Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Hormonal and histological study on the protective effect of *Moringa oleifera* against chromium toxicity

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ABSTRACT

The current study aimed to know the effects of oral administration of *Moringa oleifera* leaves powder which contains the active substances to prevent the harmful toxic effect of chromium Cr (potassium dichromate; VI) on some hormonal and biochemical tests, in addition to understand the relationship between active compound of *Moringa oleifera* on testes and liver tissues against chromium toxicity. Thirty-three healthy male white New Zealand rabbits were randomly distributed in three groups (11 rabbits in each group). The first group were orally drenched normal saline considered as a control group. The second, were treated with chromium (40 mg/ kg body weight/day) to notice experimental toxicity on some blood parameters and tissues structure., The third with 300 mg *Moringa oleifera* powder /kg diet/day with the chromium (40 mg/ kg body weight/day). Treating for all groups was continued for 60 days. The results showed negative effects as a significant decrease ($p \leq 0.01$) in testosterone and inhibin-B hormone, glutathione (GSH) and total antioxidant capacity (TAC), by the increased level of FSH, alanine amino transferase (ALT), aspartate amino transferase (AST), liver cholesterol and malondialdehyde (MDA), in addition to histological variation in liver and testes in the rabbit serum dealt with chromium (group $_2$). In contrast, a positive effect was observed as a significant raise in testosterone, inhibin-B hormone, GSH and TAC, by decreased levels of FSH, ALT, AST, liver cholesterol and MDA in the serum dealt with *Moringa oleifera* (group $_3$), in addition to the improvement effects on testicular tissue and liver to be closed to normal concentration in control group.

Keywords: Inhibin- B, Potassium dichromate, Testosterone hormone, TAC, GSH.
Article type: Research Article.

INTRODUCTION

Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases (Doisy *et al*. 2013). Chromium shows in different states, being hexavalent chromium Cr (VI), and trivalent chromium Cr (III) the most stable forms. Cr (III) is predominantly present in the salts used as micronutrients and dietary supplements (Pechova & Pavlata 2007). Potassium dichromate and chromic acid are chromium salts, widely used in leather and dyeing industry (Bagashi *et al.* 2002). Chromium-containing compounds are carcinogenic and mutagenic to humans and animals, and therefore have severe effects on tissues, especially the liver and kidneys (Fathima & Priyanka 2017) and other vital organs (Nudler *et al.* 2009). During metabolism of chromium, it is transformed to reactive chromium intermediates that produce reactive oxygen species (ROS; O'Brien *et al*. 2001). ROS can give rise to tissues injury, damage of cellular components and oxidative stress (Yam-Canul *et al*. 2008). There are many studies about chromium toxicity in the world (Alizadeh & Mirarab-Razi 2016; Mehdinia *et al.* 2017; Janbakhsh *et al.* 2018; Sobhanardakani 2019; Azizi *et al.* 2020; Saleh Ibrahim *et al.* 2022; Budovich 2021). Environmental Protection Agency (EPA) listed chromium as one of the most environmental toxic heavy metals for human (Gupta *et al*. 2015). Several findings mentioned the harmful effects

Caspian Journal of Environmental Sciences, Vol. 20 No. 3 pp. 477-489 Received: Dec. 13, 2021 Revised: March 15, 2022 Accepted: May 26, 2022
DOI: 10.22124/CJES.2022.5641

Publisher: University of Guilan, Iran

of Cr (VI) on the functions of reproductive system in male rats (Al-Mukhtar *et al*. 2016; Ibrahim *et al*. 2017). Available suggestion report that chromium has adversely affect the male reproductive system, these effects is decreases in the testicles, and the weight of some accessory glands, reduced sperm counts, sperms deformities and disturbance in sexual behavior (Afonne *et al*. 2000). Chromium have sever effects by entrance to hepatocytes resulting in ROS with tissue damages and apoptosis (Al-Saadi & Al-Kaisei 2020). Inhibin is a glycoprotein hormone secreted by gonads, comprised two subunits, termed a and b, which has an important role for inhibiting the secretion follicle stimulating hormone (FSH). The two Inhibins are formed based on a difference in their b-subunits, and these are designated inhibin-A (a = bA) and inhibin-B (a = bB). Inhibin B is the physiologically-important form of inhibin in the male. It is possible that the circulating inhibin-B level reflects the extent of the relationship between Sertoli cells and germ cells, particularly the elongate spermatid (Pineau *et al*. 1990; Illingworth *et al*. 1996). In the adult, inhibin-B production depends on both F.S.H. and spermatogenic status, but it is not known in which way germ cells contribute to inhibin-B secretion (Meachem *et al*. 2001).

However, herbal medicine is a mainstay in developing countries for major healthcare (Achinewu & Aniena, 1995). The scientists recommended in the use of natural product that characterized by a huge source of remedies that control all of human-kind insults, to avoid drugs toxicity once the treatment of any insult of kidney or liver; the site of detoxification and toxicant elimination (Abeer *et al.* 2018). It has been shown that the natural antioxidant impact plays a central role in effects associated with phyto-compounds (Mohamed & Fayed 2020). The *Moringa oleifera* is a tropical tree belonging to the *Moringaceae* family, this family contains different species (Vaknin & Mishal 2017). However, the most famous of them is *M. oleifera*. The tree grown in northern India and northern Europe, although it is also in the Red Sea area and parts of Asia and Africa, including Madagascar. However, this tree has been distributed around the world (Singh *et al*. 2019) and this fact has led to it being given many names, "drumstick tree", "benzolive tree" and "orseradish tree" (Srivastava *et al*., 2020). Historical evidence detects that ancient queens used the fruits and leaves of *M. oleifera* in their diet to maintain memory and healthy skin about 150 B.C. (Trigo *et al*. 2021), due to its versatility in medicinal use. *M. oleifera* has been considered as one of the best plants (Vinodini *et al*. 2014).

Leaves antioxidants, acting in concert with the antioxidant system occur in the epididymis preserved and promoted the sperm formation process. The basic component responsible for *M. oleifera* ability to promote libido is the flavonoids, which is believed to play an important role in changing androgen levels also be responsible for supporting male sexual behavior (Padashetty & Mishra 2007). *M. oleifera* possesses antitumor, antioxidant, antidiuretic, anti-inflammatory, hepato-protective and antidiabetic properties, reflected in the decreased stress and lipid peroxidation, as well as the upraised antioxidant activity (Sadek 2014). Therefore, this study has been to observe *M. olifera* effects against induced toxicity by chromium.

Aims of the study

Exposure to heavy metals has adverse effects on many organs of the body and thus on their roles. Therefore, we aimed to determine the ability of *Moringa* leaves which rich with polyphenol chemicals to protect rabbit testes and liver from disorders induced by chromium based on some hormonal, histopathological and biochemical elucidation.

MATERIALS AND METHODS

Chromium Cr (Potassium dichromate)

Chromium was obtained from the chemical materials store in the College of Science/ Department of Chemistry/ University of Mosul.

Rabbits

The study samples included a total of 33 individuals of healthy New Zealand male rabbits, ranging in weigh between 1250 and 1500 g and age from 10-12 months. These rabbits were fed a concentrated diet for a week in order to acclimate with experimental status.

Moringa oleifera

Packaged *M. oleifera* dry leaves powder was purchased from the *Moringa* plantation unit of the Scientific Association of *Moringa*, National Research Centre, Dokki, Egypt.

Study protocol: Thirty three healthy male rabbits were randomly distributed into 3 groups:

Group 1 (Control group): 11 male rabbits fed an ordinary diet without any supplementation for an experimental period of two months days. They were dosed with physiological saline to neutralize stress of catching rabbits.
Group 2: 11 male rabbits fed an ordinary diet and treated with Chromium 40 mg/ kg body weight/day (Momo *et al*. 019).
Group 3: 11 rabbits fed an ordinary diet mixed with 300 mg *M. oleifera* powder /kg diet/day (Khalil *et al*. 2019) and 40 mg chromium per kg body weight daily for 60 days.

Analysis kits
Commercial diagnostic kits for estimating hormones, FSH, LH and testosterone concentration was estimated using the ready-made Accubind ELISA. Microwells assay kit supplied by American Monobind Inc. Serum Inhibin and TAC were obtained by Elisa MyBioSource Company, USA. AST, ALT were determined by the method of Colorimetric test (Reitman & Frankel 1957), while GSH by the methodology of Moron *et al*. (1979); MDA by Gilbert *et al.* (1984) and liver cholesterol by Folch *et al.* (1957). Histological tests of liver and testes and microscopic slides were prepared using the method of Drury *et al*. (1985)

Data analysis
One-Way ANOVA analysis of variance using the General Linear Model, (SAS software) was employed in the statistical analysis (SAS 2004). To separate the means when there is a significant difference for this purpose, Duncan test was used. Means and standard deviations were calculated for all parameter. Significant at $p \leq 0.01$.

RESULTS

Table 1. The hormonal levels in the experimental groups.

Groups / Parameters	Group 1	Group 2	Group 3
Inhibin (pg/ mL)	40.7 ± 2.36[a]	24.34 ± 2.7[c]	36.95 ± 1.45[b]
Testosterone (ng/ mL)	2.36 ± 0.05[a]	0.94 ± 0.13[c]	1.78 ± 0.18[b]
FSH (U /mL)	1.57 ± 0.04[c]	2.50 ± 0.07[a]	1.87 ± 0.08[b]
LH (U/ mL)	5. 1 ± 0.05[a]	5.4 ± 0.1[a]	5.1 ± 0.04[a]

Note. The values is means ± SD; number of rabbits per group = 11; The numbers followed by different letters means that there is a significant difference.

Table 2. The biochemical parameter levels in the experimental groups.

Groups / Parameters	Group 1	Group 2	Group 3
AST U/ L	24.2 ± 2.36[b]	41.4 ± 2.7[a]	30.95 ± 3.45[b]
ALT U /L	12.6 ± 3.6[c]	25.4 ± 2.4[a]	20.5 ± 2.7[b]
GSH (µmol /gm) liver tissue	1.73 ± 0.04[a]	1.21 ± 0.07[c]	1.57 ± 0.08[b]
MDA (nmol /gm) liver tissue	230.1 ± 5.15b	350.72 ± 7.5[a]	300.8 ± 6.4[c]
TAC (ng /mL)	115.1 ± 5.04[a]	78.2 ± 9.06[c]	82.96 ± 6.04[b]
Cholesterol (mg /gm) liver tissue	166 ± 2.3[c]	187 ± 1.6[a]	175.1 ± 0.7[b]

Note. The values is means ± SD; Number of rabbits per group = 11, The numbers followed by different letters means that there is significant difference.

The obtained results in Table 1 showed significant drop in serum Inhibin and testosterone in group $_2$, in contrast, significant rise in FSH, while in group $_3$, they were close to the levels of control group. In Table 2, the results showed that GSH and TAC dropped significantly in group $_2$, in contrast, significant elevation of AST, ALT, MDA and cholesterol in liver, while in group $_3$, they were close to the levels of control group.

Histological findings

In the testes of second group treated with chromium, it was found that the number of layers lining the seminiferous tubules forming sperm and their thinning were reduced, so that, the thickness of the wall ranged from about 1-4 cell layers compared to the control group in which the wall thickness was about 10 layers. In the case of the third group, it was observed that the number of layers of seminiferous tubules forming sperm upraised, very similar to group $_1$. The normal histological structure of rabbit liver lobule was composed hepatic cords consist of hexagonal (cuboidal) epithelial cells which radiated from central vein (branch from hepatic vein). Among the hepatic cords, large-diameter capillaries were observed, called hepatic sinusoids. In group $_2$, treated with chromium, the formation of lesions of very severe fatty change was observed, appearing in the form of an accumulation of fat cells, which replaced the hepatocytes, as well as observing the vacuolar degeneration in the cytoplasm of the hepatic cells.

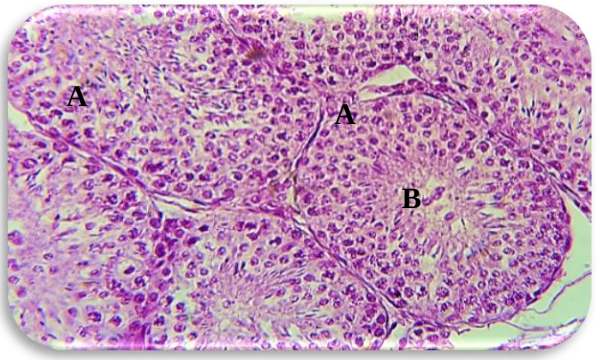

Fig. 1. Testis of control group (400 X); A: Section of rabbit testis showing seminiferous tubules, B: describes the narrowness of the lumen.

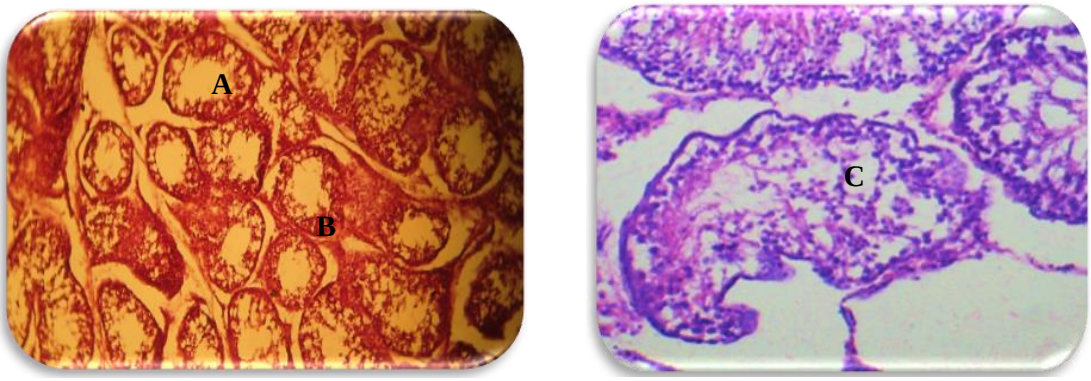

Fig. 2. Testis of group $_2$, chromium group; B: 100 X, C: 400 X; A: Lumen enlargement; and B: Fewer rows of spermatogenic cells; C: Seminiferous tubules and spermatogenic cells malformation.

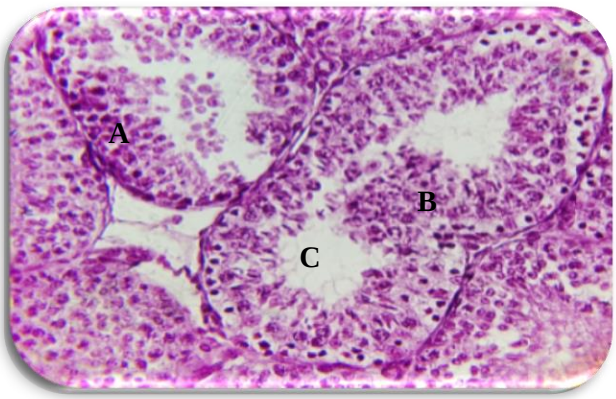

Fig. 3. Testis of group $_3$ (chromium + *Moringa* group; 400 X); A: Increase the no. of row of spermatogenic cells; B: Regularity of Seminiferous tubules; C: The cavity is narrower compared to the group $_2$ (400 X).

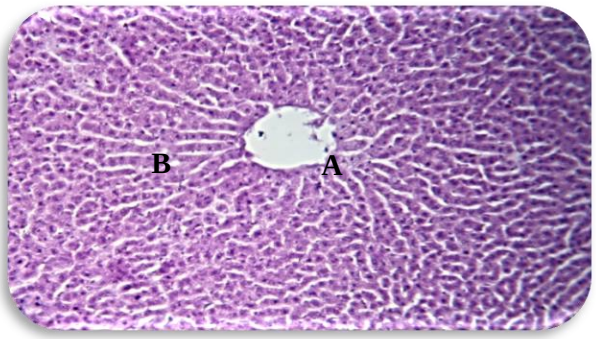

Fig. 4. The liver in control group (100 X), showing the normal shape of the central vein; A: hepatocytes and cords around the central vein; B: Cords of hepatocytes are distinct essentially normal, no fatty change, cytoplasm not vacuolated.

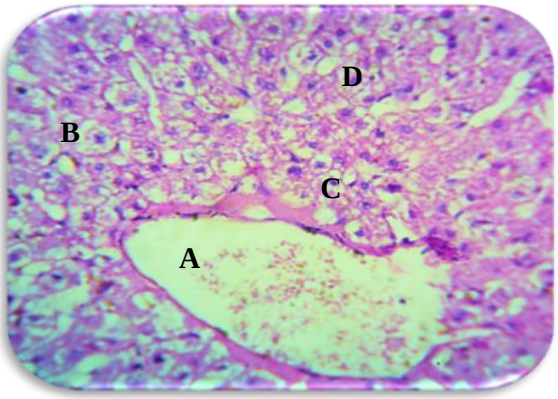

Fig 5. The liver in group $_2$ (400 X); A: Dilatation and congestion of the hepatic central vein; B: Vacuoles appear in the cytoplasm of hepatocytes; C: Irregularity in hepatic cords; D: The presence of fat cells in some venous sinuses.

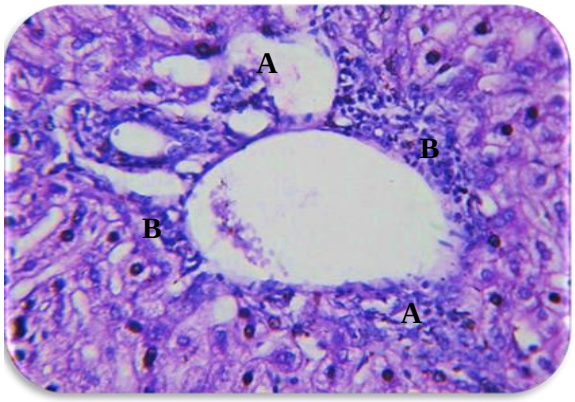

Fig. 6. The liver in group 2 (400 X); A: Hyperplasia of epithelial cells lining the bile ducts in the portal area; B: Collection of lymphocytes around the portal vein.

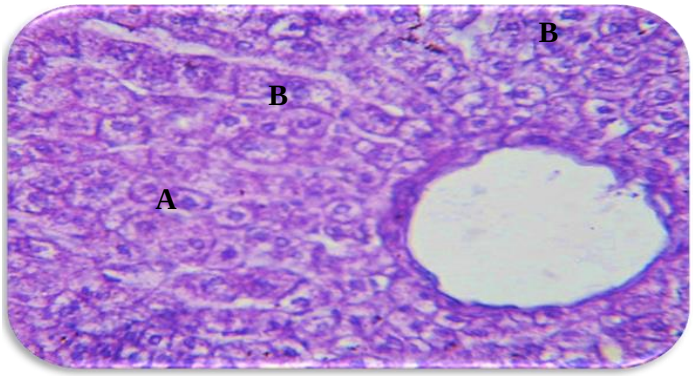

Fig. 7. The liver in group $_3$ (400 X); A: Swelling of hepatocytes, regularity of hepatic cords; B: Presence of few diploid cells and the shape is close to the control group.

DISCUSSION

The majority of metal toxicity reports on the reproductive system are coming from experimental studies on animals. In the present study, the results showed a significant decrease in the testosterone level in group $_2$, i.e., Cr (VI) treated rabbits, which may correlate to the spermatogenic impairment and depleted epididymal sperm number (Chandra *et al*. 2007), since testosterone is essential for the motivation and conservation of spermatogenesis (Singh *et al*. 1995). The elevation in FSH concentration indicates diminished negative feedback from the seminiferous tubules, and this is in agreement with our study, which reflects in a significant rise in FSH level and this increasing exhibits the reduction of inhibin-B level because of the negative feedback between them. The increased FSH level in group $_2$ is probably due to a drop in the secretion of inhibin by Sertoli cells, affecting

negative feedback on the hypothalamic pituitary axis (Weinbauer *et al*. 1989; Bashandy *et al*. 2019). Chromium also exhibits the ability to bind to the oestrogen and androgen receptors, together with other heavy metals and then could interfere with reproductive hypothalamic hormones, i.e., LH and FSH as well as with testosterone (Kortenkamp 2011). According to Jensen & colleagues (2006), occupational exposure to Cr can disrupt the production of androgens by Leydig cells or the inhibin-B by Sertoli cells (Jensen *et al*. 2006). Glutathione is normally occur in millimolar concentrations in cells and is known as the cellular system protector against lipid peroxidation and its toxic effects. It has an important role in maintaining the state of cellular redox (Rao & Shaha 2011). Oxidative stress is a sign of its depletion (Lu 1999). It was indicated by Momo *et al.* (2019), that exposure to potassium dichromate makes the most of the antioxidant enzymes inactive, either because the heavy metals have ability to rapid binding to active site of enzymes or to the displacement of metal cofactors from this active sites (Momo *et al*. 2019). The increased MDA level is considered as a sign of lipid peroxidation. In this study, a rise in cholesterol value because tissues reduced their use of this nutrient (Yousif & Ahmed 2009). Chromium has been reported to cause hepatotoxicity and oxidative stress. Chromium given to rats led to an elevation in serum alanine aminotransferase activity and hepatic lipid peroxide production (Banerjee *et al*. 2017). Previous studies have reported that oxidative stress may cause by Cr in different cells. In addition, the Cr-induced hepatic cells toxicity is related to oxidative stress also dysfunction of mitochondria is accompanied by ROS accumulation (Zhang *et al*. 2019; Fu *et al*. 2020). A high level of serum ALT and AST indicate liver damage. Their increase in this study suggests that chromium may created ROS, hence, oxidative stress leads to toxicity of liver and kidney. This might be due to the impairment their synthesis or impaired liver vitality related to oxidative stress (Soudani *et al*. 2013). Similar results on rats were observed by Saha *et al.* (2017). There is a growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank *et al*. 1982). Our study showed a significant increase in testosterone level in group $_3$, this may be due to elevated testosterone following *Moringa* administration and consequently, concurrent improvements in testicular markers of androgen synthesis (StAR protein and 3β-hydroxysteroid dehydrogenase expression), as well as the reduced lipid peroxidation in liver, which may be via reduced ROS (Mohamed *et al*. 2019). The *Maringa* ROS-scavenging effects was previously established in rats feed a high-fat diet to stimulate oxidative stress (Das *et al*. 2015). Increased ROS inhibits the expression of StAR protein and its consequent cholesterol transfer activities important for steroidogenesis (Diemer *et al*. 2003). Chlorogenic acid occurred in *Moringa*, stimulates the activity of AMPK (Ong *et al*. 2013). Activation of AMPK triggers key metabolic processes, including elevated ATP, lipogenesis inhibition and biosynthesis of cholesterol (Lopes *et al*. 2017). Modification in the activity of AMPK, affects the expression of hormone-induced StAR protein and steroidogenesis (Abdou *et al*. 2014). In rats, supplementation of *Moringa* leaf extract for four-weeks at 200-500 mg kg^{-1} increased testosterone level and steroidogenic acute regulatory (StAR) of protein expression (Mohamed *et al*. 2019). *Moringa* is an important source of zinc (Gopalakrishnan *et al*. 2016), that plays an antioxidant role in the Leydig cells (Fallah *et al*. 2018). Zinc disorder impairs the activity of angiotensin-converting enzyme that leads to drain of testosterone (Bedwal & Bahuguna 1994). Another mechanism by which *Moringa* may elevate this hormone is by Vitamin E, which known to reduce stress of testes and elevation in steroidogenesis by Leydig cells (Aybek *et al*. 2008). *Moringa* leaves are considered as an important source of Vitamin E (Ganatra *et al*. 2012). In group $_3$, the results showed a significant increase in inhibin- B with decrease in FSH level, in line with results obtained by Nayak *et al.* (2020) who reported that the use of *Moringa* before taking cyclophosphamide improved the functional properties of the sperm, reduced FSH and elevated inhibin- B values. Treatment with *Moringa* has been shown to ameliorate CP-induced damage by modulating the expression of genes specific to Sertoli cells and spermatozoa (Nayak *et al*. 2020). The result of TAC is in accordance to results obtained by Tuorkey (2016) and Luqman *et al.* (2012) reported elevation in *Moringa* concentration caused TAC level to be upraised. It can be used to stimulate the antioxidant capacity of developing rabbits, hence, *M. oleifera* may be considered as a notable source of health-protecting compounds with antioxidant power. Siddhuraju & Becker (2003) found *Moringa* leaves compounds as prebiotics and antioxidant such as chlorogenic acid and caffeic acid (Siddhuraju & Becker 2003). Similar to our study, Mbikay (2012) reported that the *M. oleifera* supplementation into diet for the developing animals reduced MDA level with upraised TAC, which may be because the reduced fat deposition by down-regulating the activities of lipoprotein lipase and malate dehydrogenase or elevating hormone-sensitive lipase activity in the fat tissue. Antioxidant capacity of *Moringa* leaves, rich source of vitamin B, calcium, protein and potassium. The results are consistent with similar findings by Oseni & Idowu (2014) who reported that *Moringa* leaves increased GSH, and TAC,

however, reduced MDA concentration in rats (Oseni & Idowu 2014). *Moringa* leaves appears to contain a package of natural vitamin E, C, carotenoids and polyphenols, which deserves further evaluation as potential antioxidant agents (Bushuty & Shanshan 2020), which is close to our results about TAC to normal level in group 3. ALT and AST are hepatocytes injury-assessment enzymes because their elevated secrete into blood then damage to the hepatocytes. The significant decrease in the levels of these enzymes in-group 3 following *Moringa* administration can be related to the plant anti-inflammatory properties which can be attributed to flavonoids such as quecetin and phenolic acid found in abundant quantity in *Moringa* leaves (Coppin *et al*. 2013). These compounds have documented anti-inflammatory properties (León *et al*. 2015). Chlorogenic acid is one of the bioactive elements occur in *Moringa* in sufficient quantities. Other authors have reported that Chlorogenic acid markedly reduces total cholesterol level in the liver (Shi *et al*. 2013), similar to our results. In addition, Cho *et al*. (2010) reported that Chlorogenic acid is a significantly activity-inhibitor of fatty acid synthase, acyl-CoA cholesterol acyltransferase and 3-hydroxy-3-methylglutaryl CoA reductase, while elevates fatty acid -oxidation and PPAR expression in mouse livers compared to group 1(Cho *et al*. 2010). Cholesterol acts as starting material for bile acids synthesis in liver. In the present study, *M. oleifera* reduced cholesterol level by cholesterol synthesis inhibition and aiding bile acid synthesis. Other authors have reported cholesterol-lowering activity in rats and rabbits, which was fed with different parts of *Moringa* (Ghasi *et al*. 2000). Cholesterol homeostasis is maintained by controlling two processes: 1- cholesterol synthesis in which HMG CoA reductase catalyses the rate limiting process controlled by nutritional and hormonal state of animals; and 2-cholesterol absorption of both dietary cholesterol and cholesterol cleared from the liver through biliary secretion (Trapani *et al*. 2012). Reddy *et al*. (2017) pointed out that the activity of the enzyme is more significantly inhibited in *Moringa*-treated group than in others, and the hypocholesterolemic role of the *Moringa* has been attributed to an upraised biliary cholesterol and bile acids concentrations as well as arise in the faecal excretion of this compounds.

Ameliorations in profile of lipid after *Moringa* administration are due to reduced reactive oxygen species (Das *et al*. 2015), reduced lipolysis and conversion of free fatty acids to phospholipids and liver cholesterol (Olayaki *et al*. 2015), and increased receptors of LDL (Fuhrman & Aviram 2001). Testis is considered as one of the main target organ for metal-induced oxidative damage because of its rich content of polyunsaturated membrane lipids (Acharya *et al*. 2006). The treated with chromium led to histological variation in rabbits testes included reduction of the number of layers lining the seminiferous tubules. Various reports in the past few decades have shown that chromium hurts the testis by forming reactive radicals, leading to cell damage such as diminished enzyme activities, lipid bilayer and DNA damage, amplifying oxidative stress damage in sperm membranes, proteins and DNA (Stohs & Bagchi 1995; Akunna *et al*. 2012). This may perhaps explains the reduced testosterone in the chromium- treated group. Germ cell apoptosis caused by ROS, which is considered as the principal causes (Rao & Shaha 2000). Concomitant treatment with *Moringa* leaf extract, which has been shown to contain bioflavonoids and other potent antioxidants, significantly enhanced the parameters of unbalanced sperm in the testis as observed in group 3. Pretreatment of *Moringa* leaf extract has been shown to protect the testes from a variety of toxic compounds (Stohs & Bagchi 1995; Saalu *et al*. 2011). Siddhuraju & Becker (2003) and Saalu *et al*. (2011) reported that *Moringa* contains phenolic compounds as essential antioxidants which help to protect the testicle from morphological, spermatogenic and oxidative modulation caused by toxic substances and some antitumor agents. *Moringa* leaves have been shown to protect spermatozoa and reduce cellular damage (Nayak *et al*. 2016). In addition, the hexane extract of *Moringa* has been related to promoted functions of the seminiferous tubule, testis, epididymis, and seminal vesicle (Cajuday & Pocsidio 2010). These actions may be consistent with the abundance of active components in *Moringa* leaves, especially polyphenols and isothiocyanates (Waterman *et al*. 2015), so administration of *Moringa* was shown for ameliorate chromium-induced damage. The liver is a very important organ in assessing the toxic potential of a substance. It is related to the metabolism and excretion of toxic substances (Mossa *et al*. 2015) such as heavy metals. The treated with chromium led to histological variation in rabbit liver included slightly congestion of central vein, fatty degeneration in the hepatocytes, and the cytoplasm of some hepatocytes appeared to be vacuolated. Our study findings in group 3 which was administrated 40 mg/ kg B.W. of chromium for experiment time were revealed slight enlargement of central vein in liver lobules which surrounded by inflammatory cells, clear vacuolization in cytoplasm of hepatocytes, and fatty degeneration which occurs in some hepatocytes. These observations were documented with recent studies (Wang *et al*. 2010), thus the oxidative stress leads to hepatotoxicity.

This may be due to drop in their synthesis or impaired liver vitality associated with stress, which promotes apoptosis and hepatocytes oxidative stress (Soudani *et al*. 2013). This is an indicator of lipid peroxidation, the oxidation of phospholipids in the cell membrane, which causes these cells to break down because the activity of free radicals cannot be resisted. This leads to an imbalance in the permeability of the cell membrane, since fatty molecules accumulate inside the cell. In addition, the accumulation of fat vacuoles in the cytoplasm of hepatocytes is the result of the breakdown of cell membranes as a result of the accumulation of free radicals. Moreover, the appearance of vacuolar degeneration lesions results from a defect in sodium pumps in mitochondria, which results in a decreased production of energy needed to form proteins. The latter leads to a deficiency in the protein necessary for the integrity of cell membranes (Al-Khafaf 2005). Furthermore, the blood congestion in the portal vein, and fibrosis around the bile ducts occur in the group of female whose ovaries removed which may be due to poor blood supply to the liver and the occurrence of a lack of oxygen, inflammatory response. This may occurs for immune reasons (Mac Sween & Whalely 1992). The bioactive compounds in the *Moringa* extract as flavonoids, phenolics and steroids (Saalu *et al*. 2011) may play an important role in the amendment of hepato-renal function and antioxidant defence system. The protective effects of flavonoids in biological systems are in association with their efficiency to inhibit oxidative stress. It was suggested that the antioxidant properties that scavenge free radicals might be the main factor responsible for protective action on chromium- induced hepatotoxicity (Bashandy *et al*. 2020). The modification observed in the ALT and AST can be reversed with *Moringa*, which plays a protective role in the permeability of membrane, since it targets oxidants and can function as a scavenger of free radicals (Sharifudin *et al*. 2012).

CONCLUSION

We conclude that *M. oleifera* is a good natural drug for its important role for recovering experimentally-induced biochemical and histological toxicity by chromium in rabbits. It fights against the upraised ALT, AST and MDA by increasing the GSH and TAC with the elevated increasing testosterone hormone.

ACKNOWLEDGMENT

The authors express their gratitude to the University of Mosul/College of Science.

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Bibliographic information of this paper for citing:

Al –Hadidy, A,A,A, Mostafa, S,O 2022, Hormonal and histological study on the protective effect of *Moringa oleifera* against chromium toxicity. Caspian Journal of Environmental Sciences, 20: 477-489.