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Research Article

RENOPROTECTIVE EFFECTS OF GINGER EXTRACT ON METHOTREXATE-INDUCED NEPHROTOXICITY IN RATS: MODULATION OF INFLAMMATORY AND INJURY BIOMARKERS KIM-1 AND LCN-2

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ARTICLE HISTORY	ABSTRACT
Received on: 29-03-2026 Revised on: 11-04-2026 Accepted on: 18-06-2026	Methotrexate is a medication that is employed to treat a variety of cancers and inflammatory diseases; however, its clinical application is restricted by its significant toxicity. Antioxidant properties and a variety of applications against a variety of maladies are possessed by natural ginger extract. The investigation focused on the protective properties of ginger extract in the context of Methotrexate-induced kidney toxicity in rodents. (25) rats were divided into five groups. The negative control (G1) received distilled water, the positive control (G2) received 25 mg/kg bw of MTX, the third group (G3) received 200 mg/kg for body weight of ginger extract, the fourth group received MTX with ginger extract together at concentration (25 mg /kg + 200mg). In comparison, the fifth group received MTX with ginger extract together at concentration 25 mg/kg bw + 400mg. Methotrexate were administered high doses via intraperitoneally one dose on the third day. After finishing of the treatment period, lipid profiles, oxidant-antioxidant, and kidney function and IL-6 level, lipocalin 2 (Lcn-2), Kidney Injury Molecule-1 (Kim-1), sod2 gene, and GPx1. G2exhibite significant decreasing in organ weights and antioxidant concentration, while increasing kidney IL-6, Kim-1, Lcn-2 gene expression and oxidative stress, while gene expression of Gpx1 and sod1 gene was low. G5 showed improved these markers compared to G2. Ginger extract demonstrated higher protective efficacy against MTX -induced kidney toxicity.
Keywords: <i>Zingiber officinale</i> , methotrexate, Oxidative stress, Nephrotoxicity, Antioxidant.	
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INTRODUCTION

Methotrexate is a folate antimetabolite that is employed to treat a variety of conditions, including acute lymphoblastic leukaemia, testicular and breast cancer, lymphoma, osteosarcoma, autoimmune diseases, and psoriasis [1,2]. Despite its efficacy, MTX's clinical application is restricted by its toxicity in numerous tissues [3]. MTX toxicity is depended on the disease, the dose and the period. Haematuria, uremia, and progressive liver fibrosis are the consequences of prolonged treatment with MTX at low concentrations, while acute kidney failure and acute hepatotoxicity are the consequences of high doses of MTX [4]. It has been emphasised that the metabolites of this substance are occure due to the phase I reaction, that causes many tissues damages in the intestine, liver and kidneyalso causes oxidative stress. The

metabolites products of MTX interact with lipids, proteins and DNA, resulting in oxidative stress, DNA damage, lipid peroxidation and protein synthesis disorder [5,6]. Despite the fact that the occurance steps of nephrotoxicity and hepatotoxicity of MTX have not akown, the studies suggest that hepatotoxicity may be oxidative stress consequence [7,8,9]. It undergoes metabolism in the liver, resulting in the formation of its primary metabolite, 7-hydroxy-MTX. Polyglutamate is the form in which this drug is stored in liver cells [10]. Because of MTX's inhibition of the cytosolic NAD-dependent dehydrogenase, liver cells accumulate MTX-polyglutamate, folic acid levels decrease, and liver necrosis occurs [11]. NADP is employed by glutathione reductase to neutralise ROS. NADP are protecting hepatocytes from the damage induced by hypochlorite radicals, anions, OH and H2O2 [12,13]. The accumulation of MTX in kidney

tissues results in renal dysfunction. The kidneys serve as accountable for the elimination of nearly 90% of methotrexate. The risk of renal dysfunction may be elevated by the elevated drug levels in plasma and urine that result from high doses. The prolonged presence of MTX in the plasma is the cause of renal dysfunction [14,15]. There are numerous applications for ginger (*Zingiber officinale*) in all medical settings [16]. It contains numerous bioactive compounds, including 6-shogaol, 6-gingerol, and zingerone, which possess a variety of medicinal benefits [17,18]. *Z. officinale* possesses antioxidant, anti-cancer, anti-diabetic, anti-viral, and anti-inflammatory properties [19,20]. The oxidative stress reduction and the inflammatory response, *Z. officinale* mitigated renal injury caused by lead [22] and carbon tetrachloride (CCl₄) in rodents [21]. In rodents, *Z. officinale* is well-tolerated and does not produce any adverse effects [23]. The protective mechanism of *Z. officinale* on DN is not completely comprehended. Consequently, the current investigation was undertaken to investigate the ameliorative impact of *Z. officinale* on renal injury induced by methotrexate in rodents.

MATERIALS AND METHODS

Animal ethics and care

The animals employed in this investigation are procured and cared for in accordance with national and international animal welfare regulations. The rodents, which were aged 4-6 weeks and weighed 180–220 grammes, were introduced to the animal house environment. This environment consisted of a chamber with a 50%:50% light/dark ratio in room temperature, and good ventilation. The rodents were provided with unlimited access to water and were fed a standard diet.

Preparation of ethanolic extract of Ginger

Ginger ethanolic extract was prepared at 65°C using 50 grammes of ginger and absolute ethanol, as determined by Soxhlet. The extract was converted into a container and deposited in an electric oven at 60°C. A dark container was used to chill the extract until it was ready for use [24,25].

Drug Doses and Preparations

Normal saline was supplemented with 50mg of methotrexate (Ebewe Pharma, Austria) in a 5ml volume. Ginger unadulterated powder was suspended in distilled water at a concentration of 0.5% carboxymethylcellulose to ensure that 1 ml of the vehicle. All medications were prepared immediately prior to administration.

Study Design

On the third day of the experiment, a kidney toxicity model was administering of methotrexate (25 mg/kg) single dose intravenously (I.P.). This was done to induce acute renal injury. Ginger was administered to the third, fourth, and fifth groups for six days, three days prior to and three days following the MTX administration. A total of 25 rodents were divided into five groups (five rats in each group), as follows:

Control group (G1): administered normal saline (1) ml by gavage for six days.

MTX group (G2): administered MTX (25mg/kg, i.p.) [26].

Ginger group (G3): administered ginger (200 mg/kg, orally) [27], in 1ml of 0.5% CMC orally for six days.

MTX+ ginger group (G4): administered MTX (25 mg/kg, i.p.), and ginger (200mg/kg, orally) 3 days before and after MTX administration [28].

MTX+ ginger group (G5): administered MTX (25 mg/kg, i.p.), and ginger (400mg/kg, orally) 3 days before and after MTX administration [28]. On the seventh day, rats were sacrificed under anaesthesia. Blood samples were collected into tubes by cardiac puncture technique. Samples of the kidneys of rodents were subsequently obtained.

Blood Samples Preparation

Clot activator was combined with blood in the tube. The blood was centrifuged at 3500 rpm for 15 minutes using a SIGMA SM7000 centrifuge (SIGMA, UK) after being allowed to coagulate for ten minutes in order to obtain serum. Prior to biochemical analysis, the serum was refrigerated at –80°C for a maximum of four weeks.

Biochemical assays

The manufacturer's reagent protocols (SOLARBIO, China) were followed to evaluate the levels of MDA, CAT, SOD, Urea nitrogen, creatinine, and BUN: creatinine ratio.

Assessment of serum IL-6, Lcn-2 and TNF α levels.

Rat IL-6, Lcn-2 and TNF α are estimate by ELISA kits (Sunlong Company, China) for the assessment of the study parameters based on the company instructions.

Molecular analysis

Gene expression of IL-6, Kim-1, Lcn-2, GPx1 and sod2 gene

Extraction of RNA done from renal tissues by Accu-Zol® total RNA Extraction solution (Bioneer, Korea). cDNA was synthesised from extracted RNA by M-MLV reverse transcriptase (Bioneer, Korea). The manufacturer's instructions were followed during the procedure.

Real-Time PCR

The IL-6, Kim-1, Lcn-2, GPx1, and sod2 genes were analysed using RT-PCR, following the procedures by housekeeping gene GAPDH (29). The fold changes were determined by the gene expression of GAPDH and the target gene [30].

Statistical Analysis

The values were expressed as mean $\pm$ SE. ANOVA test compares the data, and the Newman-Keuls test identifies marked differences ($p < 0.05$). GraphPad Prism V5 (USA) (31) was conduct the statistical analysis.

RESULTS

Relative organ's weight

The organ weights (g/100 g of BW) of the kidney and liver were elevated in MTX-exposed rats that were

not treated with ginger (G2) and those that were treated with ginger (G3) in comparison to the control rat (G1). This is illustrated in Table 1. Conversely, the control (G1) and the group that was not exposed to MTX-treated rats with ginger extract (G3) exhibited negligible differences when compared to the group that was treated with MTX-Ginger (G4 and G5).

Serum biochemical profile

In the control group, the MDA levels of MTX-exposed rodents, whether untreated (G2) or treated with Ginger (G4 and G5), are significantly elevated (Fig. 1). In contrast, CAT and SOD level were lower in the MTX-induced toxic untreated rats (G2) and the MTX-induced treated rats (G4 and G5) compared to the control (G1) and non-toxic treated rats with Ginger extract (G3). BUN, creatinine, BUN:creatinine ratio, and bilirubin were all elevated in MTX-induced rats (G2, G4, G5) compared to control rats (G1). Conversely, non-toxic induced rats (G3) treated with ginger extract exhibited a significant decrease in comparison to control rats, as illustrated in Figure 2.

Fig.3 showed T-c and LDL-c level were increased, while HDL-c was significantly decreased in (G2) as compared to (G1) and non-toxic rats and MTX-treated with gingerextract (G3,G4 and G5).

G2 exhibited the highest levels of kidney IL-6, Kim-I, and Lcn-2 gene expression, as well as the lowest levels of kidney Gpx1 and sod1 gene expression (Fig. 4). Conversely, G3 exhibited the lowest levels of kidney IL-6, Kim-I, and Lcn-2 gene expression and the highest levels of kidney Gpx1 and sod1 gene expression. In comparison to the MTX (G2) group, the rats with (GE 300 mg) and MTX (G4 group) and (GE 400 mg) and MTX (G5 group) showed the highest levels of kidney Gpx1 and sod1 gene expression, as well as the lowest levels of IL-6, Kim-I, and Lcn-2 gene expression.

Table 01: Relative organ weight (g/100gbw) of MTX- induced toxic rats treated with ginger extract

Organs wt (%)	G1	G2	G3	G4	G5
Liver	3.22± 0.25 c	6.26 ± 0.41 A	3.18 ± 0.24 c	4.10 ± 0.28 B	3.60±0.2 lc
Kidney	5.29± 0.26 c	7.73 ± 0.22 A	5.36 ± 0.18 c	5.75 ± 0.47 B	5.55± 0.38 c

The values were expressed as mean±SE, that analyzed by ANOVA-I and Newman-Keulstest. The difference express as different letters. The rats were treated with distilled water (G1), methotrexate; MTX(G2), ginger extract; GE (G3), ginger extract (200 mg)and MTX (G4), ginger extract(400 mg)and MTX (G5).

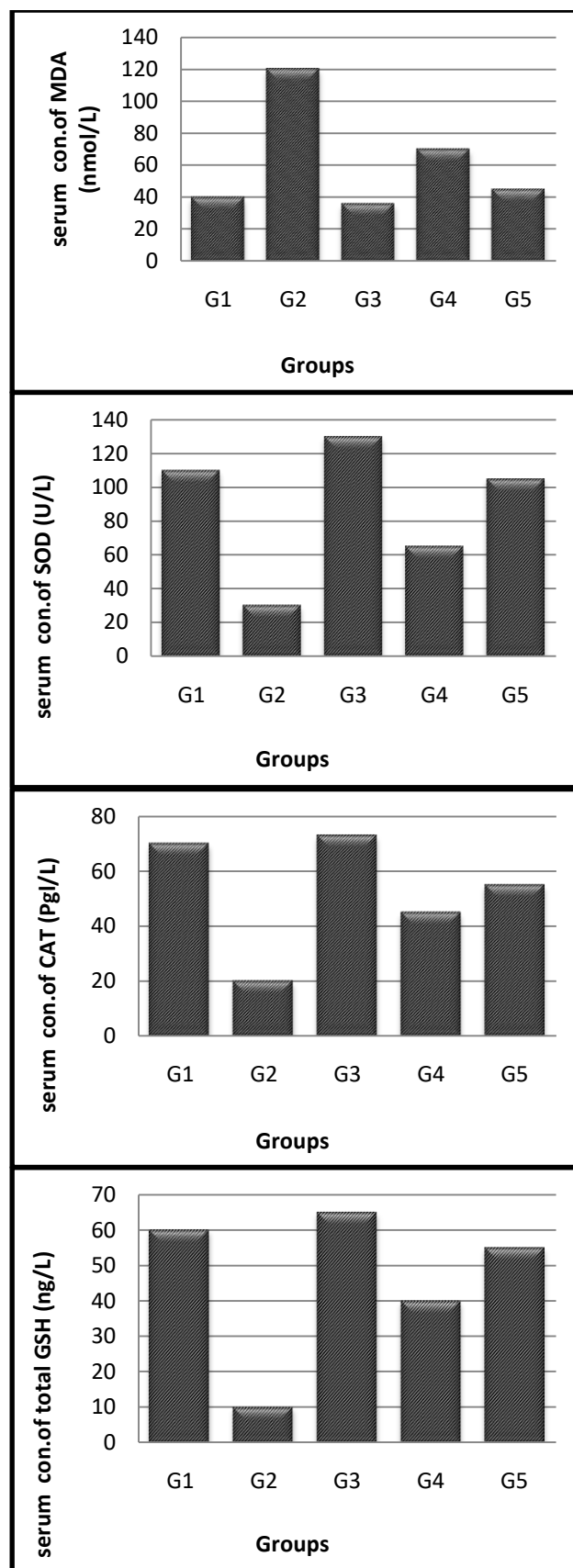


Figure 01: Serum concentration of oxidants (MDA; nmole/L) and antioxidant (CAT; pg/L, SOD; U/L, total GSH; U/L) in rats treated with distilled water (G1), MTX;(G2), Gingerextract;(G3), Ginger extract(200 mg) and MTX (G4) , Ginger extract (400 mg) and MTX (G5).

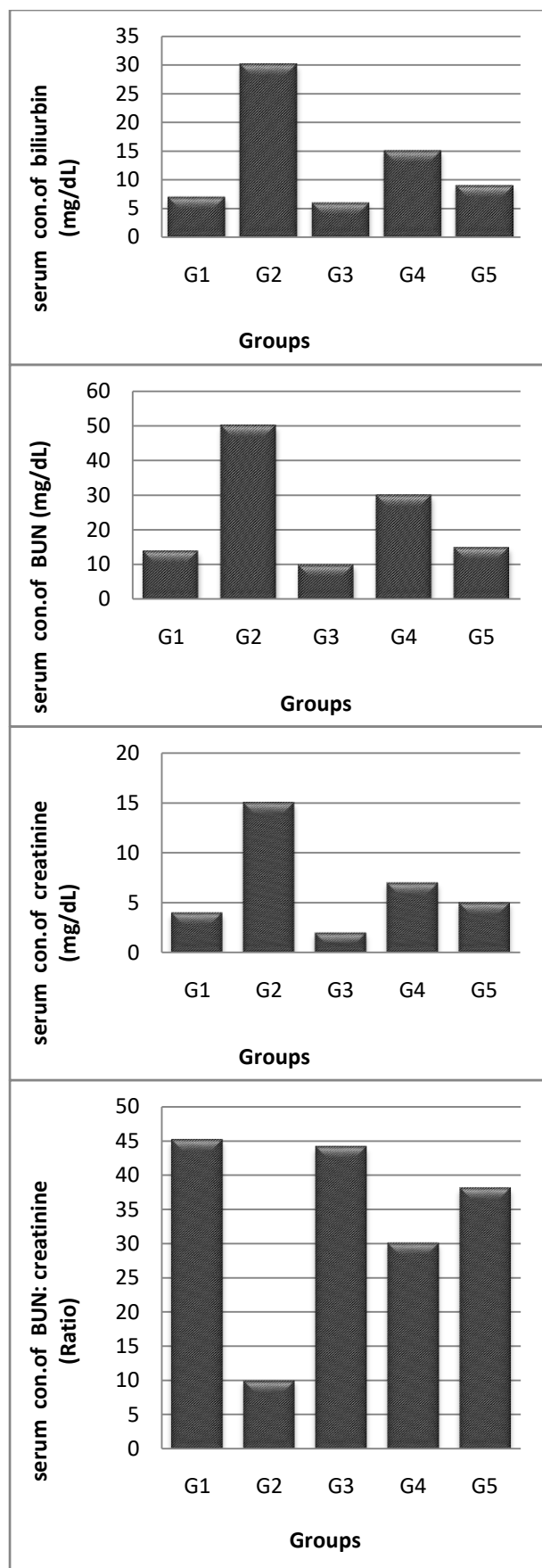


Figure 02: Kidney function tests (bilirubin; mg/dL, urea; mg/dL, creatinine; mg/dL, and BUN:creatinine ratio) in the study groups.

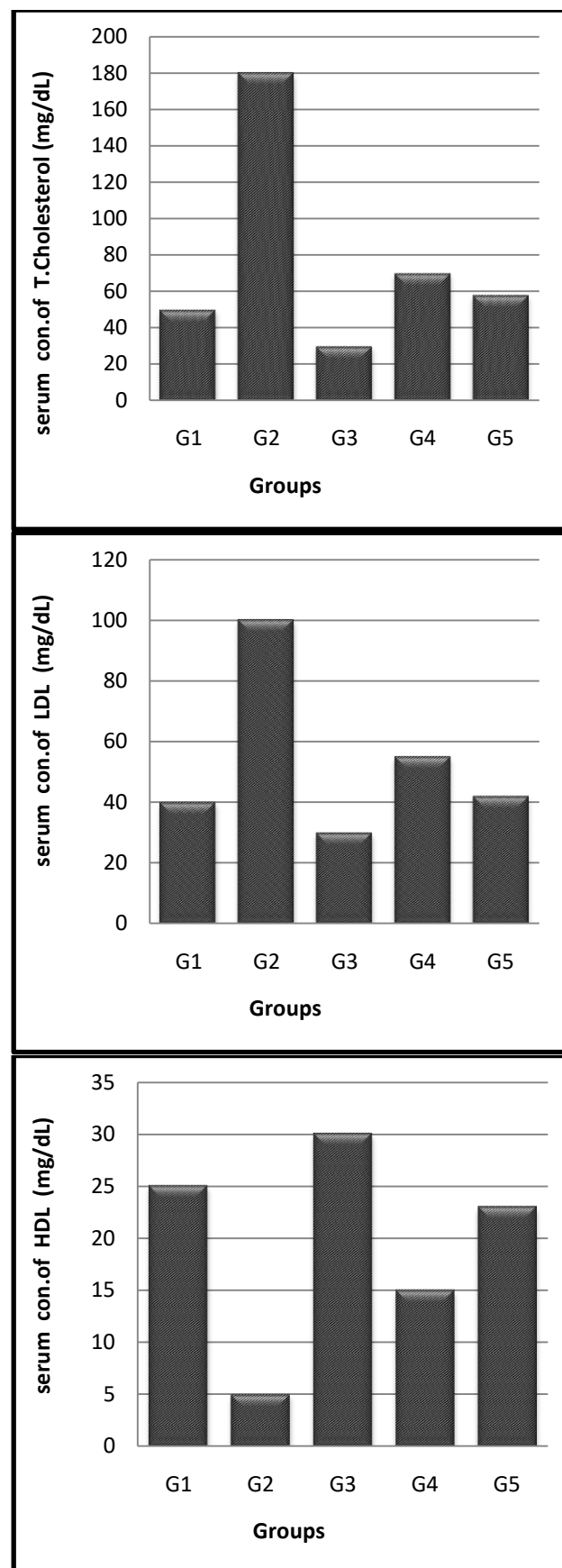


Figure 03: lipid profile (cholesterol; mg/dL, HDL-c; mg/dL, LDL-c; mg/dL; and VLDL-c; mg/dL) in the study groups.

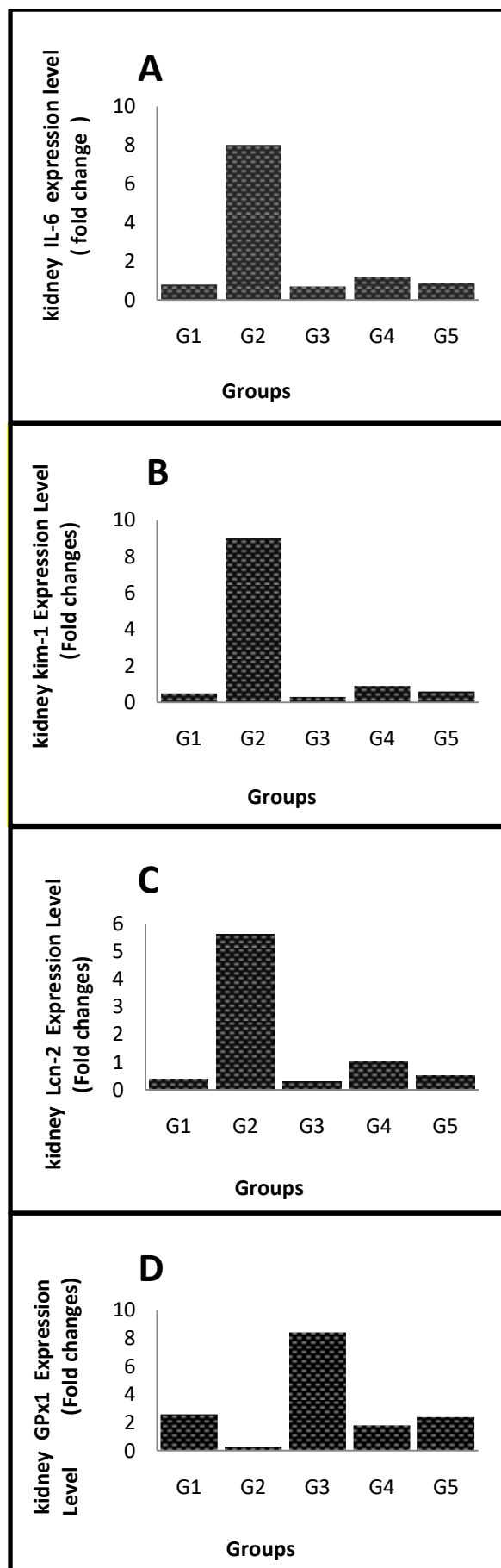


Figure 04: kidney expression level of IL-6 (A), Kim-1 (B), Lcn-2 (C), GPx1(D) and sod1 (E) genes in rats treated with methotrexate ;MTX (G2), Ginger; GE (G3), GE (200 mg) and MTX (G4),GE(400 mg) and MTX (G5) .

DISCUSSION

This study investigated the potential renoprotective effects of ginger extract (GE) against methotrexate (MTX)-induced kidney injury in rats. Our findings demonstrate that GE significantly attenuated MTX-induced nephrotoxicity, as evidenced by improvements in renal function, reduced oxidative stress and inflammation, modulation of key kidney injury biomarkers, and improvements in lipid profiles. Methotrexate-Induced Renal Injury Consistent with previous research (32), MTX administration resulted in significant renal dysfunction, characterized by elevated serum urea and creatinine levels. This was accompanied by increased oxidative stress (MDA) and decreased (SOD, CAT, and GSH) enzymes. Furthermore, MTX induced a pro-inflammatory state, demonstrated by increased serum of IL-6, Lcn-2, and TNF- α level. At the molecular level, MTX significantly upregulated the gene expression of Kim-1, Lcn-2, and IL-6 in kidney tissues, confirming the induction of renal injury and inflammation. Conversely, the expression of antioxidant genes GPx1 and SOD1 was significantly reduced, highlighting the disruption of the cellular redox balance. These results align with established mechanisms of MTX-induced nephrotoxicity, which involve increased ROS production, lipid peroxidation, and inflammatory cytokine release (33,34,35). Importantly, GE administration at the dose (400 mg/kg) mitigated the negative impacts of MTX on renal function and status of oxidative stress. GE treatment causes decreasing in urea and creatinine levels, indicating improved kidney function. GE also restored the antioxidant defense system, as evidenced by low MDA and increased SOD, CAT, and GSH. GE effectively suppressed the inflammatory response, as demonstrated by reduced serum levels of IL-6, Lcn-2, and TNF- α . The protective effects of GE were further substantiated by the modulation of gene expression. GE treatment significantly downregulated the MTX-induced upregulation of Kim-1, Lcn-2, and IL-6 gene expression, suggesting a reduction in renal tu-

bular damage and inflammation. Moreover, GE treatment restored the expression of antioxidant genes GPxI and SODI, indicating an enhancement of the cellular antioxidant capacity. These findings suggest that GE exerts its renoprotective effects, at least in part, by modulating the expression of genes involved in oxidative stress, inflammation, and kidney injury.

In addition to its effects on renal function and oxidative stress, MTX administration also significantly altered the lipid profile, leading to increased total cholesterol and LDL, and decreased HDL-c. These findings are agree with previous studies linking MTX treatment to dyslipidemia and increased cardiovascular risk (36). Notably, GE treatment significantly improved the lipid profile, reducing TC and LDL-c levels and increasing HDL-c levels. This suggests that GE may also exert a protective effect against MTX-induced cardiovascular complications. The beneficial effects of ginger on lipid profiles are occur due to increased of the liver enzymes such as cholesterol 7-alpha-hydroxylase, which is the biosynthesis of bile acids. This process leads to the elimination of cholesterol from the body (37). The renoprotective and lipid-modulating effects of GE occur due to its rich with gingerols, shogaols, and zingerone (38). These compounds have anti-inflammatory effects and antioxidant effects, which observe reduction in inflammation and oxidative stress. Furthermore, GE has been shown to modulate various signaling pathways involved in kidney injury and lipid metabolism, including the Nrf2/HO-1 pathway (39, 40) and NF- κ B pathway (41). Our findings are consistent with studies found the preventive effects of ginger against nephrotoxicity induced by various agents (42). Specifically, several studies have reported that ginger can reduce oxidative stress, inflammation, and kidney damage in animal models of nephrotoxicity [43,44,45]. Our study showed that ginger can modulate the expression of renal injury biomarkers and improve lipid profiles in MTX-induced nephrotoxicity.

CONCLUSION

Based on our findings, treatment of male rats with methotrexate was implicated in the induction of oxidative stress in the kidney via BUN, creatinine, BUN: creatinine ratio, MDA and bilirubin were increased in MTX -induced rats and total bilirubin and reduction kidney tissue antioxidants (SOD, CAT, and GPx). Post-treatment with ginger rhizome extract successfully ameliorated the deleterious biochemical induced by methotrexate. Ginger has great preventive impact against MTX -induced kidney toxicity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

INFORMED CONSENT

Not applicable. This study was conducted on animal models (Wistar rats) and did not involve human participants.

ETHICAL STATEMENT

All animal procedures were performed in accordance with national and international animal welfare regulations. The animals were procured and cared for following ethical guidelines for the use of laboratory animals. The study protocol was approved by the institutional ethics committee at the University of Al-Qadisiyah, College of Pharmacy, Iraq.

AUTHOR CONTRIBUTION

Farah Razzaq Kbyeh: Conceptualization, methodology, investigation, data collection, formal analysis, and writing – original draft. Mohamed Abdul Rida Yaseen: Supervision, validation, writing, review and editing, and project administration. Both authors have read and approved the final manuscript.

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