aimed to evaluate the protective effect of zinc oxide nanoparticles and S-Methylmethionine sulfonium chloride against D-galactose-induced liver injury in rats and explores their antioxidant, anti-inflammatory, and anti-apoptotic role. This study was carried out on 40 male Wister albino rats and divided into four groups. In the first group; the normal group did not receive any treatment, 2nd group; rats were injected intraperitoneally with a dose of 50 mg/kg daily of D-galactose for two months, 3rd group; rats received D-galactose at the same dose and received Zinc oxide nanoparticle orally by a dose (10 mg/kg/daily), 4th group; received D-galactose by the same dose and was received S-Methylmethionine by a dose (50mg/kg/daily). The present study revealed that intraperitoneal injection of D-galactose (50 mg/kg/day) leads to a marked loss of body weight, a markedly increase in lipid profile and blood glucose level, a markedly increase in malondialdehyde (MDA) level in the liver, and a markedly decrease in glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) in the liver. D-galactose has a role in elevating hemoglobin A1c (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), albumin, and fructosamine, while in the gene expression, there was an increase in inducible nitric oxide synthase (iNOS), tumor necrosis factor α (TNF- α), transforming growth factor β (TGF- β), interleukin 6 (IL-6), BAX gene expression and decrease in Bcl2 gene expression in the liver. Zinc oxide nanoparticles and S-Methylmethionine sulfonium chloride are novel treatments that play an important role in maintaining weight, managing normal lipid profile, blood glucose levels, oxidant, and antioxidant enzymes, HbA1c, ALT, AST, GGT, albumin, and fructosamine. The effect of these substances was extended to the level of gene expression of the tested genes. In conclusion, zinc oxide nanoparticles and S-Methylmethionine attenuated oxidative stress and hepatic damage induced by D-galactose treatment.

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Key words

Zinc oxide nanoparticles, MMSC, D-galactose, Oxidative stress, Anti-aging, Antioxidant

INTRODUCTION

Aging is a natural process associated with many chronic diseases, including cancer, retinopathy,

atherosclerosis, and Parkinson's disease (Franceschi *et al.*, 2018). Nowadays, worldwide suffer from the elderly population; thus, anti-aging has become an essential topic in recent years. One of the aging process's pathophysiology is thought to be oxidative damage induced by reactive oxygen species (ROS) (Liguori *et al.*, 2018). An excessive ROS can harm phospholipids and proteins and induce DNA damage, interfering with their proper function (Valiko *et al.*, 2007), leading to increased membrane lipid peroxidation and activation of apoptosis, ultimately causing cell damage (Aydm *et al.*, 2016; Xu *et al.*, 2016). Furthermore, signal transduction, gene expression, and redox regulation are all influenced by ROS (Schieber and Chandel, 2014). The most influenced organs by aging are the liver and brain (Wang *et al.*, 2012).

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Chronic D-galactose exposure has been shown to cause symptoms similar to natural aging (Hsieh *et al.*, 2009). D-galactose is a reducing sugar that can be entirely metabolized in a normal state, while a high intake of D-galactose can be transformed into aldose, hydrogen peroxide, and galactose oxidase; they operate as catalysts, converting superoxide anions and oxygen into free radicals (Xu and Zhao, 2002; Lu *et al.*, 2006, 2007) reviewed in (Wu *et al.*, 2008). D-galactose easily interacts with free amines of amino acids in proteins and peptides to create advanced glycation end products (AGEs) either *in vitro* or *in vivo* (Tian *et al.*, 2005; Lu *et al.*, 2007) reviewed in (Lu *et al.*, 2010), and AGEs can induce ROS accumulation, especially superoxide radicals and hydrogen peroxide, that induce oxidative damage linked to inflammatory damage and apoptosis in D-galactose treated rats (Hsia *et al.*, 2012). As a result, D- galactose-treated rats are increasingly being used as animal models for anti-aging and organ injury studies (Chen *et al.*, 2018).

Zinc oxide nanoparticles (ZnONPs) are nano-sized particles with a diameter of less than 100 nm that has been identified as an essential factor in biomedicine (Dawei *et al.*, 2009), and it has excellent attention in commercial and biomedical applications because its anti-bacterial, anti-inflammatory, anti-cancer, anti-diabetes properties due to its ability to release zinc ions (Jiang *et al.*, 2018). Many studies reported the cytotoxicity of ZnONPs and inducing DNA damage (Singh *et al.*, 2020). Other studies reported the beneficial effect of ZnONPs because they have antioxidant activity and free radical scavenging (Al-Mohaimed *et al.*, 2022). Most mammalian enzymes contain zinc, which serves as a structural, catalytic, and regulatory component, and it plays a crucial role in vital biological functions such as DNA replication, DNA synthesis, cell division, and oxidative stress protection (Roy *et al.*, 2013). Many previous studies documented that zinc has a protective antioxidant because of its capacity to make malondialdehyde (MDA) levels closer to normal levels (Dani and Dhawan, 2005; Malekirad *et al.*, 2010). Thus, the current study investigates the low dose of ZnONPs as anti-aging in rats.

S-Methylmethionine Sulfonium Chloride (MMSC) is a methionine derivative in many vegetables, including cabbage, kohlrabi, turnips, tomatoes, and celery (Turner and Shapiro, 1961; Hattula and Granroth, 1974). Many reports documented the anti-inflammatory, antiulcer, anti-depressant, and cytoprotective effects of MMSC (Lee *et al.*, 2012), and our previous study reported the anti-cancer and antioxidant activity of MMSC (Abouzed *et al.*, 2021). This current study investigates the effect of ZnONPs and MMSC in D-galactose-induced oxidative stress and apoptosis in albino rat liver.

MATERIALS AND METHODS

Chemicals

ZnONPs were obtained from Sigma-Aldrich (St. Louis, MO, USA) in the form of dispersion with an average <35 nm and with a concentration of 50 wt% water and were suspended in 0.9% saline before use (Torabi *et al.*, 2013). D-galactose was purchased from Sigma -Aldrich (purity <99%). MMSC was obtained from (Xi'an Realin Biotechnology Company (Xi'an, China). Easy-REDTM Total extraction kit, HiSenScriptTM RH (-) cDNA synthesis kit was supplied by INTRON Biotechnology, Inc. (Gyeonggi-do, Korea). QuantiTect SYBR Green PCR Kit was obtained from QIAGEN (Hilden, Germany). All biochemical kits were purchased from Biodiagnostics (Cairo, Egypt).

Animals

A total of 40 adult male albino rats weighing about (200-250 g) were used in the current study. They were obtained from the Egyptian Association of Biological Products and Vaccines (Aguza, Giza, Egypt). They were housed (4 animals/cage). Rats received a standard fresh pellet diet and clean drinking water *ad-libitum*. For 2 weeks before starting the experiment for acclimatization. The experimental animal was housed under standard laboratory conditions, temperature (25±2°C), humidity (60 to 65%), and lighting and dark condition (equal to 12 h light/ 12 h dark). This experiment was approved by Kafr Elsheikh University Animal Care Guidelines and National Scientific Council Guidelines for the Management and Use of Laboratory Animals.

Experimental design

After two weeks of acclimatization, the animals were divided into 4 groups (10 rats per group). Group I rats received 0.9 % normal saline as a vehicle. Rats received a D-galactose intraperitoneal dose of 50 mg/kg/ body weight daily for 8 weeks. Group III rats received ZnONPs orally daily by stomach tube by a dose (10 mg/ kg/ body weight) (Umrani and Paknikar, 2014) with an intraperitoneal injection of D-galactose by the same dose as group II. In Group IV, rats received MMCS orally at 50mg/kg/body weight/daily for 8 weeks (Abouzed *et al.*, 2021) with D-intraperitoneal galactose injection by D-intraperitoneal galactose injection by the same dose as previously mentioned in group II for 8 weeks.

Body weight and liver weight analysis

The body weight and liver weight analysis was done at the end of the experiment after mild anesthesia of the animal by intraperitoneal injection of thiopental sodium

(50 mg/kg body weight) (Abouzed *et al.*, 2021), and record the body weight and liver weight of rats in each experiment group.

Blood sample

At the end of experiments, and after exposing each experiment, rats for mild anesthesia, as mentioned previously. The blood samples were taken from the retro-orbital venous plexus of rats, kept at room temperature for 30 min in a clean, dry Eppendorf tube, and centrifuged at 3000 rpm for 20 min. After that, the clear sera samples were transferred into clean, dry Eppendorf tubes and kept at -20 °C until biochemical analysis.

Tissue sample

After 8 weeks (end of the experiment), the rats were decapitated. The livers of each rat were removed and divided into three parts. The first part of the hepatic tissue sample was taken immediately, snap-frozen in liquid nitrogen, and stored at -80 °C until RNA extraction and real-time PCR; the second part was stored at -80 °C to assess oxidative and antioxidant status. While the third part of the liver tissue was kept in 10% formalin for histopathological analysis.

Biochemical assay

Serum blood glucose level (Trinder, 1969), triacylglycerol (Fassati and Prencipe, 1982), total cholesterol (Allain *et al.*, 1974), HDL-cholesterol (Lopes-Virella *et al.*, 1977), LDL-cholesterol (Wieland and Seidel, 1983), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel, 1957), gamma-glutamyl transaminase (γGT) (Szasz, 1974), and albumin (Doumas *et al.*, 1971) were measured according to the manufacturer's instructions.

Assessment of malondialdehyde concentration and antioxidant activity in the liver tissue

One gram of liver tissue was homogenized in 5 mL cold potassium phosphate buffer (100 mM, pH 7), centrifuged for 15 min at 1520xg, and collected the supernatant in clean, dry Eppendorf tubes and stored at -80°C until the lipid peroxidation and antioxidant assay. The hepatic malondialdehyde (MDA) concentration was colorimetrically measured according to Ohkawa *et al.* method (1979). In contrast, the activity of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase were assessed according to these methods Beutler *et al.* (1963), Nishikimi *et al.* (1972) and Aebi (1984), respectively.

Histopathology analysis

The liver tissue of all animals was processed and

sectioned for histopathology analysis in all experimental groups. The technique can be stated as follows: After fixing liver tissue in 10% formalin buffer, the sample was dehydrated in ascending concentration of ethyl alcohol, washed in xylene, and embedded in paraffin blocks. The section was made by 5μ thickness, which was subsequently stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2008). The histological alterations of liver tissue among the different experimental groups were examined under a light microscope and were photographed using a digital camera computer interface (Nikon digital camera, Japan).

RNA isolation and complementary DNA synthesis

TRizol was used to extract the total RNA from the hepatic tissue of different experimental groups according to the manufacturer's instructions. The RNA pellet was dissolved in 50μl RNase-free water, and its concentration was determined using Nanodrop 2000c (ThermoScientific, USA). One strand of cDNA was synthesized using the HiSenScriptTM (-) cDNA synthesis kit by following the manufacturer's instructions, which included mixing 10μl of 2X RT reaction solution, 1μl of enzyme mix solution, 1μg of RNA, and completing to 20μl final volume by RNase free. The mixture was then incubated for 30 min at 50°C and 10 min at 85°C.

Real-time quantitative polymerase chain reaction

Quantitative real-time PCR (qRT-PCR) analysis was performed using an iQ5 Real-time PCR machine (Bio-Rad) with SYBR green and primers which MacroGen Co. manufactured in Seoul, Korea, and primer sequences were listed in (Table I). The real-time programming conditions are as follows: Denaturation at 92°C for 10 min, followed by 40 cycles at 92°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec. The gene expression difference among the experimental groups was evaluated using $\Delta\Delta C_t$ and expressed as relative mRNA levels compared to the placebo group after normalization to β-actin.

Statistical analysis

Data were expressed as means ± SEM. The statistical significance was determined by one-way analysis of variance (ANOVA) utilizing (GraphPad Software Inc., San Diego, CA, USA), and the Bonferroni test analyzed the individual difference among experimental groups. Values were considered statistically significant at $P \leq 0.05$.

RESULTS

Body weight, liver weight, and relative liver weight

Our study revealed that injection of 50 mg/kg body

weight of D-galactose in rats showed a significant decrease ($P < 0.0001$) compared to the normal group. In contrast, ZnONPs and MMSC were significantly increased ($p < 0.0001$) compared to the group treated with D-galactose. Likewise, the liver and relative liver weight was significantly increased ($P < 0.0001$) in D-galactose treated groups compared to the normal group. On the other hand, the ZnONPs and MMSC significantly ameliorated the liver and relative liver weight compared with the D-galactose treated groups, as shown in (Table II).

Biochemical parameters

D-galactose significantly increased (P -value < 0.0005) the glucose, cholesterol, HDL, TG, VLDL, fructosamine, HbA1c, ALT, AST, GGT, and albumin compared to the normal group. ZnONPs and MMSC managed to significantly lower (P -value < 0.0005) all these biochemical parameters compared to rats treated with D-galactose (Table III).

Hepatic oxidant and antioxidant

In our study, D-galactose-treated rats showed a

significant increase ($P < 0.0001$) in the MDA concentration and a decrease in the antioxidant activity, including (SOD,

Table I. Primer sequences used for Real-time PCR analysis for liver tissues.

Gene	Accession number	Primer 5'→3'
<i>TNF-α</i>	XM-110221	F GACCCTCACACTCAGATCATCTTCT R TTGTCTTTGAGATCCATGCCATT
<i>iNOS</i>	NC_005103.4	F TCTTCAAGGACCTACCTCAGGC R GCTAAGGCAAAGCTGCTAGGTC
<i>TGF-β1</i>	NM_021578.2	F TCACTTGTTTTGGTGGATGC R TTCTGTCTCTCAAGTCCCCC
<i>IL-6</i>	NW_047814.1	F TCCTACCCCAACTTCCAATGCTC R TTGGATGGTCTTGGTCTTAGCC
<i>Bax</i>	NM_007527	F CTGAGCTGACCTTGAGC R GACTCCAGCCACAAAGATG
<i>Bcl2</i>	NM_016993.2	F GGAGGGCACTTCCTGAG R GCCTGGCATCACGACT
<i>β-actin</i>	NM_031144.3	FTGTTGTCCCTGTATGCCTCT R TAATGTCACGCACGATTTC

Table II. The effect of ZnONPs and MMSC on the body weight in gram and relative liver weight in D- galactose treated rats.

	Normal	D-Galactose	ZnONPs +D-Galactose	MMSC+ D-Galactose
Body weight	281.3±9.53 ^a	212.2±4.80 ^c	262.4±5.97 ^b	271.1±6.72 ^{ab}
Liver weight	5.85±0.47 ^c	10.42±0.64 ^a	6.57±0.31 ^b	5.69±0.36 ^{cb}
Relative liver weight	0.021±0.01 ^c	0.049±0.01 ^a	0.025±0.01 ^b	0.021±0.01 ^{cb}

The Data were displayed as mean ± SE. Mean values with different superscript letters in the same raw show a significant difference at $p < 0.05$.

Table III. The effect of ZnONPs and MMSC on the biochemical parameters against D-galactose treated rats.

Biochemical parameters	Normal group	D-galactose group	ZnO NPs +D-galactose group	MMSC+ D-galactose group
Blood glucose (mg/dl)	63.28±6.19 ^c	138.1±7.52 ^a	85.96±3.95 ^b	74.5±2.99 ^{cb}
Cholesterol (mmol/L)	72.25±3.85 ^c	104.8±6.33 ^a	84.08±3.65 ^b	80.2±3.68 ^{cb}
HDL (mmol/L)	47.92±2.40 ^c	85.2±4.09 ^a	62.88±4.28 ^b	59.14±4.36 ^{cb}
TG (mmol/L)	73.25±4.05 ^c	117.4±8.43 ^a	87.75±2.32 ^b	83.75±3.66 ^{cb}
VLDL (mmol/L)	14.6±1.33 ^c	25.08±1.45 ^a	17.16±0.74 ^b	16.6±1.11 ^{cb}
Fractosamine(μmol/l)	164.7±17.38 ^c	501±12.34 ^a	280.3±16.33 ^b	234.7±12.86 ^{cb}
HbA1c (ng/l)	3.6±0.21 ^c	6.5±0.25 ^a	4.33±0.39 ^b	4.2±0.23 ^{cb}
ALT (U/l)	28.5±1.92 ^c	55.63±4.46 ^a	38.88±2.99 ^b	31.75±1.76 ^{cb}
AST (U/L)	147.3±9.49 ^c	293.8±9.56 ^a	214.3±13.29 ^b	184.2±7.71 ^{cb}
GGT (U/l)	3.88±0.21 ^c	8.28±0.299 ^a	6.52±0.36 ^b	4.44±0.23 ^{cb}
Albumin (mg/dl)	1.73±0.19 ^c	4.86±0.29 ^a	3.24±0.30 ^b	2.79±0.19 ^{cb}

The data were displayed as mean ± SE. Mean values with different superscript letters in the same raw show a significant difference at $p < 0.05$. HDL, high-density lipoprotein; TG, triglycerides; VLDL, very low density lipoprotein; HbA1c, hemoglobin A1c; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, Gamma-glutamyl transferase.

GSH, and CAT) when compared to the normal group. ZnONPs and MMSC treatment showed a significant decrease in the MDA level, significantly increasing the activity of SOD, GSH, and CAT compared with D-galactose treated groups, as shown in (Fig. 1).

Histopathological findings

The histopathological analysis of liver tissue showed in all experimental groups was the normal hepatocytes arranged in cords around the central vein structure of liver tissue in the normal group (Fig. 2A, B). In comparison with the normal group, the liver of rats treated with D-galactose showed severe periportal hepatic degeneration (arrow indicates numerous apoptotic cells with nuclear pyknosis and marked cytoplasmic eosinophilia) and periportal mononuclear cells infiltration that confirms the pathological changes in the liver tissue, including severe vascular lesion, fatty change, fibrosis, hepatic degeneration, necrosis, and inflammatory lesions. Furthermore, all hepatic pathological changes induced by D-galactose injection were ameliorated by ZnONPs and MMSC treatments. MMSC treatment showed mild vascular lesion, fibrosis, moderate periportal hepatic apoptosis, and fatty changes (Fig. 2G, H). However, ZnONPs treatment showed mild vascular lesions, apoptosis, and fatty changes and alleviated hepatic fibrosis and necrosis (Fig. 2E, F). All pathological changes in all experimental groups were summarized in (Fig. 2I).

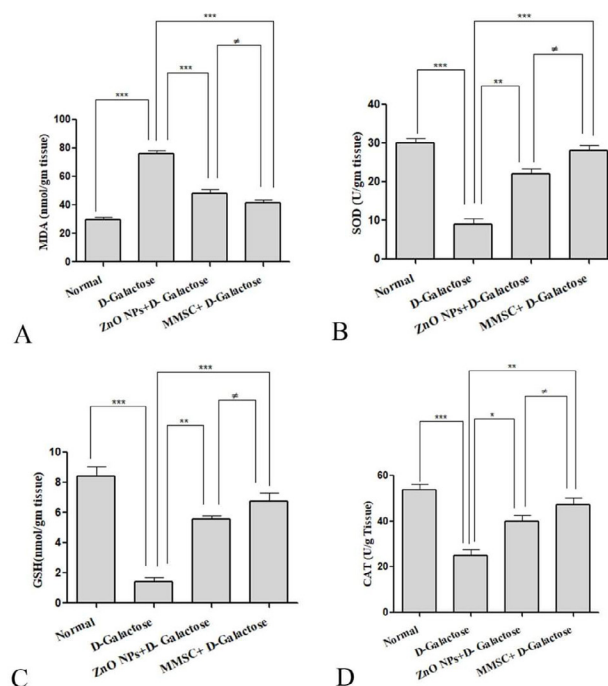


Fig. 1. The hepatic malondialdehyde (MDA) (A), superoxide dismutase (SOD) (B), reduced glutathione (GSH) (C), and catalase (CAT) (D) in all experimental groups. Data were displayed as mean $\pm$ SE *significantly different at $p < 0.05$ using ANOVA followed by Bonferroni's as a post-hoc test.

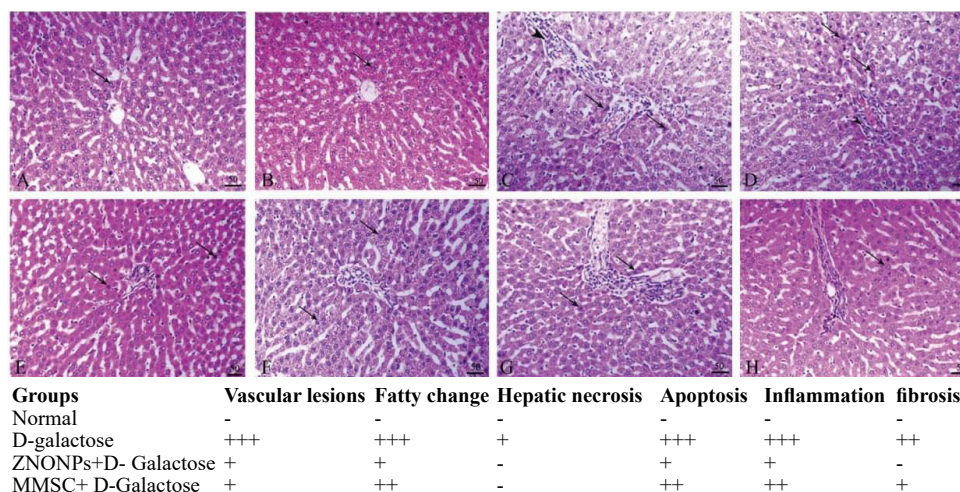


Fig. 2. Histopathology analysis of hepatic tissue in all different experimental groups. Panel A and B (normal group) show normal hepatocytes arranged in cords around the central vein (arrow) which appear to have normal architecture. Panel C, D (D- galactose treated group) showed periportal hepatic degeneration (arrow indicates numerous apoptotic cells with nuclear pyknosis and marked cytoplasmic eosinophilia) and periportal mononuclear cells infiltration (arrowhead). Panel E, F (D- galactose + ZnONPS) shows mild periportal hepatic apoptosis and inflammation (arrows). The panel G, H (D- galactose + MMSC) shows a marked decrease in the periportal hepatic apoptosis and inflammation (arrow indicates few apoptotic hepatocytes). Panel I shows the summary of the pathological changes in all experimental groups. H & E, X200, bar= 50 μ m.

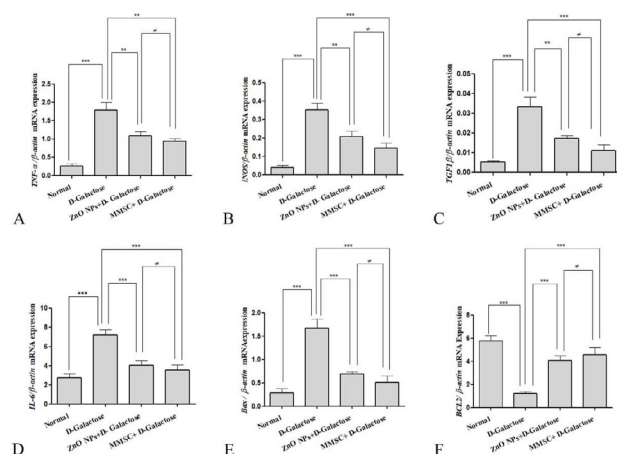


Fig. 3. mRNA expression of genes *TNF-α* (A), *iNOS* (B), *TGF-β1* (C), *IL-6* (D), *Bax* (E), and *BCL2* (F) in the liver tissues. The mRNA expression levels were measured by qRT-PCR and normalized to β -actin-binding protein. Following primer sets were used for the detection (Table I), data are the mean $\pm$ SE, *significantly different at $p < 0.05$ using ANOVA followed by Bonferroni's as a post-hoc test.

Gene expression in the liver

The gene expression level of investigated genes was measured using RT-PCR and normalized to β -actin. Our findings revealed that the expression of inflammatory-related genes, including (*TNF-α*, *iNOS*, *TGF-1β*, *IL-6*), and apoptotic gene (*Bax*), was significantly up-regulated, and the anti-apoptotic gene (*BCL2*) was significantly down-regulated in the group treated with D-galactose in comparison with those in the normal rats. While ZnONPs and MMSC administration significantly ameliorated this gene expression in comparison with the positive control group, as shown in (Fig. 3).

DISCUSSION

Modern science strives to replace traditional medicines with more effective medicines with fewer side effects. This research highlights ZnONPs and MMSC as safe and modern active substances. This study revealed the effect of ZnONPs and MMSC against glycation induced by D-galactose. The D-galactose rat model is widely used to induce glycation and accelerate aging (Wei *et al.*, 2005; Li *et al.*, 2015). D-galactose reduces monosaccharides to bind with free amines for AGEs production (Hegab *et al.*, 2012) reviewed in (Bo-Htay *et al.*, 2018). The animals that received D-galactose daily showed a reduced body weight due to a defect in glucose metabolism (Liu *et al.*, 2019). Many

studies confirmed that D-galactose is vital in increasing blood glucose levels (Omidi *et al.*, 2020). Hyperglycemia may lead to glucotoxicity in many diabetic patients (Jha *et al.*, 2018). D-galactose is one of the reducing sugars; when it increases, it increases reactive oxygen species (ROS) then glycation occurs (Wang, 1999), reviewed in (Hsieh *et al.*, 2009). Based on that, D-galactose causes oxidative stress that significantly reduces antioxidant expression and increases liver damage biomarkers (ALT, AST, GGT, Albumin) (Sofy *et al.*, 2014; Hadzi-Petrushev *et al.*, 2015). Therefore, D-galactose induces liver damage due to unstable liver metabolism that occurs after changes in the activity of serum enzymes (Gao *et al.*, 2018). D-galactose increases lipid profile (Ahangarpour *et al.*, 2018) and gene expression of inflammatory factors (*TNF-α*, *IL-6*, and *NF-κB*), apoptosis-related genes (*Bcl-2* and *Bax*) (Akbari *et al.*, 2022). The lipid profile surge is due to galactose reductase, which converts excess D-galactose to galactitol. The body cannot metabolize the new product and then accumulate galactitol in the cells, leading to ROS generation. ROS generation affects protein, lipid, and DNA, leading to increased DNA, protein, and lipid peroxidation (Bo-Htay *et al.*, 2018). Fructosamine (FRA) is a compound formed during the glycation process (Mawhinney, 2010). D-galactose can increase Fructosamine concentration in the liver, which agrees with (Weng and Wang, 2018), which showed an increase in FRA levels by D-galactose injection in rats.

In our study, ZnONPs significantly manage the liver enzyme biomarkers alternated by D-galactose injection. According to another study, ZnONPs have reduced liver enzymes (ALT, AST, GGT) compared to the positive control group with D-galactose (Bashandy *et al.*, 2018). The liver enzyme's improvement may be due to the ZnONPs antioxidant activity and free radical scavenging (Al-Mohameed *et al.*, 2022). Our findings show that ZnONPs treatment significantly increases the antioxidant activity of SOD, CAT, and GSH and decreases MDA compared to the positive control group with D-galactose (Bashandy *et al.*, 2018). The ZnONPs hepatoprotective effect was confirmed by histopathological analysis that refers to its antioxidant property.

On the other hand, MMSC treatment significantly improves the serum hepatic enzymes and biomarkers, including (AIT, AST, GGT, and Albumin) inconsistent with another study (Abouzed *et al.*, 2021). MMSC has a hepatoprotective effect due to its ability to reduce oxidative damage, which matches many studies (Celik *et al.*, 2021). MMSC plays a vital role in regulating oxidants and antioxidant enzymes (MDA, GSH, SOD, and CAT) (Sokmen *et al.*, 2012; Abouzed *et al.*, 2021) in comparison with D-galactose-treated rats (Parameshwaran

et al., 2010). MMSC is a novel free radical scavenger, as confirmed in many studies (Sokmen *et al.*, 2012; Tunali *et al.*, 2015; Abouzed *et al.*, 2021). The damage induced in the liver occurs due to the activation of pro-inflammatory cytokines by ROS (Cichoż-Lach and Michalak, 2014). ROS is responsible for many pathological conditions (Shields *et al.*, 2021).

Nitric oxide synthase (NOS) is an enzyme that produces Nitric oxide from L-arginine. Nitric oxide has an essential role in many physiological processes and immune defense mechanisms as NO is a free radical with unpaired that plays an essential role in septic shock and may function in autoimmune disease. Inducible nitric oxide synthase (iNOS) gene expression is increased during the aging process caused by D-galactose injections (Fan *et al.*, 2009; Woo *et al.*, 2014). β -actin is considered an endogenous housekeeping gene. β -actin gene expression is always affected by many biological factors, such as growth, differentiation, and pathological conditions (Ruan and Lai, 2007). TNF- α is a part of the immune system as an inflammatory response in case of inflammation. TGF- β 1 is a family of cytokines that has many cellular functions. IL-6 is an interleukin that acts as a pro-inflammatory cytokine and anti-inflammatory myokine encoded by the IL_6 gene. Apoptosis regulator BAX is a member of the Bcl2 gene family responsible for releasing cytochrome c. Our study measured all these gene expressions using RT-PCR in liver homogenate. D-galactose has a role in significantly elevating the gene expression of (iNOS, TNF- α , TGF- β 1, IL-6, BAX) and reducing Bcl2 gene expression (Akbari *et al.*, 2022).

CONCLUSION

The current study shows the protective impact of ZnONPs and MMSC administration against hepatic injury due to D- galactose administration. Through decreasing oxidative damage and increasing the hepatic antioxidant. Furthermore, decreasing the expression of inflammatory and apoptotic-related genes and improving the liver structure.

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IRB approval

Ethical approval was obtained from the Research, Publication, and Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. All experimental procedures are carried out per Kafrelsheikh University's animal care guidelines and the National Science Council's Guide for the Care and Use of Laboratory Animals. All procedures were done and designed to alleviate the suffering of experimental animals.

Data availability

Data are available upon request.

Statement of conflict of interest

The authors have declared no conflict of interest.

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