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Appraisal Role of Watery and Acetonic Leaves Extract of *Moringa oleifera* Growing in Egypt against Cd-induced Toxicity in Male Albino Rats

Kamal Mahmoud
Biochemistry Department, Faculty of Agriculture, Menoufia University, Menoufia, Egypt.
E-mail kamalmahmoud129@gmail.com.

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ABSTRACT

Cadmium (Cd) is an environmental pollutant that negatively affects various organs of the organism, especially the liver and kidneys. Using plant species to reduce the harmful effects of heavy metals has gained popularity, due to its low cost in parallel with fewer side effects than the physical and chemical methods. Here, we evaluate the role of *Moringa oleifera* (*M.O*) leaf extracts (aqueous and acetone) at two different doses (300 and 600 mg/Kg b.wt) from each extract against cadmium toxicity. Thirty male albino rats (190±10g) were divided into six groups and treated for 30 days. Group 1: control, Group 2: treated by Cd alone (5.4 mg/Kg b.w/day), Group 3 and 4: received *M.O* aqueous extract at 300 and 600 mg/Kg b.w/day respectively in parallel with Cd as in group 2, Group 5 and 6 received *M.O* acetonic extract at 300 and 600 mg/Kg b.w/day respectively in parallel with Cd as in group 2. In comparison with the control group, cadmium treatment showed a significant increase ($P \leq 0.05$) in the levels of liver, renal biomarkers, and oxidative stress parameters, but showed a significant decrease in the antioxidant indices. In contrast, groups treated with aqueous and acetones' *M.O* extract in parallel with cadmium, showed a significant reduction in the previously mentioned parameters except for antioxidant indices which recorded significant ($P \leq 0.05$) elevation. The result revealed that *M.O* leaf extracts successfully improved the undesirable effects of cadmium and restored almost all variables to near their control levels and using Moringa leaf extracts as natural supplementary besides their health benefits can protect against toxicity caused by cadmium.

INTRODUCTION

Nowadays, heavy metals form a serious pollution problem and are increasingly predominant in our daily life, which should be noticed in our drinking water, air, and soil. For anyone, it is complicated to avoid exposure to any of the harmful heavy metals that are so common in our environment (Masindi and Muedi, 2018). They can disrupt cellular processes by binding metals to DNA and nuclear proteins (Flora *et al.*, 2008), displacement of essential metals from their respective sites e.g., calcium (Ca^{+2}) in bones or iron (Fe^{+2}) in erythrocytes (Jaishankar *et al.*, 2014; Puerto-Parejo *et al.*, 2017). In addition, some metals motivate the production of reactive forms of oxygen, as a result, they alter the activity of the antioxidant system (Tandon *et al.*, 2003; Mao *et al.* 2018). In contrast to organic pollutants, heavy metals are non–biodegradable, so they remain longly in their host organism, causing

long-term problems. There are multiple factors that control the toxicity of heavy metals like their dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals (Mahmoud *et al.,* 2011).

Cadmium (Cd) is one of the most detrimental to health due to its high toxicity, even at relatively low doses. In the last few years, its toxic levels have increased dramatically in the environment because it is naturally released into the environment through volcanic activities, forest fires, and the generation of sea salt aerosols (Yurtsever & Sengil, 2009; Ali *et al.*, 2019). This environmental toxicant adversely affects various organs such as lungs, bones, kidneys, liver, pancreas, and testes. The kidney is the main target organ of cadmium action. During chronic and sub-chronic exposure, the metal may overlap with the metabolic process via the renal cortex resulting in the malfunctioning of kidneys (Stohs *et al.,* 2000; Siddiqui, 2010).

Using plant species to reduce the harmful effects of heavy metals has gained popularity, due to its low cost in parallel with fewer side effects than the physical and chemical methods (Ali *et al.,* 2013). *Moringa oleifera* (*M.O*), a medicinal plant, has been used both *in vitro* and *in vivo* to overcome the toxicity of heavy metals (Jiraungkoorskul & Jiraungkoorskul, 2016).

Moringa oleifera (*M.O*) plant is a natural species of the *Moringaceae* family, grows rapidly, a drought-resistant tree, native to sub-Himalayan tracts of Northern India, however, it is distributed worldwide in the tropics and subtropics (Oluwole *et al.,* 2013). It has a wide range of medicinal applications due to its high nutritional value. Mostly, every part of the moringa tree can be used as food or medicine or for industrial purposes in various countries (Khalafalla *et al.,* 2010). The distinct parts of the moringa tree are rich in both macro-and micronutrients, such as protein and many vitamins (Richter *et al.,* 2003), as well as specific plant pigments., α-carotene and β-carotene, lutein, and phytochemical constituents such as flavonoids, polyphenols, alkaloids, tannins, saponins and sterols (Amaglo *et al.,* 2010; Coppin *et al.,* 2013; Saini et al., 2016). Multiple studies revealed a hepatoprotective effect of *M.O* extracts against various hepatotoxic drugs and chemicals. It has also been noted that Moringa and its various parts play a role in preventing and treating a wide range of afflictions, like inflammation, cardiovascular disorders, gastrointestinal, hematological, and hepatorenal diseases (Minaiyan et al., 2014; Misra et al., 2014; Toppo et al., 2015; Kou et al., 2018). The therapeutic effects of *M.O* could be due to the combined actions of various bioactive components found in it, including polyphenols, alkaloids, vitamins, flavonoids, and other compounds (Verma et al., 2009) and they collectively act on broad physiological processes including metabolism and immunity. In this study, we introduce the aqueous and acetonic extracts of *M.O* leaves as a potential therapy against the induced toxicity by cadmium.

MATERIALS AND METHODS

1.Chemical Reagents:

The kits for GSH, SOD and GST were obtained from Cayman chemical, E. Ellsworth Road, Ann Arbor, USA. kits for ALT, AST, ALP, albumin, urea, and creatinine were obtained from a gamma trade company, in Egypt. Cadmium chloride ($CdCl_2$), thiobarbituric acid (TBA), and Trichloroacetic acid (TCA) were obtained from Sigma, St. Louis, MO, USA. Other chemicals and reagents were obtained from a high commercial company in Egypt.

2. Plant Material:

Fresh leaves of *M. oleifera* were collected from the faculty of agriculture, Menoufia University, Shebin El-Kom, Menoufia, Egypt. The leaves were cleaned and air-dried for 24

hours, then dried at 50 °C. The dried samples were ground into a fine powder and kept in the refrigerator for analysis (Fig.1).

2.1. Preparation of Plant Extracts:

Of the total (1 Kg) dried powdered leaves, (½ Kg) was extracted by distilled water, and (½ Kg) was extracted by acetone at room temperature for 3 days. The resulting extracts were filtered using Whatman № 1 filter paper and the residues were re-extracted by the same process until plant materials were exhausted. The collected filtrates were pooled and evaporated to dryness under reduced pressure to give a semisolid residue, which was then lyophilized to get the powder and were stored at - 20 °C until used. The yields were 99.6 and 74.5 g per 500 g of dried powdered leaves of aqueous and acetone extracts respectively.

Fig. 1: *Moringa oleifera* fresh, dry, and powder leaves (from left to right).

3. Experimental Animals:

Thirty healthy adult male albino rats were obtained from the Memorial Institute of Ophthalmology in Giza Egypt. They were fed on commercial pellets (carbohydrate 80%; protein 10%; fats 5%; salt mixture 4%, and vitamins mixture 1%) and water *ad libitum*. After two weeks of adaptation, rats were weighed (190±10 g) and randomly divided into six groups each having five rats as follows:

Group (A)	Served as a negative control.
Group (B)	Rats were received 5.4 mg Cd /Kg b.wt/day ($1/10^{th}$ of LD_{50}) in the form of cadmium chloride ($CdCl_2$) orally by stomach tube for 30 consecutive days.
Group (C)	Rats were treated as group B followed after one hour by *M.O* aqueous extract at 300 mg/Kg b.wt/day for 30 consecutive days.
Group (D)	Rats were treated as group B followed after one hour by *M.O* aqueous extract at 600 mg/Kg b.wt/day for 30 consecutive days.
Group (E)	Rats were treated as group B followed after one hour by *M.O* acetonic extract at 300 mg/Kg b.wt/day for 30 consecutive days.
Group (F)	Rats were treated as group B followed after one hour by *M.O* acetonic extract at 600 mg/Kg b.wt/day for 30 consecutive days.

All experimental investigations were performed according to “humane animals” as stated in the “Guide for the Care and Use of Laboratory Animal Resources” (NRC, 2011)

3.1. Blood sampling and biochemical estimation:

After 20 and 30 days from the exposure period, rats were deprived of food overnight and blood samples were collected from the eye veins in heparin-containing tubes, then centrifuged at 4000 rpm for 10 min at 4 °C to obtain plasma, which was used for hepatorenal marker assays. The plasma albumin (Alb) level was assayed by the colorimetric method according to Cannon *et al.,* (1974). The activities of transaminases ALT and AST were measured colorimetrically in plasma by using the procedure described by Young (1990),

while ALP was measured colorimetrically in plasma by using the method developed by Moss *et al.*, (1987). The plasma urea and creatinine levels were assayed by the colorimetric method according to Young (2001).

After 30 days of the experiment, rats were sacrificed, and the liver has been removed immediately. At the same time, one lobe has been perfused with normal saline (0.9%, w/v) for the determination of the oxidative stress indices. In the liver tissues, the activities of SOD and GST have been estimated according to Marklund (1980) and Habig et al. (1974), respectively. Additionally, the levels of GSH and MDA have been detected based on Tietze (1969) and Burlakova et al. (1975), respectively.

4. Statistical Analysis:

Data were expressed as mean ± SD and statistical analysis was carried out by analysis of variance (ANOVA) followed by Duncan's multiple tests. *P-Value* less than 0.05 ($p < 0.05$) was statistically significant. All calculations were performed using Statistical Package for the Social Sciences (SPSS) version 25.

RESULTS

Effect of Cadmium and *M. oleifera* Leaf Extracts on Hepatic Function Tests:

It is clear from **Table-1** that the activity of AST, ALT, and ALP enzymes in plasma was significantly ($p \leq 0.05$) elevated in the Cd-group (B) after 20 and 30 consecutive days of exposure period compared to the control group (A), which indicates hepatotoxicity. Co-treatment with watery and acetonic leaves' extract of *M. oleifera* at 300 and 600 mg/kg b.wt from each extract (groups C, D, E and F) significantly ($p < 0.05$) prevented the hepatic dysfunctions, which is reflected by a reduction in their activities when compared to the group B. In contrast to this, there was significantly ($p < 0.05$) depletion in plasma level of albumin after 20 and 30 days of the exposure period in the group B compared with A, while the treatment with *M. oleifera* extracts resulted in increased albumin concentration compared to the group B. The effect of *M. oleifera* extracts was time and dose-dependent.

Table-1: Effect of Cd and moringa (watery and acetonic leaf extract) on the plasma hepatic indices

	AST activity (U/l)		ALT activity (U/l)		ALP activity (U/l)		Alb. level (g/dl)	
Period / Treatment	20 days	30 days	20 days	30 days	20 days	30 days	20 days	30 days
Group (A)	33±0.71 100%	35.8±0.84 100%	30.4±1.52 100%	33.4±1.14 100%	96.6±1.14 100%	99.4±1.52 100%	4.16±0.02 100%	4.09±0.01 100%
Group (B)	99.8±1.30 302.4%	153.4±1.14 428.5%	95±1 312.5%	147±1.87 440.1%	258.4±1.52 267.5%	384±2.45 386.3%	3.11±0.03 74.8%	2.86±0.02 69.9%
Group (C)	68.6±0.89 207.9%	75.4±1.14 210.6%	65±1.58 213.8%	71.6±0.89 214.4%	189.8±1.30 196.5%	218.6±1.95 219.9%	3.74±0.03 89.9%	3.56±0.01 87%
Group (D)	47.6±1.14 144.2%	52.2±0.84 145.8%	44.8±1.30 147.4%	49±0.71 146.7%	158.8±1.30 164.4%	187.4±1.14 188.5%	3.92±0.03 94.2%	3.76±0.01 91.9%
Group (E)	72.6±1.34 220%	84.2±1.30 235.2%	69.2±1.30 227.6%	77.6±1.34 232.3%	196.6±1.52 203.5%	242.4±1.34 243.9%	3.64±0.04 87.5%	3.47±0.02 84.8%
Group (F)	54±1.58 163.6%	63±1 176%	52.4±1.14 172.4%	59.6±1.34 178.4%	174.2±1.30 180.3%	201.4±1.14 202.6%	3.84±0.02 92.3%	3.62±0.02 88.5%

Group (A): Control; **Group (B):** rats received 5.4 mg Cd/Kg b.wt/day orally; **Group (C):** rats treated as group B beside *M.O* watery extract at 300 mg /Kg b.wt/day; **Group (D):** rats treated as group B beside *M.O* watery extract at 600 mg /Kg b.wt/day; **Group (E):** rats treated as group B beside *M.O* acetonic extract at 300 mg /Kg b.wt/day; **Group (F):** rats treated as group B beside *M.O* acetonic extract at 600 mg /Kg b.wt/day .

Effect of Cadmium and *M. oleifera* Leaf Extracts on Renal Function Tests:

A significant elevation ($p< 0.05$) in urea and creatinine levels was observed in group B compared to group A after 20 and 30 consecutive days of the exposure period (Fig. 2). Co-treatment with watery and acetonic leaves' extract of *M. oleifera* at 300 and 600 mg/kg b.wt from each extract (groups C, D, E and F) prevented the renal dysfunctions, which is reflected by a significant decrease ($p< 0.05$) in their level compared to the group B.

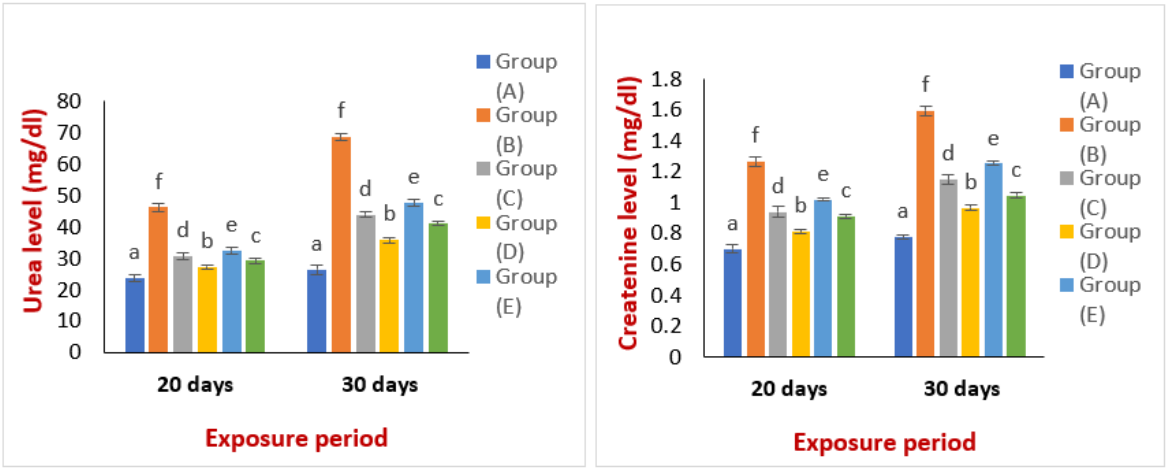

Fig.2: Effect of Cd and moringa (watery and acetonic leaf extract) on the plasma renal indices

Group (A): Control; **Group (B):** rats received 5.4 mg Cd/Kg b.wt/day orally; **Group (C):** rats treated as group B beside *M.O* watery extract at 300 mg /Kg b.wt/day; **Group (D):** rats treated as group B beside *M.O* watery extract at 600 mg /Kg b.wt/day; **Group (E):** rats treated as group B beside *M.O* acetonic extract at 300 mg /Kg b.wt/day; **Group (F):** rats treated as group B beside *M.O* acetonic extract at 600 mg /Kg b.wt/day .

Effect of Cadmium and *M. oleifera* Leaf Extracts on Oxidative Stress Parameters:

Compared with group A, group B exhibited significantly ($p< 0.05$) raised MDA levels in the liver but significantly ($p< 0.05$) reduced the activity of SOD and GST and levels of GSH in the liver after 20 and 30 consecutive days of the exposure period (Fig 3). Conversely, in the groups C, D, E and F which were treated with watery and acetonic leaves' extract of *M. oleifera* at 300 and 600 mg/kg b.wt from each extract, a significant decline ($p< 0.05$) in the MDA content and significant raise ($p< 0.05$) in the activity of SOD and GST and levels of GSH in the liver were observed when compared to the group (B).

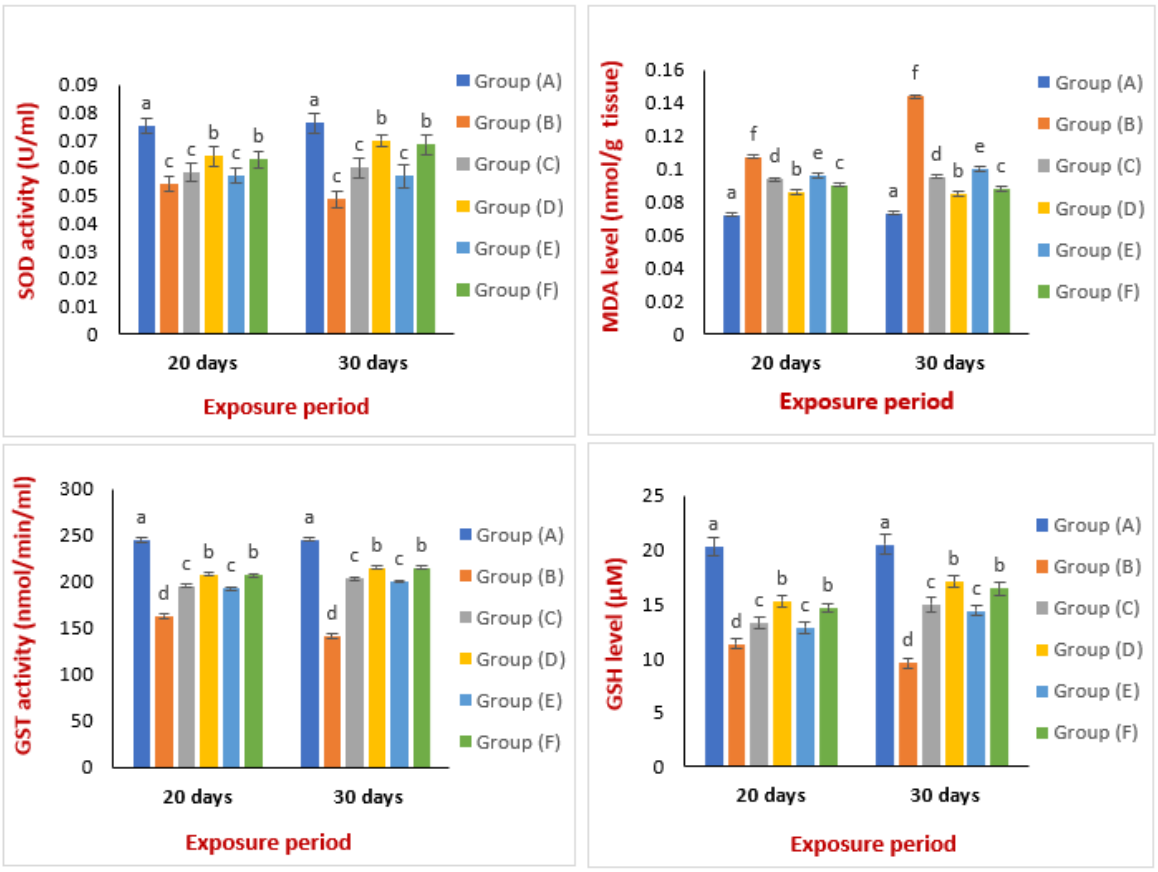

Fig. 3: Effect of Cd and moringa (watery and acetonic leaf extract) on the hepatic SOD, GST, GSH, and MDA levels

Group (A): Control; **Group (B):** rats received 5.4 mg Cd/Kg b.wt/day orally; **Group (C):** rats treated as group B beside *M.O* watery extract at 300 mg /Kg b.wt/day; **Group (D):** rats treated as group B beside *M.O* watery extract at 600 mg /Kg b.wt/day; **Group (E):** rats treated as group B beside *M.O* acetonic extract at 300 mg /Kg b.wt/day; **Group (F):** rats treated as group B beside *M.O* acetonic extract at 600 mg /Kg b.wt/day .

DISCUSSION

Environmental contamination by cadmium is a worldwide problem (Sandbichler and Hockner 2016). It is a mineral that is non-essential to the body's metabolism and is toxic to all types of organisms. Cadmium is absorbed and accumulated in various parts of the body, the most important of which are the liver and kidneys, so they are exposed to Cd-toxicity (Kowalewska 2001; Matović *et al.*, 2015; Andresen *et al.*, 2016; Kumar *et al.*, 2017). Keeping this in view, the present study focused on evaluating the role of Egyptian Moringa leaf extracts against experimental Cd-toxicity in male albino rats. The obtained result showed that Cd-caused severe toxic effects on the liver and kidney as revealed by levitation in their function indices as well as, higher oxidative which was revealed by decreasing the activity of antioxidant parameters and elevating the lipid peroxidation.

Numerous studies indicate that cadmium causes hepatotoxicity by increasing the production of ROS that attack essential cell components and thus cause their contents to be released into the circulation. An increase in the activity of liver function enzymes is one of the major effects of Cd-induced hepatotoxicity. In addition, the resulting hypoproteinemia in the Cd-treated group may be due to dietary insufficiency and excessive excretion and/or may be caused by hepatitis (Shaikh *et al.*, 1999; Metwally & Hashem 2009; Renugadevi & Prabu 2010; Choudhary and Devi, 2014; Mohammad *et al.*, 2018; Seif *et al.*, 2019).

In this study, elevated levels of ALT, AST, and ALP activities in group B (Cd-treated group) indicate liver damage, and this may be due to hepatocellular necrosis, which causes an increase in the permeability of the cell membrane resulting in the release of these enzymes in the bloodstream, However, rats in group C, D, E & F which treated with Cd in parallel with watery or acetonic M.O extract at 300 and 600 mg/kg bw from each extract respectively, showed a significant decrease in the levels of ALT, AST, and ALP activities when compared to Cd-treated group. Noting that the effect of the higher dose of both extracts in reducing the damage caused by Cd was greater than the effect of the small dose for them. This might be due to the hepatoprotective property of M.O which may contribute to the stabilizing activity of the cell membrane preventing enzyme leakage.

Simultaneous treatment of *M. oleifera* extracts with cadmium reduced Cd-induced hepatotoxicity by improving liver function as indicated by reducing ALT, AST, and ALP activities and raising albumin levels. The hepatoprotective property of Moringa is attributed to its high antioxidant activity and free radical scavenging activity which caused stabilization of cell membrane activity preventing enzyme leakage as earlier postulated which is consistent with Toppo *et al.*, 2015; Aml Salem Saleh, 2019; Alshubaily & Almotairi 2020. A previous study by Selvakumar & Natarajan, 2008 reported that the hepatoprotective effect of *M. oleifera* was due to the presence of phenolic compounds such as Quercetin and kaempferol. The aqueous *M.O* extract was more effective than the acetonic extract in protecting the hepato-renal from cadmium toxicity, which may be due to its higher content of total phenolics and flavonoids, which agrees with Hussain *et al.*, 2017 who reported that the content of Moringa aqueous and acetone extracts of total phenolics and flavonoids was 243.8 and 145.22 mg/g; 142.51 and 124.64 mg/g respectively.

The kidneys are severely affected by heavy metals such as cadmium, as these toxins damage kidney cells and fail in their functions, which is evident in severe changes in the indicators and indications of kidney functions in the blood such as urea and creatinine (Prozialeck & Edwards, 2012; Stohs & Hartman, 2015). Several studies suggest that the mechanism of action of cadmium in damaging the kidneys depends on raising oxidative stress and increasing free radicals significantly. Therefore, the researchers suggested the possibility of using different plants that contain high content of natural antioxidants, especially phenolic compounds, and flavonoids, to combat free radicals caused by heavy metals, which protects kidney cells (Prozialeck & Edwards, 2012; Saleh, 2018; Seif *et al.*, 2019).

The results of this study indicate that the use of watery and acetonic extracts of *M. oleifera* led to a significant improvement in urea and creatinine levels in Cd-treated animals. This can be explained by the high content of those extracts of phenolic compounds and flavonoids, which in turn work to combat oxidative stress resulting from treatment with cadmium and thus protect kidney cells.

Cadmium-caused toxicity indirectly generates free radicles such as hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), and nitric oxide ($^{\bullet}NO$), which raises the oxidative stress and consequently disintegrate cell walls of the hepatic tissues (Stohs *et al.*, 2001; Tandon *et al*.2003; Mao *et al.* 2018). From the obtained results, we found that Cd-induced oxidative stress through a marked rise in lipid peroxidation indices (MDA levels) accompanied by a decrease in antioxidant levels (SOD, GST, and GSH). These data are consistent with Renugadevi & Prabu 2010; Adaramoye & Akanni 2016; Saleh, 2018. On the other hand, treating animals with watery or acetonic moringa extract ameliorated the changes that occurred with Cd by reducing the elevation in the level of MDA accompanied by a decrease in antioxidant levels (SOD, GST, and GSH). This great effect of these extracts can be explained by their high content of bioactive components, which increase their efficiency in preventing damage to oxidative stress of the cell membrane of biological cells. These results are in line with

previous studies by Sreelatha & Padma 2011; Coppin *et al.,* 2013; Toppo *et al.,* 2015; Saini *et al.,* 2016; Aml Salem Saleh, 2019; Elblehi *et al.,* 2019.

Looking at the overall results of the current study, we can come up with a specific vision about how moringa extracts work in protecting liver and kidney cells in Cd-treated rats, as it can be said that these compounds rich in natural antioxidants protect liver and kidney cells from cadmium by resisting oxidative stress, which is a key deterioration in liver and kidney cells, which if possible to combat and resist free radicals, results in successively protecting cells from the impact of those free cracks that destroy living cells.

Conclusion

Simultaneous administration of watery or acetonic extract of Moringa at two different doses significantly reduced the abnormal changes induced by cadmium as evidenced by decreasing levels of hepatorenal and lipid peroxidation indices and increasing in the antioxidant parameter. This went on because *M. oleifera* has a high antioxidant content, the ability to scavenge free radicals, and the chelating property of minerals. All these properties of moringa make it relieve hepatotoxicity caused by cadmium.

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