Histopathological changes in kidney and liver with oxidative stress and protection by plant extracts

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Abstract. *Potassium permanganate ($KMnO_4$) is utilized to cleanse pathogenic microbes in vegetables and fruits. Humans may be exposed to this compound from vegetables and fruits. This study aims to evaluate the impact of oxidants such as potassium permanganate in kidney and liver tissues and assess protective roles of aqueous extracts of H. sabdariffa flowers and M. parviflora leaves against the oxidizing substance. For this purpose, 28 female Swiss albino mice were distributed into four groups: each group contained seven animals. Group I recruited control animals. Group II received a daily $KMnO_4$ (0.5 mg/kg/BW) dose. Group III received a daily oral dose containing $KMnO_4$ and an aqueous extract of H. sabdariffa flowers (0.5 mg/kg/BW + 500 mg/kg/BW). Group IV received a daily $KMnO_4$ and an aqueous extract of M. parviflora leaves (0.5 mg/kg/BW + 300 mg/kg/BW). The treatment in all groups lasted for 30 consecutive days. Mice exposed to $KMnO_4$ showed severe histopathological changes in the kidney and the liver. The treatment with aqueous extracts of H. sabdariffa flowers and M. parviflora leaves prevented the damage of tissues induced by $KMnO_4$ and exhibited a better protective role.*

Introduction

Potassium permanganates ($KMnO_4$) are oxidizing factors utilized in aquaculture (Franca et al., 2013). KMnO4 is utilized to wash vegetables and fruits for its ability to sterilize pathogenic microbes (Subramanya et al., 2018). Free radicals induce damage that become the main concern because of its serious consequences. Free radicals may result in different degenerative diseases such as rheumatoid arthritis, aging and tumors. There are two species of free radicals which are formed in the organisms, i.e., reactive oxygen species and reactive nitrogen species. These are released in the human body through pathophysiological pathways. Normally, free radicals are scavenged by antioxidants, but this neutralization is not completed leading to a phenomenon called oxidative stress that is responsible for the damage. Free radicals affect lipids, proteins, and DNA. Together with endogenous antioxidants, food components are excellent sources of natural antioxidants. Most vegetables and fruits are rich sources of antioxidants (Dhaliwal and Singh, 2015). $KMnO_4$ is known by its oxidant and irritant properties and by the acute toxicity of manganese. Swallowing a little dose (4–20 mg/kg) of $KMnO_4$ can lead to gastrointestinal ordeal; however, bolus ingestion causes respiratory arrest followed by coagulative necrosis and hemorrhage in the esophagus, the stomach, and the liver (Willhite et al., 2013). Potassium permanganate is seldom utilized for suicidal trail. Swallowing it can cause positional and systemic toxicities like damage and necrotic tissues that result from permanganate toxicity. A patient with a lethal dose of $KMnO_4$ has narrowing of the superior airway, bringing about complicated intubation in addition to liver inhibition and coagulopathy as systemic manifestation (Agrawal et al., 2014).

Hibiscus sabdariffa (Family: malvaceae) is a perennial and herbal plant distributed in Iraq. It is used in various foods in addition to herbal medicine (Balarabe, 2019). Different therapeutic benefits are found in the hibiscus plant, including anti-positive and gram-negative bacteria, anti-tumor, anti-apoptosis and anti-oxidant activity (Puro et al., 2017). This plant is a rich source of antioxidants and prevents DNA breakdown (Adeoye et al., 2019). It works to increase the capacity of antioxidants inside the cell and reduce oxidative stress (Soto et al., 2016). It protects the kidney tissue and improves its functions against toxins such as lead (Okonkwo, 2020). The ethanolic extract of *Hibiscus sabdariffa* calyces has hypolipidemic effects that reduce serum cholesterol and triglycerides (Umoren et al., 2020; Gaffer and Mustafa, 2019). Many natural compounds are presented in calyx extract of *Hibiscus sabdariffa*, such as anthocyanin, flavonoid, carotenoid, phenol, and vitamin C (Jamini et al., 2019).

Malva parviflora L. (malvaceae family) is a perennial herb distributed in all regions of Iraq. It has a pharmaceutical importance containing phenolics, flavonoids, flavonols and fatty acids (Rasheed et al., 2017). The polyphenols in the leaves of this plant show high antioxidant activity (Abd El-Salam and Morsy, 2019). Certain compounds, such as oleanolic acid, scopoletin and tiliroside, have the ability to lower blood pressure and prevent oxidative damage and inflammation in the kidneys (Lagunas-Herrera et al., 2019). Other compounds in this plant have

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anti-bacterial activity, especially bacteria that infect the urinary tract (Kidane et al., 2019). *M. parviflora* extracts possess analgesic and anti-inflammatory compounds (Ramirez-Serrano et al., 2019). *M. parviflora* has shown its pharmacological activities in various sicknesses. Leaves are utilized in the arrangement of wounding and bulging. A lotion manufactured from leaves is utilized to treat trauma and broken limbs. These leaves are utilized to draw bulging, inflamed festering wounds. *M. parviflora* has various activities like antidiabetic, antifungal, hepatoprotective, neuroprotective, anti-irritant, antioxidant, and anti-ulcerogenic activity (Singh and Navneet, 2017).

Materials and methods

This study was approved by the ethical committee at the University of Anbar. The granted authorization No. 1 was issued in 11/1/2021. The flowers of *H. sabdariffa* and leaves of *M. parviflora* were collected from the area (Anbar-Iraq), were washed with water and dried, and then stored in tight polythene bags.

Hibiscus and Malva extract preparation

Dried *H. sabdariffa* flowers and *M. parviflora* leaves were ground to obtain powder. A magnetic stirrer device was used to mix the powder of each plant with 10 volumes (w/v) of pure water for 2 hours. This 10% solution was then clarified by gauze, sanitized via filtration on 0.1-micron filters (Millipore), and the drying was carried out at 50°C using an incubator. This dried extract was suspended in water to the final concentration for *H. sabdariffa* 50 mg/mL (Okonkwo, 2020) and for *M. parviflora* 30 mg/mL (Rasheed et al., 2017).

Stock solution production of $KMnO_4$

Potassium permanganate used in this study was obtained from the Department of Chemistry, College of Science, University of Anbar. The stock solutions were acquired and serial dilutions were done.

Animals

Twenty-eight (28) Swiss albino mice (female) aged between 2 and 3 months were purchased from the Iraqi Center for Cancer Research and Medical Genetics, Al-Mustansiriyah University. They were kept in cages for 4 weeks for environmental adaptation. Twenty-eight mice weighing between 25–30 g were divided into 4 groups, I, II, III, and IV of 7 mice per group. Group I was control, group II had KMnO4 (0.5 mg/kg/BW) daily, group III had an aqueous extract of *H. sabdariffa* flowers (500 mg/kg BW) and $KMnO_4$ (0.5 mg/kg/BW) daily, and group 4 had an aqueous extract of *M. parviflora* leaves (300 mg/kg/BW) and $KMnO_4$ (0.5 mg/kg/BW). The experiment lasted until the end of thirty days.

Histological section

The tissue specimens of kidneys and the liver were abstracted from the mice and located in formalin. Pieces of the organs were prepared for microscopic examination. The tissues were dehydrated for 2 hours in a graduated level of alcohol (ethanol 50% to absolute) in the ascending stage, and then they were submerged in xylene for 30 minutes. The tissue was impregnated with wax of paraffin and cut at 4-micron thickness. The tissue sections were floated on a water bath at 50°C less than the melting grade of paraffin wax. They were dried (20–30 minutes) and dyed with hematoxylin and eosin stain, hydrated, cleared and mounted (DPX) in a mountant, averting blebs of air. Pictures were taken by an electronic camera at 40X and 10X magnification powers in a light microscope.

Results

The histopathological changes in the mice organs were microscopically examined. The results indicated various stages of structural changes in the kidney and the liver.

Kidney

The kidneys of the control group (I) demonstrated intact renal corpuscles and tubules in cortex and medulla (Fig. 1). The kidneys in the second group (administered with $KMnO_4$) showed blood vessel congestion, destruction of renal tubules, cellular infiltration and necrosis, large urinary space in renal corpuscles (Fig. 2). However, the kidneys in groups III and IV showed normal renal corpuscles and tubules (Figs. 3 and 4) compared with damaged kidney tissues as demonstrated in Fig. 2.

Liver

The control liver (group I) shows intact hepatic lobules, central veins and sinusoidal capillaries that ordinarily conserve the normal architecture viewed in this organ (Fig. 5); meanwhile, animals of group II show edema, blood vessel congestion, cellular necrosis, chromatin condensation in the nucleus, cellular infiltration, and nucleus like a ring (Fig. 6). There are no plausible changes in the hepatic cells, micro- and macro-vasculature, and hepatic plates in the mice (group III and IV) treated with $KMnO_4$ and with aqueous extracts of plants (Figs. 7 and 8).

Discussion

The mechanism of action of $KMnO_4$ in the body is through the generation of strong oxidative free radicals that attack the building blocks inside the cell, such as proteins, fats and nucleic acids (Dhaliwal and Singh, 2015). The histopathological observations of kidney sections of mice in the current study showed blood vessel congestion, destruction of renal tubules, cellular infiltration and necrosis, and a large urinary space in renal corpuscles. The histopathological observations of liver sections of mice in the current study showed edema, blood vessel congestion, cellular necrosis, chromatin condensation in the nucleus, cellular infiltration, and nucleus like a ring (circular

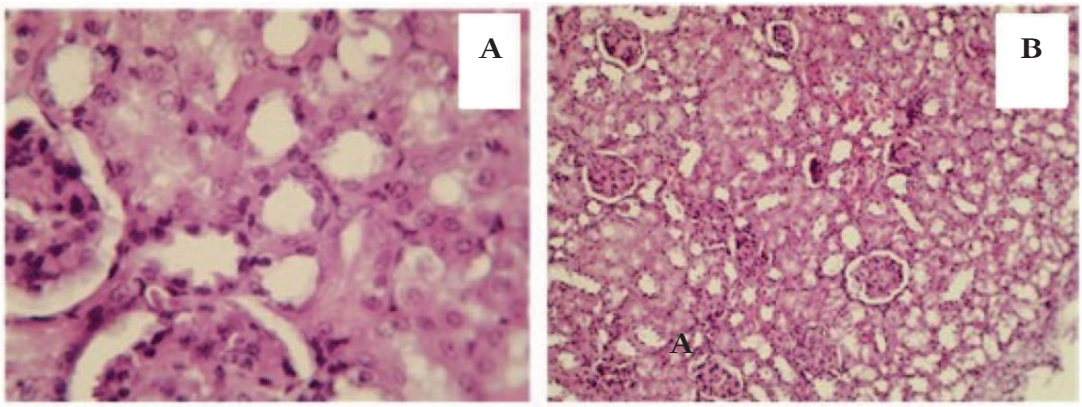

Fig. 1. Cross-sections of the kidney in control mice.
A: 10X and B: 40X (H & E).

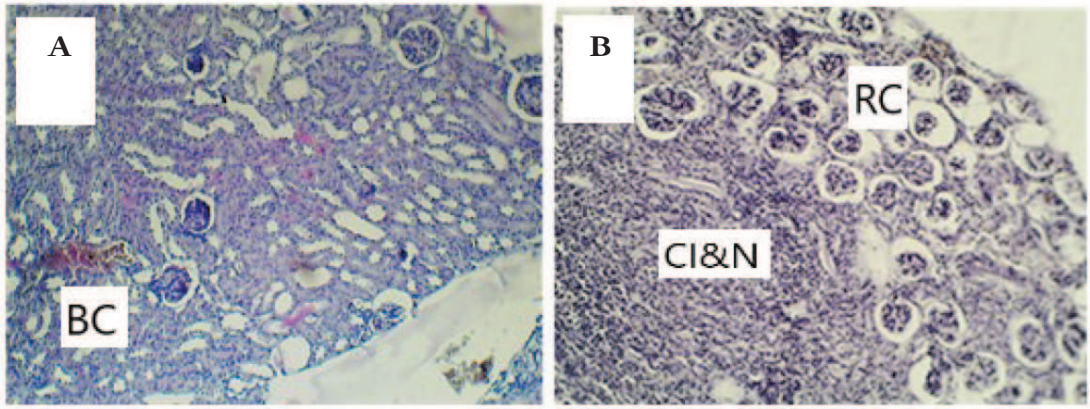

Fig. 2. Cross-sections of the kidney in mice exposed to $KMnO_4$.
(A) BC (blood vessel congestion) and (B) RC (renal corpuscle),
CI&N (cellular infiltration and necrosis). A: 10X and B: 40X (H & E).

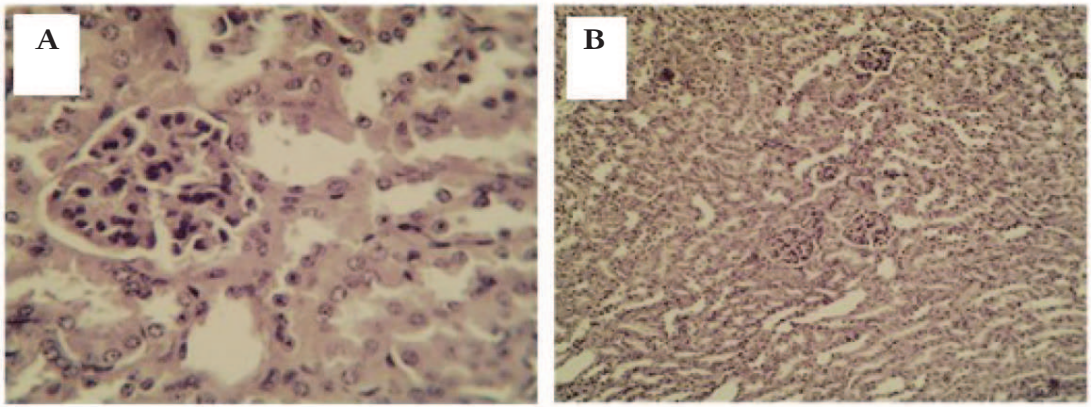

Fig. 3. Cross-sections of the kidney in mice administrated $KMnO_4$
and treated with an aqueous extract of *Hibiscus*.
A: 40X and B: 10X (H & E).

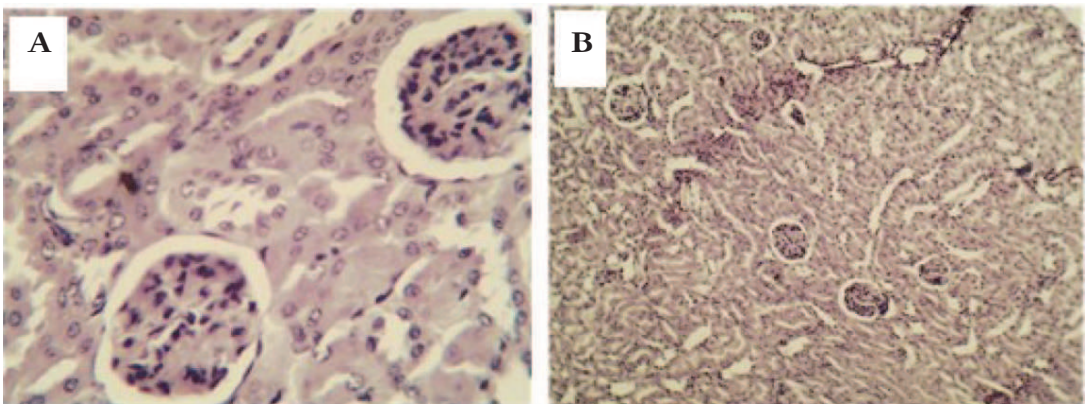

Fig. 4. Cross-sections of the kidney in mice administrated $KMnO_4$ and treated with an aqueous extract of *Malva*.
A: 40X and B: 10X (H & E).

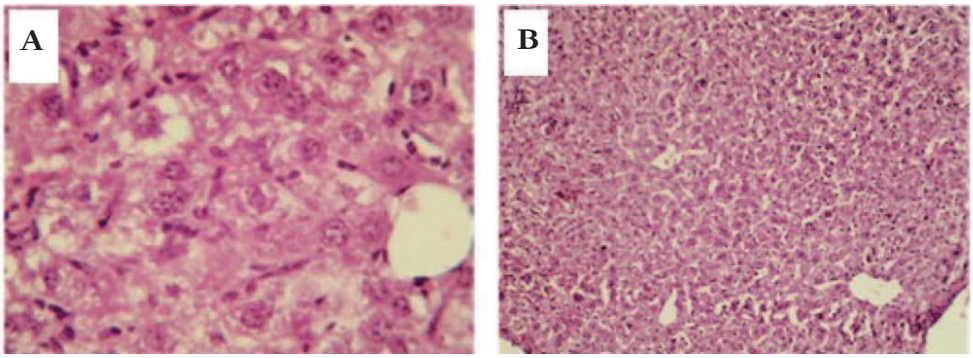

Fig. 5. Cross-sections of the liver in control mice.
A: 40X and B: 10X, (H & E).

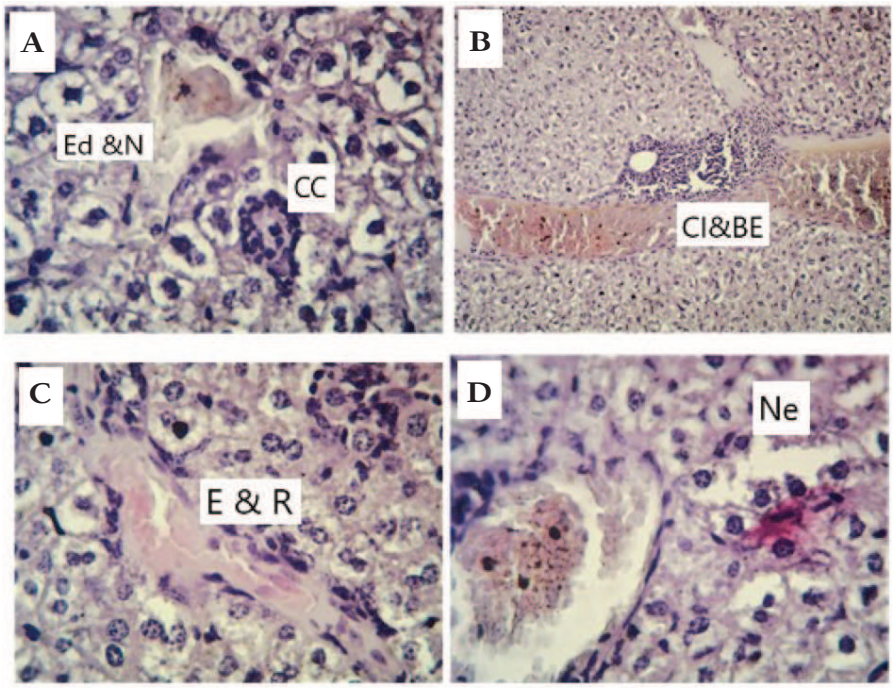

Fig. 6. Cross-sections of the liver in mice exposed to $KMnO_4$.
(A) Ed (edema), N (necrosis) and CC (chromatin condensation); (B) CI (cellular infiltration), BE (blood vessel edema); (C) E (edema), R (nucleus like a ring or circle); and (D) Ne (necrosis).
A, C, and D: 40X and B: 10X (H & E).

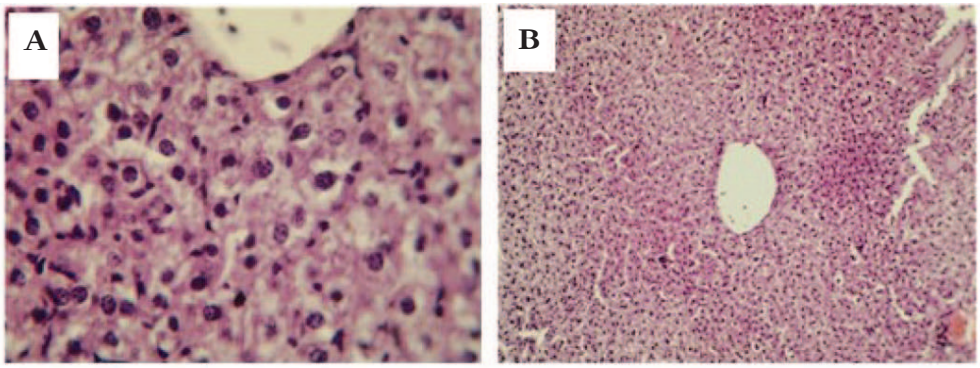

Fig. 7. Cross-sections of the liver in mice administrated $KMnO_4$ and treated with an aqueous extract of *Hibiscus*.
A: 40X and B: 10X (H & E).

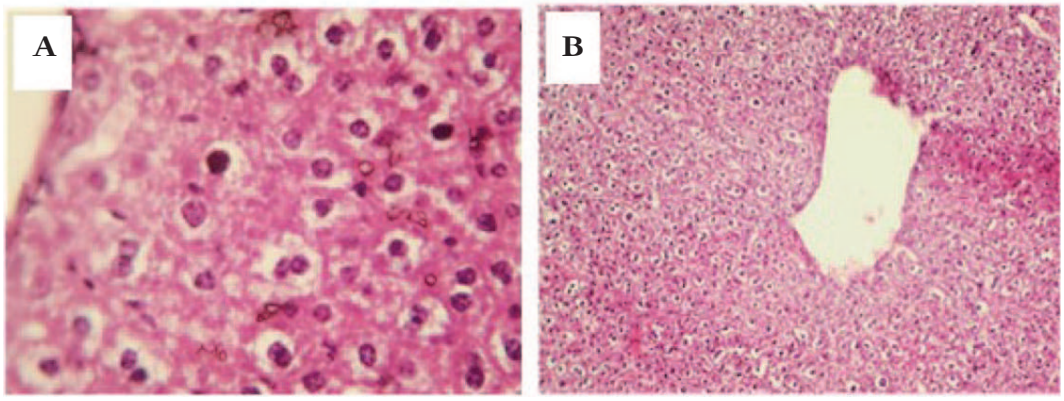

Fig. 8. Cross-sections of the liver in mice administrated $KMnO_4$ and treated with an aqueous extract of *Malva*.
A: 40X and B: 10X (H & E).

shape). These pathological changes may act as an indicator to kidney and liver toxicity, and oxidative stress with $KMnO_4$. The oxidant materials lead to the development and progression of kidney and liver damage, so it is considered the major pathological mechanism (Marins et al., 2020). Ejikeme et al. (2016) have found that $KMnO_4$ leads to a histopathological change and injuries in the tissues of the kidney (like cystic spaces, necrotic tubules, and destruction of renal tubules) and the liver (like enlargement of the sinusoids, disintegration of hepatic chords, and liver steatosis); besides, $KMnO_4$ affects urea and creatinine levels in the body. Al- Zwean (2017) has found that $KMnO_4$ induced significant changes in hepatic enzymes (like ALP, AST, and ALT) and proteins. Ali (2017) has observed various histopathological changes in the renal tissue (like aggregations of mononuclear cells and congestion of blood vessels), and coagulative necrosis in the hepatic tissue. Another study has shown that $KMnO_4$ affected biochemical molecules in the kidneys and the liver of mice (Hussein and Kata, 2008).

Medicinal plants are considered sources of natural productions and the most remarkable of the functional compounds are antioxidants. Antioxidant compounds finish the chain reactions resulting from free radicals (Rani et al., 2015). The kidneys in the groups treated with $KMnO_4$ and with aqueous extracts of plants showed normal renal corpuscles and tubules being intact without any structural damages. There are no plausible changes in the hepatic cells, micro- and macro-vasculature, and hepatic plates in the mice treated with $KMnO_4$ and with aqueous extracts of plants. Pacome et al. (2014) have shown that the components that form the petals of *H. sabdariffa* like alkaloids, anthocyanins, phenols, flavonoids, saponins, steroids, sterols and tannins contribute to the antioxidative effectiveness and have a scavenging ability (around 97%). However, the extracts of *H. sabdariffa* are possible sources of natural antioxidants, and this substantiates their utilities in herbal medicine. The results of improving the liver from the effect of hibiscus agree with Adeyemi et al. (2014). Therefore, it can be concluded that antioxidants in the *H. sabdariffa* extract, especially the anthocyanins, preserve the kidneys and the liver against oxidative factors.

Malva parviflora prevents inflammation, oxidative damage, and hypertension in the kidneys of mice because it contains some compounds such as oleanolic acid, scopoletin and tiliroside (Lagunas-Herrera et al., 2019). Another study has shown that the extracts of *M. parviflora* protect the liver from toxins (Mallhi et

al., 2014). Lowering tissue damage is exhibited in the histopathologic valuations of the current study. Treating diabetic rats with *M. parviflora* reduces oxidative stress and fat oxidation and protects the kidneys and the liver (Gutierrez, 2012). The results of the *M. parviflora* that has antioxidant activity agree with those of Farhan et al. (2012) and Ridh et al. (2018). Using the *M. parviflora* extract leads to improvement of all these injuries, which shows the protective effects of *M. parviflora* against histopathologic damage due to $KMnO_4$. Because this damage is caused by activated inflammatory and oxidative factors following $KMnO_4$ usage, it appears that *M. parviflora* decreases the histopathologic damage by lowering oxidative stress and inflammation. In advowson of these findings, it is exhibited that the utilization of antioxidants can weaken renal and hepatic injuries due to $KMnO_4$ (Ejikeme et al., 2016).

Conclusion

The current study explains for the first time that using aqueous extracts of *H. sabdariffa* and *M. parviflora* can prevent strongest oxidants ($KMnO_4$) that induce renal and hepatic injuries. The histological changes in the kidney and the liver may be a direct cause of renal toxicity and hepatotoxicity at high $KMnO_4$; and in other cases, *H. sabdariffa* and *M. parviflora* have to some extent protect the kidneys and the liver as well. The protective mechanisms of plant extracts may be due to reduced oxidative stress, reduced inflammation, or increased effectiveness of antioxidants in the body, or possibly other unknown mechanisms, which require further research studies. These herbal extracts can also be utilized as food additives for aquaculture.

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Conflict of interests

Authors declare no conflict of interest.

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