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Protective effect of *Momordica charantia* against Hepatorenal toxicity induced by Potassium bromate ($KBrO_3$) in rats

Efecto protector de *Momordica charantia* contra la toxicidad hepatorrenal inducida por el bromato de potasio ($KBrO_3$) en ratas

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ABSTRACT

This study aims to evaluate the effects of *Momordica charantia* on hepatorenal toxicity caused by potassium bromate ($KBrO_3$) in rats. Wistar rats were divided into 4 groups as control, $KBrO_3$, bitter melon (MC), and $KBrO_3$+MC. Examining the antioxidant enzyme grade of the kidney tissues, it was found that the enzyme activities of catalase (CAT) (P<0.05), glutathione peroxidase (GSH–Px) (P<0.01), and superoxide dismutase (SOD) (P<0.01) decreased in the $KBrO_3$ group in comparison to the control. There was a significant decrease (P<0.001) in glutathione (GSH) levels and an increase (P<0.01) in malondialdehyde (MDA) level in the $KBrO_3$ group in comparison to the control. Examining the antioxidant enzyme activities in liver tissue, it was determined that CAT, GSH–Px, and SOD enzymes reduced significantly (P<0.05, P<0.01, and P<0.001, respectively) in the $KBrO_3$ group in comparison to the control, and the enzyme activity of decreased CAT, GSH–Px, and SOD enzymes significantly elevated (P<0.01) in the MC group. There was a reduction in GSH level in the $KBrO_3$ group in comparison to the control (P<0.01), while an increase was recorded in the $KBrO_3$+MC group (P<0.05). MDA level in liver tissue increased in $KBrO_3$ group in comparison to the control (P<0.01) and MC decreased the MDA level. Histopathological analysis results indicate severe degenerative and necrotic lesions in hepatorenal histoarchitecture of $KBrO_3$ rats in comparison to the control. However, application of MC+$KBrO_3$ significantly reduced the induced hepatorenal injury with a concomitant increase in histopathological lesions. From the immunohistochemical aspect, MC revealed apoptosis concomitant with the suppression of necrosis in the $KBrO_3$-treated rats as demonstrated by the caspase-3 activity.

Key words: Potassium bromate; *Momordica charantia*; hepatotoxicity; nephrotoxicity; rat

RESUMEN

Este estudio se evaluo los efectos de la *Momordica charantia* sobre la toxicidad hepatorrenal causada por el bromato de potasio ($KBrO_3$) en ratas. Las ratas Wistar fueron asignadas a 4 grupos como control, $KBrO_3$, melón amargo (MC), $KBrO_3$+MC. Al examinar el grado de enzimas antioxidantes de los tejidos renales, se observó que las actividades enzimáticas de catalasa (CAT) (P<0,05), glutatión peroxidasa (GSH–Px) (P<0,01) y superóxido dismutasa (SOD) (P<0,01) disminuyeron en el grupo KBrO3 en comparación con el control. Hubo una reducción significativa (P<0,001) en los niveles de glutatión (GSH) y un aumento (P<0,01) en los niveles de malondialdehído (MDA) en el grupo $KBrO_3$ en comparación con el control. Al examinar los niveles de enzimas antioxidantes en el tejido hepático, se determinó que las enzimas CAT, GSHPx y SOD se redujeron significativamente (P<0,05, P<0,01 y P<0,001, respectivamente) en el grupo $KBrO_3$ en comparación con el control, y la actividad enzimática de las enzimas CAT, GSHPx y SOD disminuyó y se elevó significativamente (P<0,01) en el grupo MC. Hubo una reducción del nivel de GSH en el grupo $KBrO_3$ en comparación con el control (P<0,01), mientras que hubo un aumento en el grupo $KBrO_3$+MC (P<0,05). El nivel de MDA en el tejido hepático aumentó en el grupo $KBrO_3$ en comparación con el control (P<0,01) y MC redujo el nivel de MDA. Los resultados del análisis histopatológico indican graves lesiones degenerativas y necróticas en la histoarquitectura hepatorrenal de las ratas KBrO3 en comparación con el control. Sin embargo, la aplicación de MC+$KBrO_3$ redujo significativamente la lesión hepatorrenal inducida con un aumento concomitante de las lesiones histopatológicas. Desde el punto de vista inmunohistoquímico, la MC reveló una apoptosis concomitante con la supresión de la necrosis en las ratas tratadas con $KBrO_3$, como demostró la actividad de la caspasa–3.

Palabras clave: Bromato de potasio; *Momordica charantia*; hepatotoxicidad; nefrotoxicidad; rata

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INTRODUCTION

Potassium bromate ($KBrO_3$) is widely utilized in cosmetic products (including permanent waving solutions and textile dyeing) [1], as well as, in enhancing the quality of flour, making dough in the baking industry, and adjusting beverages, cheese, and fish paste [2]. $KBrO_3$ is present in tap water samples due to the ozone disinfection process [3].

In most countries, including the USA, it is still utilized as a stabilizer for bread and cake, even though it is related to the increase in damage to certain organs [4]. Many studies reported that $KBrO_3$ has a detrimental effect on the dietary characteristics of bread by decreasing the most important vitamins of bread, named vitamins A1, B1, B2, and E, and niacin, and it also has the potential to cause deafness, eye and skin redness, and pain [5]. $KBrO_3$ was reported to be a possible human and animal carcinogen, exposure to $KBrO_3$ leads to multiorgan toxicity, and the main target organ is the kidney [6].

As reported in previous studies, $KBrO_3$ was shown to have severe destructive effects on various organs and central nervous system [7]. Elevated production of reactive oxygen species (ROS) and free radicals is thought to play a mediatory role in $KBrO_3$-caused toxicity. These radicals react with macromolecules such as proteins, nucleic acids, and membrane lipids, disrupting the balance in homeostasis, which in turn results in a wide range of potential tissue damage [8]. Consequently, safe and effective synthetic and/or natural ROS scavengers and antioxidants are therapeutically important. Some of them, such as rutin, taurine, *Nymphaeaalba L*, and vitamin C, showed potential protective effects against $KBrO_3$-induced nephrotoxicity and hepatoxicity [1, 9]. *Momordica charantia* (MC), also known as bitter gourd or bitter melon, is a plant widely grown in the most tropical and subtropical parts of the world and is widely used as a food and medicinal plant in South Asia and the East. MC is also used in treating many diseases, such as diabetes mellitus [10]. Previous studies reported that MC has antioxidant activity and antidiabetic and anticancer effects [11, 12], and that it also has protective effects on injured tissues (including liver, brain, stomach, and heart) [13, 14, 15].

Given these remarkable properties of MC, this study aims to investigate the protective effects of *Momordica charantia* on $KBrO_3$-induced hepatoxicity and nephrotoxicity in rats.

MATERIALS AND METHODS

Preparation of the MC fruit extracts

The juice of *Momordica charantia* was produced using the method developed Singh *et al.* [16]. Fresh fruit (1 kg) was meticulously cleaned. A professional juicer (Moulinex Ju650, France) was used for juice extraction. The fresh juice was centrifuged (Hettich rotina 420 R, Germany) at 5700 G for 30 min, and the resulting clear supernatant was designated as 100%. *Momordica charantia* juice was autoclaved and then diluted with distilled water to achieve a 50% concentration, as per Sitasawad *et al.* [17]. It was stored at 4°C (Ariston ENXTG (-24°C), Italy) and delivered by gavage at a daily [d] dosage of 300 $mg \cdot kg^{-1}$ of body weight.

Animals and experimental design

Before the initiation of research, approval was obtained from the local ethics committee for animal experiments at Bingöl University (BUHADEK: 02.09.2021-2021/04-04/04). Wistar albino rats were kept in the Animal Experiments Unit until they reached the desired weight (Denver instrument SI–234, Germany) (200 – 300 g), and they were given food and water ad libitum. A consistent light/dark photoperiod of 12 hours light and 12 hours dark was maintained.

Throughout the experiment, the temperature was kept at 20 ± 3°C. In this study, 24 rats (*Rattus norvegicus)* were assigned to 4 groups as control (0.5 mL, i.p. isotonic water), potassium bromate (10 $mg \cdot kg^{-1} \cdot d^{-1}$ oral), bitter melon extract (MC) (300 $mg \cdot kg^{-1} \cdot d^{-1}$ oral), potassium bromate + bitter melon extract (MC) groups with 6 rats in each group. On the 21st d, rats were anesthetized using (60 $mg \cdot kg^{-1}$ ip) Ketamine–HCl and 10 $mg \cdot kg^{-1}$ ip Xylazine. Liver and kidney tissues were harvested after median laparotomy, rinsed with phosphate–buffered saline, stored in a deep freezer (WiseCryo South Korea) (-80°C) for analysis (SOD, MDA, and CAT), and fixed for histopathological and immunohistochemistry analyses for 72 h.

Preparation of liver and kidney homogenates

Homogenization of samples (500 mg) was performed using 5000 L of an ice–cold buffer consisting of 10 $nmol \cdot L^{-1}$ Tris–HCl (pH 7.4) and 1 $mmol \cdot L^{-1}$ EDTA with 0.32 $mol \cdot L^{-1}$ sucrose, utilizing an IKA Ultra Turrax T25 homogenizer (Germany) and a Bandelin Sonopuls 20 KHz ultrasonic homogenizer (Germany) for 8 min. The homogenate was then centrifuged (Hettich rotina 420 R, Germany) at 10000 G for 30 min. All procedures were conducted at 4°C, and the resultant clear supernatants were utilized for the assessment of tissue antioxidant enzymes (SOD, GSH–Px, and CAT), as well as GSH and MDA concentrations [18].

Antioxidant enzymes, MDA, and GSH determination

Glutathione in tissues was determined by using the method introduced by Rizzi *et al.* [19]. MDA in tissues was measured by utilizing the method specified by Jain *et al.* [20]. GSH–Px enzyme activity of tissues was examined at 340 nm according to the method specified by Paglia and Valentine [21]. SOD was determined at 505 nm using the method of Sun *et al.* [22]. CAT activity was assessed at 240 nm by the decomposition of H_2O_2 in accordance with the method developed by Aebi [23]. The total protein concentration of liver and kidney tissues homogenates were measured by using method of Lowry *et al.* [24].

Histopathological and Immunohistochemical Investigations

To investigate pathological lesions, the liver and kidney tissues were put on an automated tissue–tracking device (Leica TP 1020, Germany). As part of routine tissue monitoring, all tissues were dehydrated using the alcohol series, made transparent using the xylol series, and then blocked in paraffin. Serial sections of 5µm thickness were obtained by microtome (Leica RM 2135, country). They were stained using Hematoxylin–Eosin (H&E) dye. Slides were investigated using a light microscope (BX51, Olympus Corp., Tokyo, Japan) with a DP72 camera attached. Ten randomly chosen microscopic fields were analyzed at 20x-40x magnification. The ratings were semi–quantitative, assessment the quantity of

microscopic areas exhibiting necrotic and degenerative lesions. The score was assigned a number value: none: – (0 lesion), mild: + (degeneration), moderate: ++ (degeneration+necrosis), and severe: ++++ (degeneration+extensive necrosis) [25, 26]. The liver and kidney tissue sections were subjected to the standard avidin–biotin–peroxidase procedure (ABC) by following the manufacturer's protocol against the anti–caspase–3 antibody (sc–56053). All the sections were incubated with 3.3.9–diaminobenzidine chromagen (DAB) (Histostain–Kit, Invitrogen, USA) and counterstained using Mayer's hematoxylin [27].

Statistical analyses

The results were statistically analyzed using software (SPSS, version 20.0). The variations in the measured parameters across groups were assessed using Kruskal–Wallis, whereas comparisons between two groups with significant results were conducted employing the Mann–Whitney U test (P<0.05).

RESULTS AND DISCUSSION

When examining the antioxidant enzyme levels in rat kidney tissue, it was found that CAT (P<0.05), GSH–Px (P<0.01), and SOD (P<0.01) activities significantly decreased in the $KBrO_3$ group in comparison to the control. These enzyme activities increased in the $KBrO_3$ + MC group in comparison to the $KBrO_3$ group, but not significantly. Examining the GSH level, a very important antioxidant substance protecting cells from the toxic influences of ROS such as free radicals, peroxides, and heavy metals, a significant decrease (P<0.001) was observed in the $KBrO_3$ group in comparison to the control, while there was a significant increase (P<0.05) in the $KBrO_3$ + MC group in comparison to the $KBrO_3$ group. MDA levels in rat kidney tissue significantly elevated (P<0.05) in the $KBrO_3$ group in comparison to the control (P<0.01). However, it was found that the elevated MDA values in the $KBrO_3$ group was significantly decreased (P<0.05) in the MC group (FIG. 1).

The changes in the antioxidant enzyme activities in the liver tissue of rats were observed to be similar to those in the kidney tissues, and it was observed that the CAT, GSH–Px, and SOD enzymes reduced significantly (P<0.05, P<0.01, and P<0.001, respectively) in the $KBrO_3$–treated group in comparison to the control group. It was reported that the same enzyme activities increased significantly in the MC group (P<0.01, P<0.01, P<0.01, and P<0.01, respectively). It was also determined that antioxidant enzyme activities increased in the $KBrO_3$ + MC group, but not significantly. It was found that the GSH level in the liver tissue decreased in the $KBrO_3$ group in comparison to the control (P<0.01), and the decreased GSH level increased in the $KBrO_3$ + MC group (P<0.05). Finally, the MDA level, an important marker of lipid peroxidation, elevated in the $KBrO_3$ group in comparison to the control (P<0.01), and the application of *M. charantia* reduced the elevated MDA level (FIG. 2).

It was reported in the literature that the $KBrO_3$-caused damage in liver and kidney tissues occurs via oxidative stress [8]. The harmful products of oxidative damage originating from oxidative stress increase lipid peroxidation, which causes damage to macromolecules. The toxic substance $KBrO_3$ causes moderate to severe toxic influences during its ROS–mediated biotransformation in vivo. Cellular redox balance and structural integrity in target

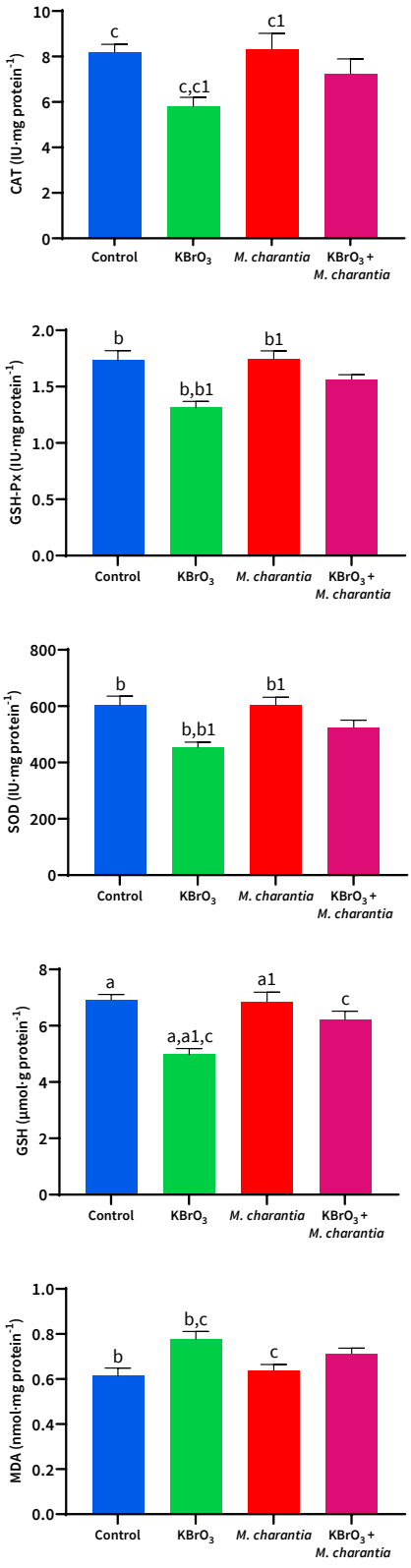

FIGURE 1. CAT (Catalase), GSH–Px (Glutathione Peroxidase), SOD (Superoxide Dismutase), GSH (Glutathione), MDA (Malondialdehyde) in the kidney tissues by groups. Significant differences between the groups due to different letters (a:P<0.001, b:P<0.01, c:P<0.05)

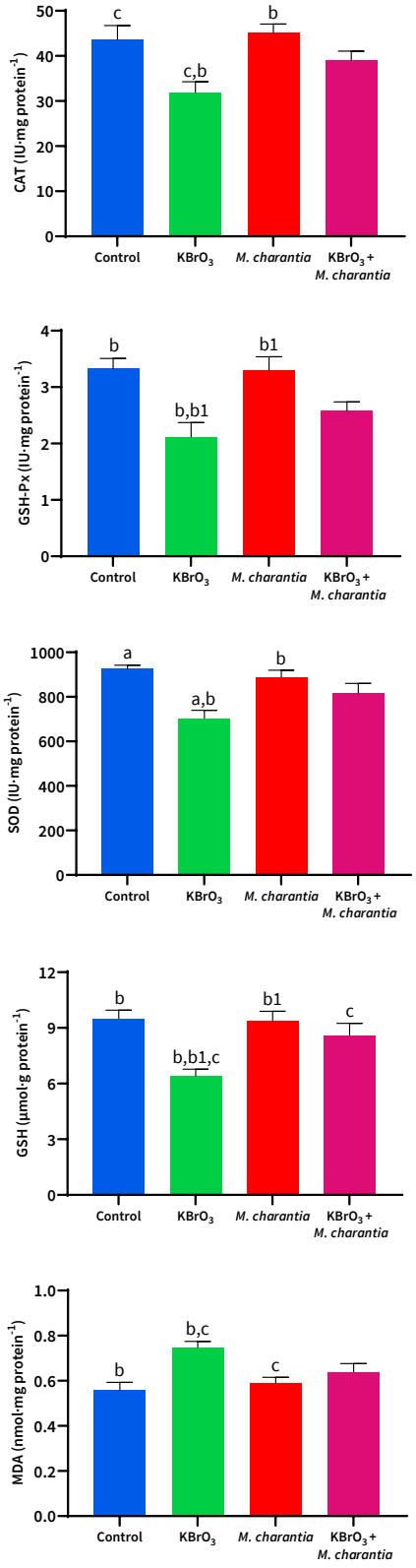

FIGURE 2. CAT (Catalase), GSH–Px (Glutathione Peroxidase), SOD (Superoxide Dismutase), GSH (Glutathione), MDA (Malondialdehyde) in the liver tissues by groups. Significant differences between the groups due to different letters (a:*P*<0.001, b:*P*<0.01, c:*P*<0.05)

tissues are disrupted by these species [28]. ROS increases MDA levels and affects GSH levels in target tissues [29]. MDA was used as a marker for lipid peroxidation in this study, whereas GSH, SOD, CAT, and GSH–Px served as indications of cellular defense against oxidative stress. Oxidative damage is perpetually mitigated by radical scavengers and antioxidant enzymes, including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH–Px) [30]. Oxidative stress significantly impacts renal damage. This effect occurs after the interaction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with lipids in cellular and mitochondrial membranes, a process referred to as lipid peroxidation, which modifies many signaling pathways. Elevated concentrations of lipid peroxidation products, including malondialdehyde (MDA), and protein oxidation often signify oxidative injury in renal tissues [31]. The influence of ethanol extract of Aframomum angustifolium seeds on kidney and liver damage induced by $KBrO_3$ was investigated in a previous study. In this study, MDA, creatinine, electrolyte (Na^+ and K^+) concentrations and CAT, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were analysed as biochemical parameters.

The study demonstrated that MDA concentration in kidney tissue rose remarkably from day (d) 3 to d 10 in all groups, but was higher in the second group to which $KBrO_3$ was added. In the same study, liver tissue demonstrated similar results, but a dramatic rise was observed in the third group that received the plant extract along with $KBrO_3$. Catalase activity was inhibited in both kidney and liver tissues in the second group and reduced remarkably compared to the control group. ALP enzyme activity was remarkably risen in the third group on d 3 (P<0.05), while no significant difference was found on d 10 (P>0.05).

In this study, it was reported that bromate caused hepato – and renotoxicity in Wistar rats and ethanol extract of *Aframomum angustifolium* seeds could accelerate in vivo repair [32]. In another similar study, the influence of $KBrO_3$ application on lipid peroxidation (LPO), SOD, and GSH markers in rat liver tissue and the protective effect of vitamin C against this influence were investigated. It was reported that MDA, the end product of LPO concentration, significantly increased (P<0.01) in the liver of the $KBrO_3$ group. Vitamin C was reported to effectively prevent the oxidative damage caused by $KBrO_3$ in the $KBrO_3$ group and significantly decrease the MDA concentration in comparison to the $KBrO_3$ group, and elevate the decrease in SOD and GSH levels (P<0.05) caused by $KBrO_3$ in the liver.

In their study, it was suggested that lipid peroxidation was the cause of both the increased MDA concentration and reduced GSH and SOD concentrations in rat liver tissue and the failure of the body's antioxidant defense mechanisms to inhibit the overproduction of free radicals [1]. In another study investigating the influence of riboflavin on $KBrO_3$–induced nephrotoxicity in rats, it was shown that there was a significant decrease in marked toxicity and renal function markers after riboflavin treatment, as well as increased activity of key antioxidant enzymes and proteins.

It was also reported that riboflavin prevented target cell necrosis in liver cells, just as $KBrO_3$-induced programmed cell death. The results of this study also support the renal toxicity of $KBrO_3$, as evidenced by an increase in oxidative stress parameters, as well

as alteration in the function of key biomolecules and disruption of structural components of target cells.

In this study, MDA levels increased at different rates in the $KBrO_3$ group and decreased in the $KBrO_3$+Riboflavin groups. Analyzing the CAT activity, it was found that there was a reduction in the $KBrO_3$ group and an increase in the group, to which two different doses of riboflavin were added, in comparison to the $KBrO_3$ group. Moreover, SOD and glutathione reductase (GR) enzyme activities were reported to demonstrate a similar trend to the CAT enzyme at different rates.

The reduced GSH level, which is one of the cellular reductants for assessing oxidative stress, was reported to demonstrate a significant decrease in the $KBrO_3$ group and an improvement in the riboflavin group [33]. In terms of the toxic influence of $KBrO_3$, the present study shows similarities with the literature. For instance, it was found that the MDA level, an important marker of lipid peroxidation, increased (P<0.01) in the $KBrO_3$ group in comparison to the control in both kidney and liver tissues (FIGS. 1 and 2). When examining the enzymes (CAT, GSHPx, SOD), which are indicators of antioxidant defense, similar to the literature, it was determined that these enzyme activities decreased significantly (P<0.05, P<0.01, and P<0.001, respectively) in the $KBrO_3$ group in comparison to the control (FIGS. 1 and 2).

In addition, when evaluating the level of GSH, another important antioxidant defense mechanism protecting cells from toxic effects, a significant reduction (P<0.001, P<0.01) was observed in the $KBrO_3$ group in comparison to the control (FIGS. 1 and 2). MC is a plant commonly utilized as an antioxidant, anti–diabetic, anti–hepatoma, nutraceutical, and antimalarial [34].

Previous studies showed that MC has a strong antioxidant capacity both at the cellular level and in animal models [35, 36]. Other studies validated that MC extracts contain phytochemicals exhibiting anti–diabetic and antioxidant effects in diabetic rat models by enhancing pancreatic insulin secretion and insulin sensitivity in peripheral tissues, reducing insulin resistance and hepatic gluconeogenesis, and regulating glycolysis, gluconeogenesis, and antihyperlipidaemic effects. The extract of this plant was also determined to reduce oxidative stress and have anti–peroxidant properties that protect cells from ROS. Therefore, this plant is a good candidate against diabetes in mammals, particularly humans. It is also commonly utilized in traditional medicine [37].

In another study, the correlation between phenolic acid concentration and anti–glycation activity of MC fruit extract was examined and reported to be directly related with increasing suppression of the glycation process [38]. In a study in which MC juice was administered orally, it was reported that it could ameliorate histopathological changes in the pancreas by reducing MDA levels and increasing serum total antioxidant capacity (TAOC) and pancreatic GSH levels [39]. In another study in which *Momordica charantia* polysaccharide (MCP) was used, it was reported that MCP significantly elevated the antioxidant capacity by elevating SOD levels and reducing MDA levels, thereby alleviating streptozocin (STZ)–induced pancreatic damage and repairing pancreatic β–cells [40].

In another study, MCP was given to mice tih STZ–induced diabetes. As a result, it was reported that the antioxidant enzyme defense system was strengthened and lipid peroxidation was attenuated in a dose–dependent manner with this application, and this result might be related to the antiglycation ability of MCP [41]. In many other studies employing MCP, MCP improved total antioxidant capacity by improving SOD levels and decreasing MDA levels [40]. MCP treatment was reported to be able to reduce MDA in diabetic kidneys while increasing GSH, SOD, and CAT levels [42].

Moreover, MCP in high–fat–induced obese rats caused a significant reduction in insulin resistance by decreasing lipid and oxidative stress levels [43]. Considering the results of the in vitro experiments, it was reported that MCP has a dose–dependent and linear relationship in scavenging hydroxyl radicals [35, 36, 44, 45]. It was suggested to increase the activity of antioxidant enzymes such as SOD, glutathione peroxidase (GSH–Px), and cataloxidase (CAT) by acting indirectly on free radicals, and reduce MDA levels by chelating metal ions required for the ROS production. As can be seen, the protective effects of *Momordica charantia* L. against the degenerative influences of toxic substances were examined in previous studies, and the results achieved in the present study are consistent with those reported in previous studies. In the present study, both kidney and liver tissues demonstrated an increase in MDA caused by the toxic influence of $KBrO_3$ and a decrease in GSH, CAT. It was found that *Momordica charantia* L. had a positive effect on GSH–Px, SOD levels. Despite the fact that the data obtained in this study are consistent with the literature in general, the curative influence of *M. charantia* against the destructive influence of $KBrO_3$ has not been studied in previous studies. In this respect, the present study is thought to contribute to the literature.

Even though the exact mechanism of action is not yet known, the hepatorenal toxicity caused by $KBrO_3$ causes oxidative stress by triggering the production of ROS and lipid peroxidation. Moreover, due to the loss of balance between free radical production and the antioxidant defense system, $KBrO_3$ was reported to cause various lesions leading to pathological conditions in many tissues and organs, particularly the liver and kidney [7, 46]. The impairment of liver and kidney functions to varying degrees occurs as a direct result of changes in the histological structures of these organs depending on the degree of exposure to $KBrO_3$. These results indicate the need for further studies to elucidate the hepatorenal toxic effects of $KBrO_3$ [1].

The histological appearance was normal in both liver and kidney tissues of the control and MC groups (FIGS. 3a,c and 4a,e), and there was no significant change (P>0.05). Sinusoidal dilatation and congestion in the central veins and partial impairment of hepatic helix structure were determined in the liver tissues of rats in the $KBrO_3$ group. There was parenchymal and hydropic degeneration in the hepatocytes of this group, and degenerative and necrotic changes were observed in some hepatocytes, most of which were cetrilobular. Some of the necrotic hepatocytes were karyolytic and there were some pyknotic nuclei. Comparing the $KBrO_3$ and the $KBrO_3$ + MC groups, it was found that the results observed in the MC group were significantly milder in terms of severity and frequency (P<0.05). Histopathological analysis of kidney tissue demonstrated diffuse degenerated and necrotic cells in the tubular epithelium in the $KBrO_3$ group. In addition, glomerular atrophy, widened Bowman's space, tubular and lymphatic dilatation and hyaline cylinders in the tubular lumen were also observed in the kidney tissues of this.

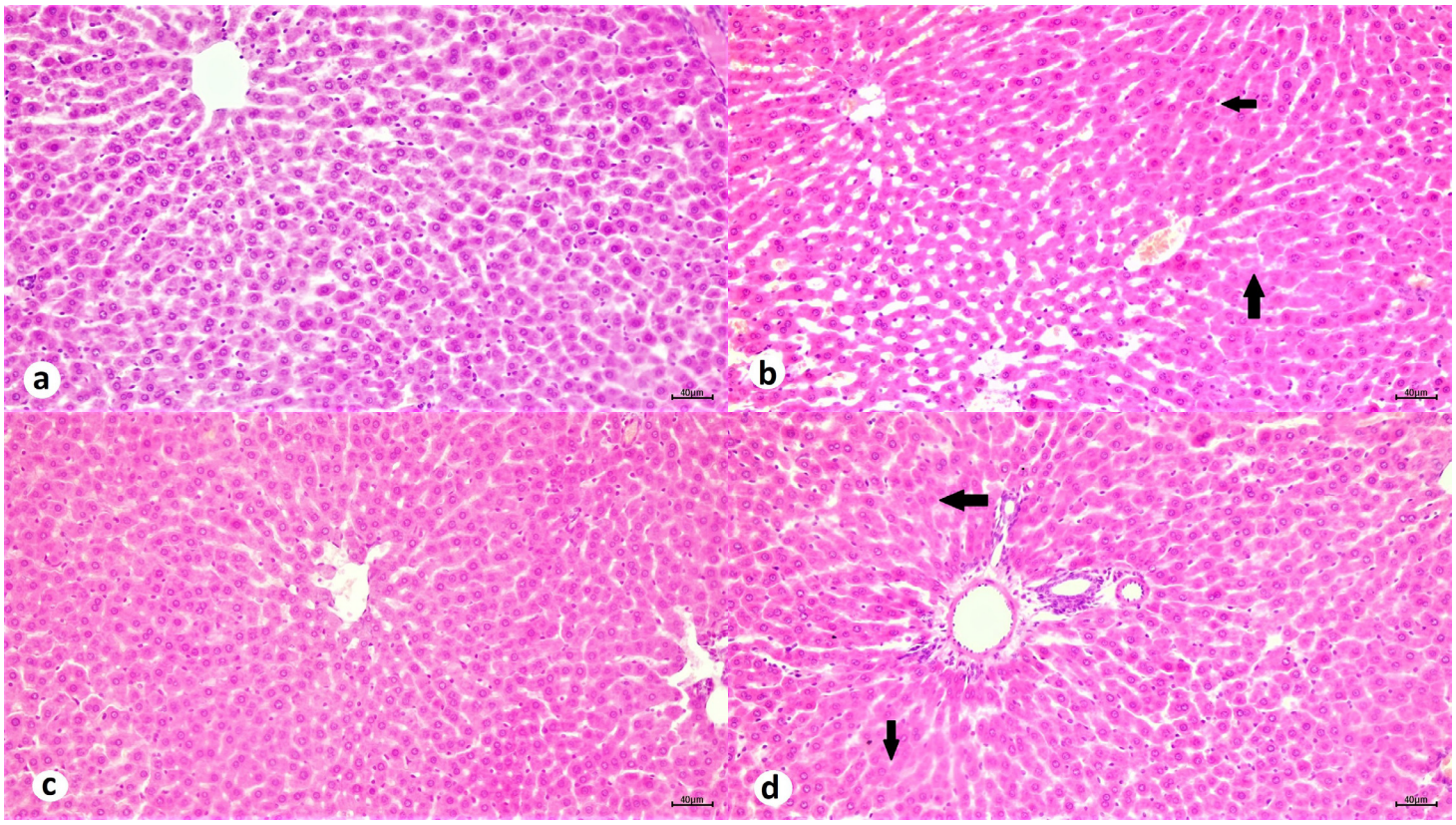

FIGURE 3. a) Control group, normal histological appearance of liver tissue (H&E, 40 µm). b) $KBrO_3$, severe necrotic hepatocytes (arrows), (H&E, 40 µm). c) MC group, normal histological appearance of liver tissue (H&E, 40 µm). d) $KBrO_3$+MC, mild necrotic hepatocytes (arrows), and hydropic degeneration. (H&E, 40 µm)

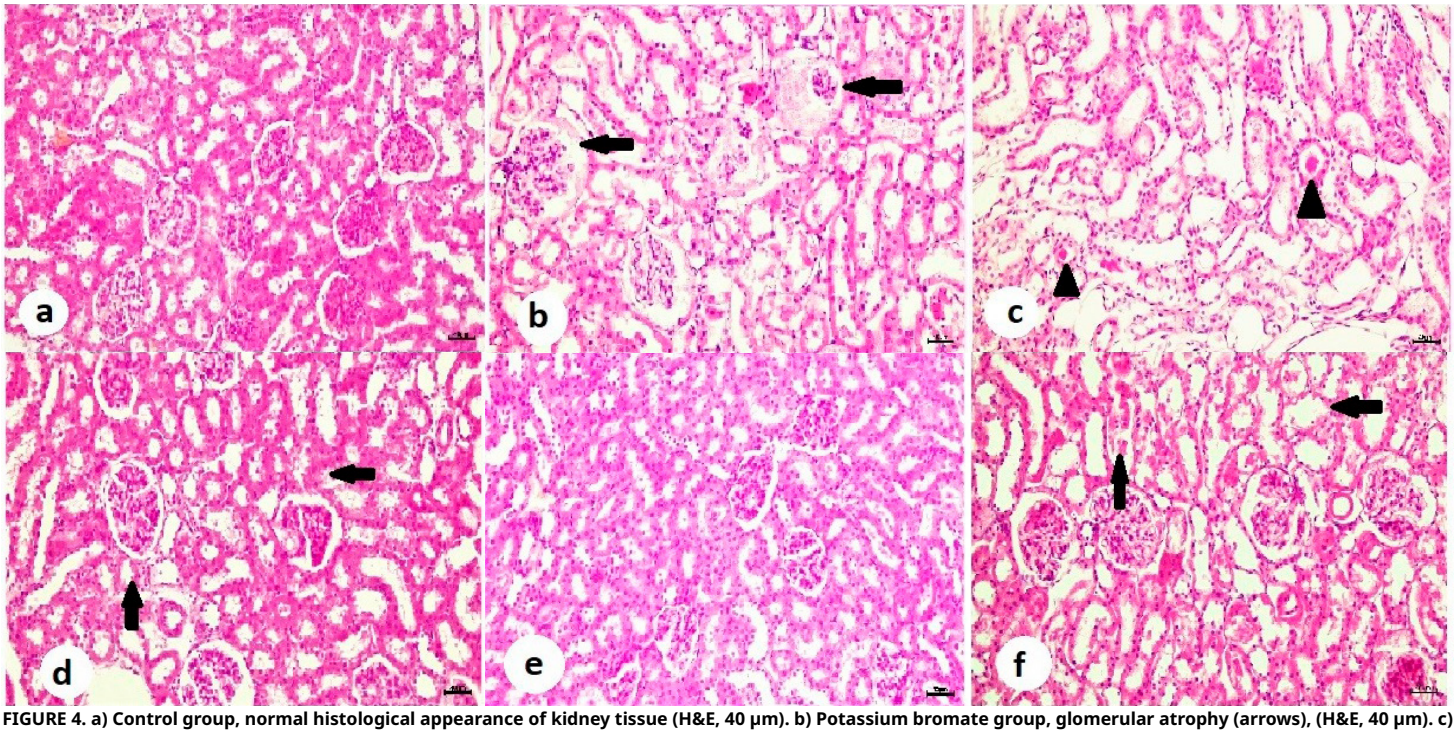

FIGURE 4. a) Control group, normal histological appearance of kidney tissue (H&E, 40 µm). b) Potassium bromate group, glomerular atrophy (arrows), (H&E, 40 µm). c) Potassium bromate group, Hyaline cylinders in tubules (arrow heads), (H&E, 40 µm). d) Potassium bromate group, severe necrosis in the tubular epithelium (arrows), (H&E, 40 µm). e) MC group, normal histological appearance of kidney tissue (H&E, 40 µm). f) Potassium bromate+MC, necrotic epithelium (arrows). (H&E, 40 µm)

Evaluating the staining results of liver and kidney tissues, no positive immunoreaction was observed in the control and MC groups, except for some weak and rare immunopositive reactions (FIGS. 5a,c and 6a,c). Strong and diffuse positive immunoreactivity for caspase-3 was observed in the $KBrO_3$ group (FIGs. 5b and 6b).

The frequency and intensity of the positive reactions in the $KBrO_3$ + MC group were significantly lower than in the $KBrO_3$ group. In other words, the number of positive cells reduced with the administration of MC in the $KBrO_3$ group (FIGS. 5d and 6d).

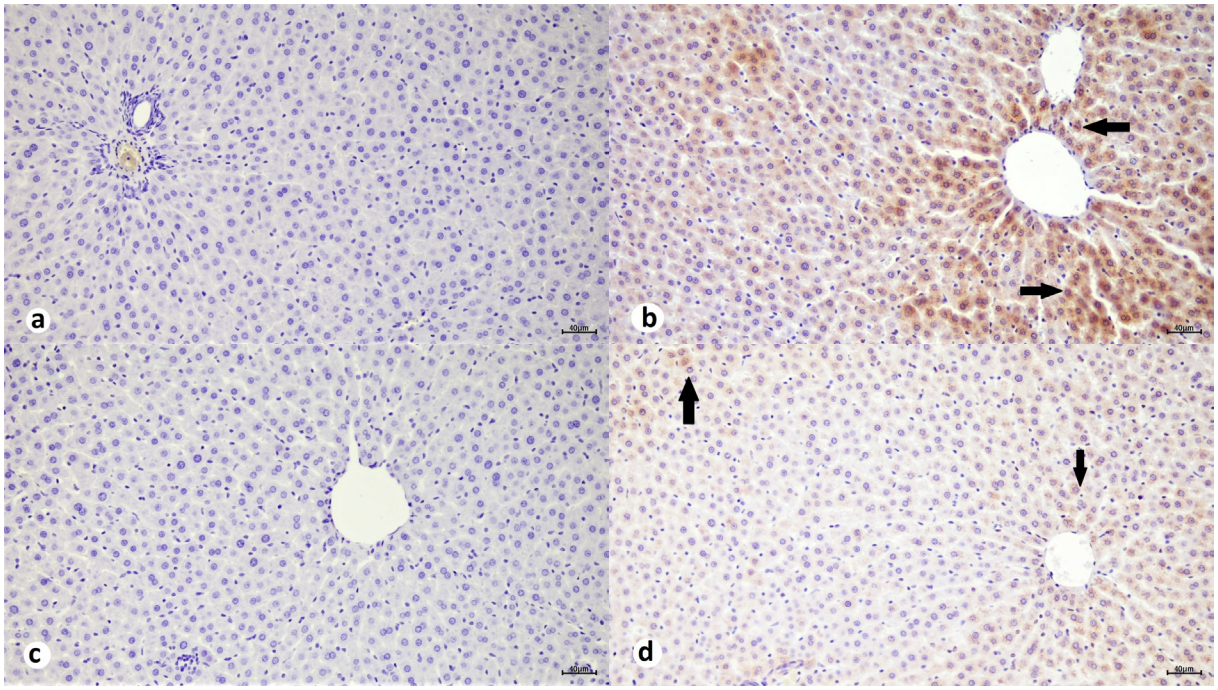

FIGURE 5. a) Control group liver tissue, Caspase – 3 negativity (IHC, 40 µm). b) Potasium bromate group. Strong Caspase – 3 positivity (IHC, 40 µm). c) MC group, Caspase – 3 negativity (IHC, 40 µm). d) Potassium bromate+MC, weak Caspase – 3 positivity (IHC, 40 µm)

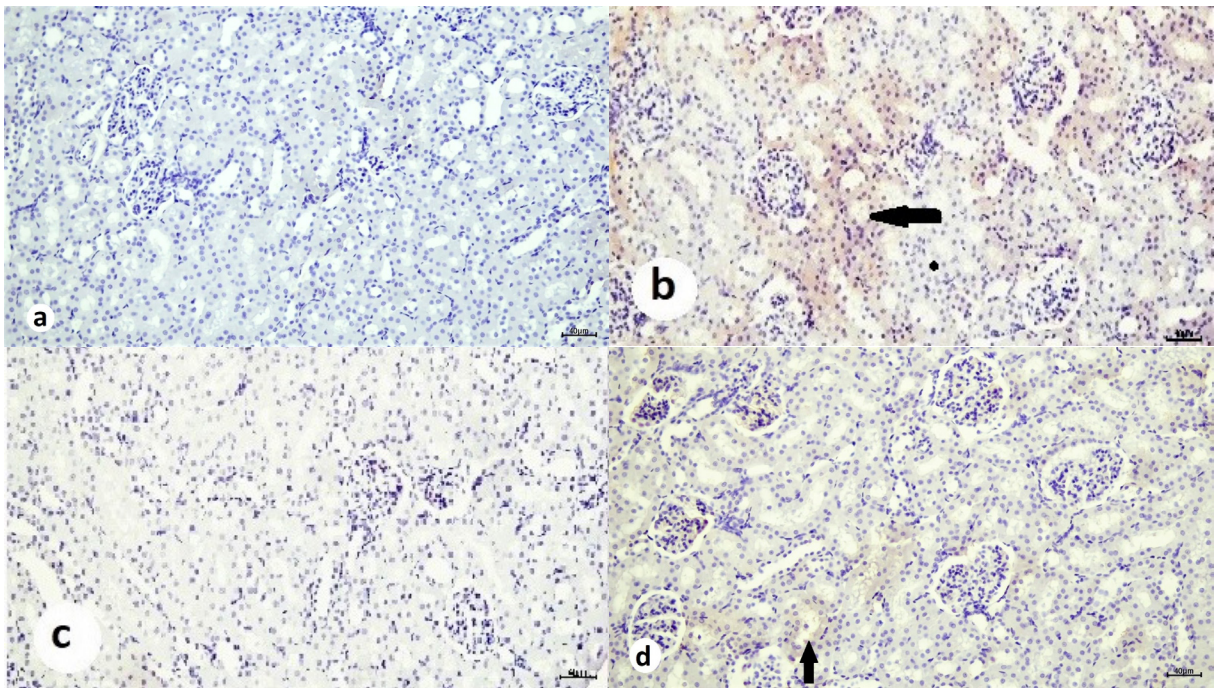

FIGURE 6. a) Control group kidney tissue, Caspase – 3 negativity (IHC, 40 µm). b) Potassium bromate group. Strong Caspase – 3 positivity (IHC, 40 µm). c) MC group, Caspase-3 negativity (IHC, 40 µm). d) Potassium bromate+MC, weak Caspase-3 positivity (IHC, 40 µm)

In the present study, the lesions that occurred as pathological responses of liver tissues to $KBrO_3$ were generally dilatation of sinusoids and congestion in the central veins and partially degenerative and necrotic changes in some hepatocytes with disruption of the hepatic cord structure, similar to previous studies [1, 5]. Even though $KBrO_3$ was reported to cause histological changes such as inflammatory cellular infiltration and a marked elevation in collagen deposition in the portal region [1] and vacuolation in hepatocytes [47], none of these findings were observed in this study.

This might be because lipid peroxidation and free radical oxidative stress were partially mitigated by the lower dose and duration of exposure to $KBrO_3$ in this study, resulting in fewer lesions in the liver. In most renal diseases, oxidative damage caused by chemical poisoning causes various lesions in the nephrons, resulting in the kidney losing its filtering capacity and urea levels increasing. In general, elevated urea levels are related to nephritis, renal ischemia, urinary tract obstruction, and exposure to certain chemicals that are toxic to the kidneys [48].

It was reported that severe histological lesions were seen in the renal cortex of rats subjected to $KBrO_3$, and these lesions were generally observed in both glomerular and tubular structures, sometimes only in tubular structures [4]. Glomerular damage was reported as glomerular atrophy, thickening or disorganization of the glomerular basement membrane, decrease in glomerular cellularity, and congestion in glomerular capillaries [48], as well as, dilatation of lymphatic vessels. However, it was emphasized that the occurrence of all these lesions varies with the dose and duration of exposure to $KBrO_3$, and that exposure to low doses does not cause lesions, whereas exposure to high doses causes the severe lesions reported above [4]. In the present study, tubular and glomerular damage due to nephrotoxicity caused by $KBrO_3$ was similar to results achieved in previous studies, but thickening of the glomerular basement membrane and congestion of the glomerular capillaries were not observed.

Recent studies employed various plant–derived agents to prevent hepatorenal injury caused by $KBrO_3$. In a study by Deng *et al.* [15] using *Momordica charantia* against the harmful effects of $KBrO_3$ on the liver, it was found that MC was very effective in preventing lesions in the liver, which was also used to prevent GSH. It was reported that *M. charantia* exhibits its protective effects by increasing GSH–Px activity and GSH levels and reducing the overproduction of lipid peroxidation products and NO in the liver and the whole body, or by reducing mitochondrial ROS production or mitochondrial membrane potential. *M. charantia* may offer significant advantages in developing functional foods [15]. Abdel–Latif *et al.* [48], in their study investigating the effects of metformin on $KBrO_3$-induced nephrotoxicity, highlighted that tubular and glomerular lesion were significantly reduced by metformin administration, which could be explained by the antioxidant, anti–inflammatory, and anti–apoptotic characteristics of the agent used.

CONCLUSION

In this study, it was concluded that *Momordica charantia* used in hepatorenal lesions caused by $KBrO_3$ reduced oxidative stress and prevented lipid peroxidation to a large extent. *Momordica charantia* can ameliorate $KBrO_3$–induced hepatotoxicity and nephrotoxicity associated with oxidative stress in rats thanks to its antioxidant, anticancer, and anti–inflammatory effects. Thus, the results achieved in this study suggest that *Momordica charantia* could be a potential dietary supplement for individuals consuming as a food additive ($KBrO_3$) to prevent oxidative damage and the increase of caspase–3 expression in liver and kidney tissues. These positive effects of *Momordica charantia* may be the inhibition of hepatic and nephrotic caspase-3 expression and oxidative stress.

Conflict of interests

The authors declare no conflict of interest regarding the publication of this manuscript.

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