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Chemical impact of *Moringa oleifera* seeds in preventing cadmium toxicity in internal organ tissues

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Abstract
With toxic effects that ranges some extent to the ecosystem and human health, cadmium is a heavy metal. This work assessed the effects of *Moringa oleifera* seed powder on renal and hepatic toxicity due to cadmium acetate in the Kidney and liver of Sprague-Dawley rats. In the experimental design, *Moringa oleifera* seeds powder in different doses was given to test its protective effect against histopathological changes brought by cadmium such as liquefactive necrosis and blood vessel congestion in the renal tissues. The findings showed equally that oral use of *Moringa oleifera* seeds powder remarkably alleviated the toxic impact of cadmium as the structure of both renal and hepatic tissues was restored. This study is novel because it aims to establish whether *Moringa oleifera* seeds could afford liver and kidney tissues some protection against cadmium-induced damage.

Keywords: Cadmium toxicity, moringa seed extract, renal injury, hepatic injury, heavy metal contamination, natural antioxidants, chemical herbal remedies, organ protection

Introduction
Moringa oleifera commonly known as the miracle tree has received much attention in the past few years due to its nutrients and healable properties. This plant contains flavonoids, phenolic acids, and vitamins, and has an antioxidant, anti-inflammatory, and hepatoprotective pharmacological profile (Albrakati, 2017; Naw *et al*., 2023; S, 2024) [15, 16]. Through the years, man has relied on the *Moringa oleifera* to serve as his source of medications which include the seed, the leaves, and the pods, especially in areas where the incidence of malnutrition and chronic ailments are rife. The pharmacological application of *Moringa oleifera* is most needed as antidote to heavy metal and particularly cadmium (Cd) corpused in human body due its nephrotoxicous and hepatotoxicous effects (Ali, 2014; Ali *et al*., 2017) [17, 18].

Cadmium is another heavy metal existing mainly in the environment due to various industries, the use of fertilizers in agriculture and condemned dumping of industrial wastes. Cadmium exposure can be associated with organ disorders which include kidney damage, liver injury and enhanced oxidative pressure (Hassan *et al*., 2020; Ventiyaningsih *et al*., 2020) [19, 20]. The nephrotoxicity of cadmium manifested histologically as tubular and glomerular dysfunction, and biochemically as changes in the renal function index including creatinine and urea levels (Kanwel *et al*., 2020) [21]. Likewise, cadmium affects the hepatocytes and causes hepatotoxicity by increasing the liver enzymes which define the impaired liver cells function (Saleh, 2023) [22]. Identifying protective measures against cadmium intake becomes more important as issues of cadmium exposure have received so much concern due to its adverse effects on human health.

Some current research have shown that *Moringa oleifera* extracts have ability to reduce the toxic effect of cadmium. The phytochemical compounds of *Moringa Oleifera*, especially the antioxidant compounds, have been found to reduce oxidative stress and free radicals that threaten the cell structure and membrane (Naw *et al*., <2023>, B.O <2022>) [16]. For example, the aqueous extracts *Moringa oleifera* have proved to possess noticeable anti-nephrotoxic effects against several nephrotoxic agents such as gentamicin and acetaminophen by decreasing the renal injuries as well as the biochemical rating of kidney functions (Tayib & Badwi, 2016) [23]. Further, hepato-protective effects of MO has been supported by studies showing decreased levels of liver enzymes and reestablishment of hepatic tissue structure after treatment with hepatotoxic substances. They include; (Saleh, 2023; Tayib & Badwi, 2016) [22, 23].

These evidences suggest that *Moringa oleifera* has multiple ways to offer protective effect against cadmium induced toxicity. It believed that by virtue of the antioxidant content of *Moringa Oleifera*, it was able to effectively counteract with reactive oxygen species (ROS) produced during cadmium exposure (Naw *et al*. 2023) [16]. In addition, Due to its anti-inflammatory properties the leaf extract of *Moringa oleifera* may also be protective by acting on inflammation signaling post WT and decreasing the level of tissue inflammation and damage (B.O, 2022). Apart from containing anti-inflammatory properties, *Moringa oleifera* has been shown to possess other important chemical compounds which have bioactivity such as kaempferol and querceln since it treat inflammation and oxidation induced by heavy metal toxicity (S, 2024; Wang *et al*., 2016) [24].

Beside its role in protection, *Moringa oleifera* is known to possess nutritive benefits since it contains vitamins; minerals and proteins that can help the body in its healing process after being toxic. With the addition of *Moringa oleifera* into diet, its ability may prove useful in preventing the detrimental effects of cadmium in populations at risk of heavy metal toxicity resultant from environmental or work activities.

As part of opinion on the prospect of its use in food chemistry and safety, this research aims to determine the chemical protective effect of *Moringa oleifera* seed extract against cadmium induced hepatotoxicity and nephrotoxicity. This study will address the possible role of *Moringa oleifera* in the amelioration of cadmium induced toxicity and its possible mode of action. In doing so it aiming at the development of natural pharmacological resources that can be employed in the treatment or even in the prevention of health complications resulting from heavy metals.

Materials and Methods

The employment of animals and normal procedure of experimentation. As pointed out earlier, many of the internal organs are vital and the impact of environmental pollution especially by lead is alarming based on the present study, therefore, it becomes important to find out if the seeds of *Moringa oleifera* plant mentioned in the introduction have the ability to shield liver and kidney tissues from the ill effects of cadmium. This study was conducted at the labs of University Of Tehran using 28 Male Sprague Dawley rats weighing nearly 90-100 g used in this study were obtained from Razi Vaccine and Serum Research Institute. Each animal was put in individual polypropylene cage (380 mm × 200 mm × 590 mm) and observed while it was being weighed. Locally bred albino rats were housed in a standard environmental condition a temperature of 22±2 °C and light/dark cycle of 12/12 for a period of eight weeks [16, 17]. The rats were grouped according to their feeding regime into four groups. The first group, T_1, had ad libitum access to standard diet and distilled water, while the second group, T_2, had standard diet (Deeper, Alborz Province, Karaj, Hesarak, Iran), distilled water, 0.46% cadmium (Merck, Germany 539924) were provided, and access to tap water. T_3, T_4 and T_5 groups received distilled water containing 0.46 cadmium on average. Its unique diet consists of its usual fare plus powdered *Moringa oleifera* seeds at varying concentrations: 4% *Moringa oleifera* seeds and (T_4) 2%.

Preparation of *Moringa oleifera* seeds powder

This research employed seeds from *Moringa Oleifera*, which were purchased from local markets in Najaf, Iraq as a functional food. Following distilling them in water, the seeds and leaves of *Moringa oleifera* were arranged thinly on a piece of perforated aluminum foil, measuring 5.50 m×7.50 m. This was placed on the floor of the room and maintained at a controlled temperature of 22 °C for five days until the last check of the drought condition was conducted. Details of this experiment were conducted in the industrial area in Eshtehard in Iran. The leaves were dried before being ground using a local mill. It was then added to the normal proportions of the food ingredients as a powder. As for the recipe involving *Moringa oleifera* seeds (T_3 *Moringa oleifera* seeds 4%): *Moringa oleifera* seeds powder – 120 g–2880 g/ Ordinary food powder. Subsequently to the addition of 4250 ml of distilled water each powder combination was kneaded with fingers shaped form and then put into the drying chamber. For this investigation, the specimen was collected after 4 days of a culture being grown. On average one developed experimental group of animals was provided twenty grams per week of one of the four types of special diets.

Preparation of Histological sections

The rats were anesthetized with diethyl ether obtained from Merck KgaA, 64271 Darmstadten Germany before removal of liver and kidney tissues at Tehran University-Iran, Karaj Unfortunately the specimens submitted were in form of slides. Method [18] proposed the preparation and visualization of tissue slices in order to detect leads in kidney and liver tissues. These sections were photographed with a digital camera mounted on a light microscope with 10 magnification and stained with Hematoxylin and Eosin (H&E). Supervised by 10 microscopes, H&E staining is then applied to the segment.

Results

Liver tissue Results

A series of experiments have been carried out to examine the impacts of cadmium on liver tissue and the findings have been helpful in comprehending changes that the heavy metal brings to the healthy tissue structure. The result of this comparative study shows that in the current study, T_2 which is treatment with cadmium has caused significant pathological changes in liver tissue with identifying of multiple necrotizing areas positive for liquefactive necrosis and positive intralobular structure less, pinkish, diffused, homogeneous materials which define amyloidosis. This is in concordance with earlier works that described cadmium ability to prompt necrotic alterations in hepatic tissues by portraying it as a strong hepatotoxic agent (Okwuonu *et al*., 2020; Ağır & Eraslan, 2019) [8, 1].

Additional evaluation showed that, there was marked congestive phenomenon in the blood vessels, indicated by the enlargement of hepatic vein diameters and increased thickness of the vein walls. Mild liver fibrosis characterized by fibrous connective tissue in the liver parenchyma echoes previous investigation and confirmation of equivalent vascular and fibrotic alterations consequent to cadmium exposure (Zhai *et al*., 2013; Mafulul & Okoye, 2012) [14, 5]. On the other hand, the control group (T_1) had normal liver parenchyma texture without a high density of occupied lesions indicating that histological changes of the liver due to cadmium exposure are quite distinct (Okwuonu *et al*., 2020; Hyder *et al*., 2013) [8, 3].

The ability of Cadmium to attenuate cadmium induced liver damage was also studied on the therapeutic level. The

results of treatment groups T_3 and T_4 were significantly more indicative of liver tissue recovery with improvement to near normal hepatic architecture without significant occupied lesions seen in T_3. The most marked regeneration took place in T_4, at which the liver tissue was sharply outlined with no gross pathological changes in the liver. This result supports data obtained from previous studies indicating that different natural substances, such as herbal extracts, may produce a preventive impact on cadmium-caused hepatotoxicity (Toppo *et al*., 2015; Mohammad *et al*., 2013) [12, 6]. These treatments show that liver structure can be preserved, thus the need to look for supportive measures that help deal with the effects of heavy metals.

The current study is unique in the way it investigates the histopathological impact of cadmium on liver tissues with regard to the use of natural remedies for protection. Past investigations have confirmed that cadmium contributes to toxic liver effects and numerous studies have documented that cadmium impairs antioxidant protection thus inducing oxidative stress and tissue injury. The results of this research bring into the existing literature concerning the necessity to study the protective agents against cadmium toxicity more thoroughly, especially with reference to the growing Ec exposure to heavy metals.

Therefore, the findings of the current study support other related studies on cadmium hepatotoxicity that include necrosis, fibrosis and vascular congestion. The protective effects observed with Cadmium treatments gives a lead for future interventions to prevent cadmium induced liver injury. The relevance of these results is not limited to the laboratory, indicating possible approaches to reducing the threat of environmental cadmium influence on health.

Renal tissue Results

Research on nephrotoxicity of cadmium (Cd) in renal tissue has produced promising results especially concerning untreated and treated rats. Cad revived without therapeutic intervention applied to groups of animals (T_2) described significantly more serious histopathological changes when compared with the control (T_1). Especially, there is congestion in the renal tissue collect together in the renal vein and artery. Moreover, minor changes were observed, including thickening of the Bowman's capsule in some areas, entailed with prominent glomerular tuft atrophy, and a general increase in glomerular space. In continuation, distal convoluted renal tubules depicted severe hydropic degenerative changes. These findings corroborate with previous works where cadmium exposure was proven to cause severe renal dysfunction, glomerular congestion and tubular degeneration Mohammed *et al*. Mohammed *et al*. (2014) [7] Satarug *et al*. 2022. [9]

Additional microscopical analysis of the renal tissue in the T_2 group only clearly demonstrated hemorrhagic events in which the glomerular tuft atrophy and increased glomerular size was also evident. Indeed, the observation of hemorrhage in the renal tissue sections Being consistent with previous studies that have reported histological alteration in renal tissues exposed to cadmium it has been ascertained that the metal has toxic effects on kidney morphology and function (Satarug *et al*., 2022; Yimthiang *et al*., 2023) [9, 13]. The observed changes strengthen the importance of therapeutic interventions in reducing nephrotoxicity in cadmium exposure.

Among them, the mortality caused by infection or other factors was observed in T1 group, while the immediate and delayed renal injury related to refeeding was acquired in T2 group; in contrast, the therapeutic feeding initiation in T_3 group led to the decrease of renal damage incidence. A normal texture of renal tissue was observed: properly sized and shaped glomeruli and the existence of the glomerular capsule confirmed its function. Furthermore, the size and structure of proximal convoluted tubules were also normal with only mild pathological changes. This improvement aligns with previous observation by (Smereczański *et al*., 2023) [11] that natural extracts provide protection against cadmium-induced nephrotoxicity, which suggest that dietary modification can enhance nephrotoxic effects.

The best effect in the T_4 group was observed, where the histological picture of the renal tissues can be regarded as normal because all the structural and textural features of the glomeruli and the size of the renal tubules were normal. This treatment group had least damage pointing to higher concentration of the therapeutic agents may provide better protective impact against cadmium toxicity. These findings of the present study are aligned by literature which has evidenced that certain nutrients are capable of ameliorating the oxidative stress and inflammation Mediated by cadmium, the renal function and morphology (Smereczański *et al*., 2023; Kamt *et al*., 2023) [11, 4].

The findings of this study further the understanding of cadmium nephrotoxicity and the possibility of a diet based solution. Various cadmium-induced renal effects proposed by earlier investigations mainly include cadmium deposit in the kidney in tubules associated with tubular cell damage and nephron size reduction and lowered GFR (Satarug *et al*., 2022; Yimthiang *et al*., 2023) [9, 13]. The outcomes of the current study not only support these previous observations but also can extend them by indicating the possible involvement of therapeutic feeding in kidney sparing and recovery from cadmium-mediated injury.

Finally the outcomes of the current investigation support that cadmium exposure cause nephrotoxicity and provides a glimpse into the possibility of dietary modulation in this pathophysiology. The large gaps between the untreated and treated groups emphasize the need for understanding the options that might help to preserve renal function considering the effect of cadmium. More research is needed to understand how these protective effects occur and which dietary constituents could minimize the risk for nephrotoxicity.

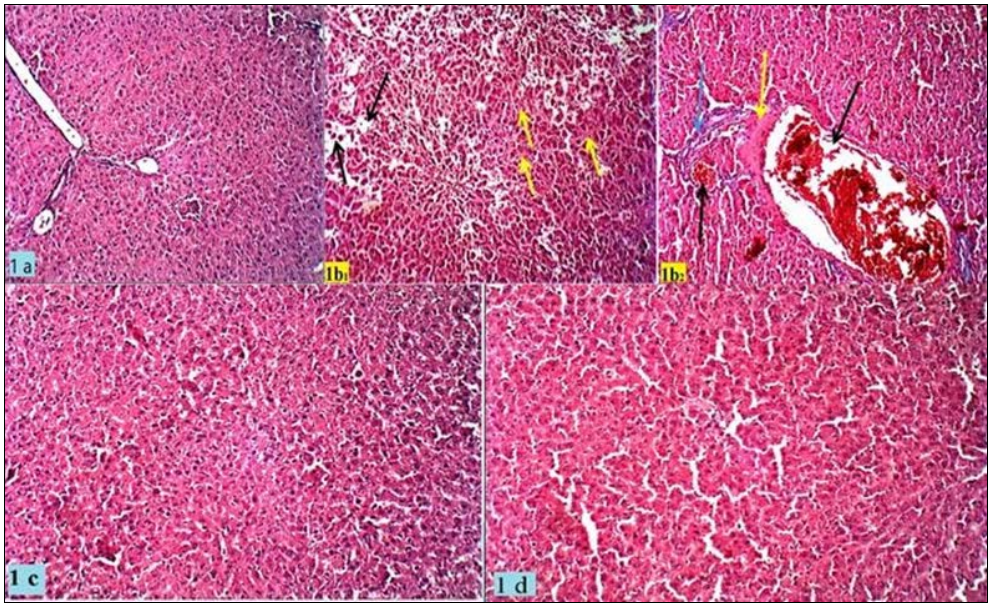

Fig 1: a. Histological section for rats in the control group shows the normal texture of liver parenchyma without any significant occupied lesion (SOL). 1b1 the histopathological section of the liver (Hepatic tissue) in rats of the T_2 group shows multiple necrotizing areas (Liqu effective necrosis) (Black arrow) and infiltration of structure less, pinkish, diffused, homogenous martial (Amyloidosis, amyloid degeneration) (Yellow arrow). 1b2 the histopathological section in the liver of rats in the T_2 group shows severe blood vessel congestion with dilatation of hepatic vein diameter (Black arrow) and increase of vein wall thickness (Yellow arrow). The fibrous connective tissue can be seen in the liver parenchyma (Mild liver fibrosis) (Blue arrow). 1c the histopathological section in the liver of rats in the T_3 group shows normal hepatic tissue without any significant occupied lesion (SOL). 1d Histopathological section for rats in the T_4 group shows the normal texture of liver parenchyma without any significant occupied lesion (SOL). The sections are stained by hematoxylin and eosin (H&E) stain. The sections are captured using a light microscope with the digital camera via ten magnifiers

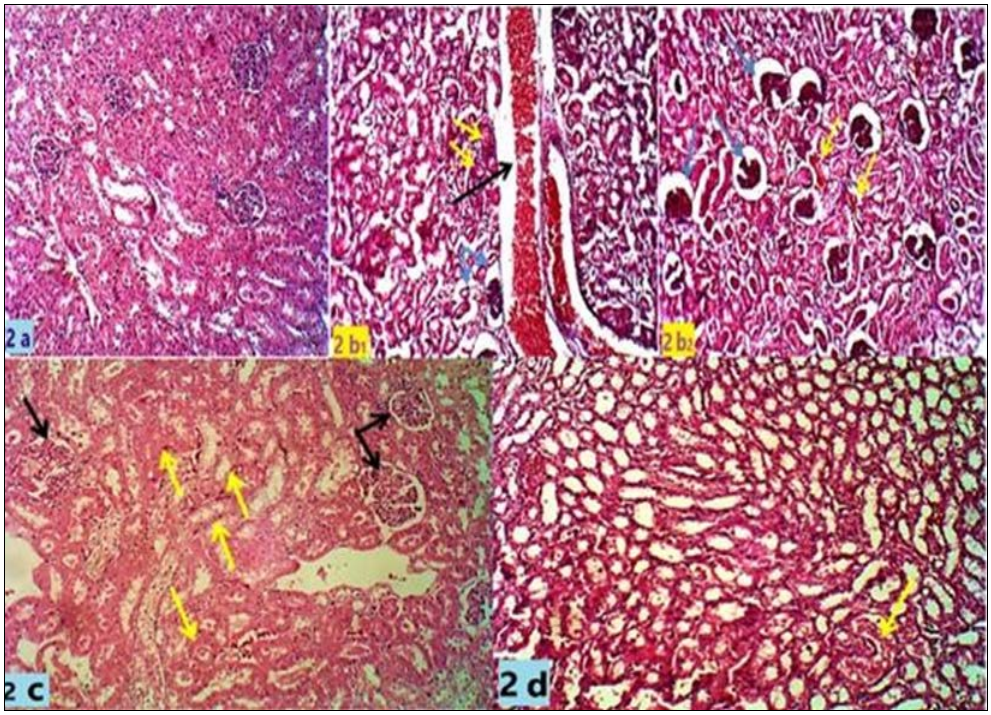

Fig 2: a. Histological section for rats in the control group shows the normal texture of renal tissue without any significant occupied lesion (SOL). 2b1 the T_2 group shows severe blood vessel congestion (Renal vein and artery, Black arrow) with glomerular tuft atrophy and increasing glomerular space (Blue arrow), and the distal convoluted renal tubules show severe hydropic degeneration lesion (Yellow arrow). 2b2 the T_2 group shows the glomerular tuft atrophy (Glomeruli atrophic lesion, blue arrow) with increasing of glomerular space. There is a clear hemorrhage in the renal tissue section (Yellow arrow). 2c the T_3 group shows the normal texture of renal tissue, the glomeruli are normal in size, and the glomerular tuft shows normal size and texture with a normal glomerular capsule (Black arrow). The proximal convoluted tubules are normal in size, diameter, and texture with mild degenerative changes (Yellow arrow). 2d the T_4 group shows the normal glomeruli (Yellow arrow) structure and texture with normal tuft size and capsule. The renal tubules are also normal in their size and texture. The sections are stained by hematoxylin and eosin (H&E) stain. The sections are captured using a light microscope with a digital camera via ten magnifiers

Conclusion

This study elucidates the severe hepatotoxic and nephrotoxic effects of cadmium exposure, highlighting significant histo-pathological changes in liver and renal tissues. The findings confirm the association of cadmium with necrosis, fibrosis, and vascular congestion in the liver, while renal tissue exhibited congestion, glomerular atrophy, and tubular degeneration. Importantly, therapeutic interventions demonstrated potential for recovery, with natural remedies significantly mitigating tissue damage. These results underscore the necessity of exploring protective dietary strategies against cadmium toxicity, contributing valuable insights for future research aimed at preserving organ function amidst increasing environmental exposure to heavy metals.

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