

Role of *Moringa oleifera* Leaves Extract Against Cadmium in Mice

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Abstract— The present work was designed to study the possible protective effect of *M. oleifera* Lam. leaves extract against the toxicity of cadmium acetates on serum biochemical parameters and histology of liver and kidney in rats. On studying the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were increased after treatment with *M. oleifera*, cadmium when compared with control group. The levels of the enzymes AST, ALT and ALP activities were increased after administration of cadmium and then decreased approximately to the control level when administering leaves extract of *M. oleifera* to rats administered cadmium. The results of the histopathological examination, after administration of cadmium in the present study revealed some changes in the hepatocytes like cytoplasmic vacuolation with pyknotic nuclei. In some cells, there were aggregation of chromatin materials inside nuclear envelope and some cells showed degeneration and necrosis. Also, the central vein appeared engorged with blood, hepatocytes appear to be swollen, dilatation and congestion of central vein, Kupffer cell hyperplasia, distorted ‘lobular’ architecture of liver parenchyma also seen. Histopathological studies in the liver of rats treated with cadmium clearly indicated an extensive liver parenchymal damage like swollen hepatocytes, congested and dilated central vein and Kupffer cell hyperplasia. Examination of kidney in rats treated with cadmium, showed aggregation of hemolytic blood cells in between tubules, and beginning of cellular damage that include pyknosis of some nuclei. Also, there was congestion in some blood vessels, which appeared engorged with blood cells. In some sections found cytoplasmic vacuolation and appearance of tubular cast, and some tubules showed necrotic cells with pyknotic nuclei. In some tubules, there were swollen cells, which appeared with cytoplasmic vacuolation. Histological examination of kidneys of rats received lead showed severe changes in proximal and distal convoluted tubules and collecting tubules, thick limb of Henle’s loop which represented by appearance of vacuolation of the cells, and there were necrotic cells with pyknotic nuclei. Blood vessel in between the tubules and the loops of Henle appeared engorged with blood and appearance of many necrotic cells. There was a significant improvement in the histopathology of the liver and kidney in rats treated with *M. oleifera* + cadmium. Hepatocytes appear to be little swollen, mild distortion of ‘Lobular’ architecture of liver parenchyma, mild widening of sinusoidal spaces observed in *M. oleifera* + cadmium treated group. Hence, it could be concluded that cadmium is toxic heavy metals, which affects the serum biochemical profile and causes degenerative histopathological changes in rat liver and kidney.

Simultaneous treatment with leaves extracts of *M. oleifera* partially improved the hepatocellular damages induced by cadmium.

Index Terms—About four key words or phrases in alphabetical order, separated by commas.

I. INTRODUCTION

The term “heavy metal” applies to a group of metals with similar chemical properties. A heavy metal is a metallic element, which is toxic and has a high density, specific gravity or atomic weight. There are many heavy metals in our environment both naturally and from pollution. People may be exposed to small amounts of heavy metals through food, water, air, and commercial products. People can also be exposed in their workplace, as several industries use or produce these metals. Acute heavy metal poisoning usually occurs when people are exposed to large amounts of a metal at one time. There is growing evidence that “chronic” or long-term exposure to lower levels of heavy metals also causes health problems. The symptoms of chronic heavy metal poisoning can be severe, but are often less obvious and develop much more slowly over time than the symptoms caused by acute exposure (Hu, 2002).

Chronic heavy metal poisoning can be challenging for both health care providers and patients because there are often many more questions than answers. Many of the symptoms of chronic heavy metal toxicity can include Headache; Weakness; Muscle and joint pains; Constipation; Feeling tired (Hu, 2002).

Vinodini et al. (2014) reported that cadmium (Cd) is one of the naturally occurring metallic toxicants, affecting various organs. Liver is one of the organs affected by Cd toxicity (acute and chronic exposure). Disruption of the cellular antioxidant system, generation of reactive oxygen species and oxidative stress, etc., are one of the few mechanisms by which cadmium affects the liver.

Hu (2002) reported that an individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache. Chronic exposure to metals at a high enough level to cause chronic toxicity effects.

Moringa oleifera Lam. is a multipurpose and exceptionally nutritious vegetable tree with a variety of potential uses. It is a sub-tropical species that is known by different regional names as benzolive, drumstick tree, kelor, marango, mulangay, nébéday, saijhan, mooringai and sajna. In addition, *M. oleifera* tree also known as drumstick tree is a rapid growing deciduous shrub or small tree of about 13 m tall and 35 cm in diameter with an umbrella-shaped open cap (Anjorin et al., 2010). It has very high nutritional properties that would be

useful as a food supplement, especially in those relegated communities. Besides its nutritional and medicinal applications, *M. oleifera* is very useful as an alley crop in the agro-forestry industry. It is useful not only for human beings but also for animals and also in various industrial applications. Besides *M.oleifera* being processed into a medicine, it contains acetone, which can be prepared into herbal formulation, which is an effective anti-malaria bio agent (Patel et al., 2010).

It has also been reported that, *M. oleifera* oil and micronutrients contain antitumor, antiepileptic, antidiuretic, anti-inflammatory and venomous bite characters (Hsu et al., 2006). *M. oleifera* contains specific plant pigments with demonstrated powerful antioxidative ability such as vitamins C, E, A, caffeoylquinic acids, carotenoids, lutein, α -carotene and β -carotene, kaempferol, quercetin and rutin (Ho, 1994; Siddhuraju and Becker, 2003; Aslam et al., 2005).

Plants as medicinal agents were mentioned in historic documents dating back many thousands of years (Rasonavivo et al., 1992). Currently, medicinal herbs as a whole were reported to be used against a wide range of health problems such as cough, cold, stomach, cataract, constipation and many other ailments (Jimenez-Arellanes et al., 2003).

The plant *M. oleifera* as one of these herbs was reported to prevent effectively, morphological changes and oxidative damage in lens of rats by enhancing the activities of anti-oxidant enzymes, reducing the intensity of lipid peroxidation and inhibiting generation of free radicals (Sreelatha and Padma, 2009).

The healing properties of *M. oleifera* oil have been documented by ancient cultures. *M.oleifera* oil has tremendous cosmetic value and is used in body and hair care as a moisturizer and skin conditioner. *M. oleifera* oil has been used in skin preparations and ointments since Egyptian times (Ramachandran et al., 1980; Monica, 2005).

Aim of the work

1-The current study was designed to test the secure use of *Moringa oleifera* leaves extract, a known medicinal plant in Saudi Arabia, to study its protective role in reducing the toxic effects caused by these heavy metals on the liver and kidney functions and on blood in mice.

2-To examine the toxic effect of cadmium acetates on the liver and kidney functions and blood in mice after different periods of administration.

3- To study the effect of *Moringa oleifera* leaves extract - a medicinal plant known in Saudi Arabia - on liver and kidney functions and blood in mice after particular duration of administration.

II. REVIEW OF LITERATURES

A. Effect of Heavy Metals on Health:

Heavy metals are individual metals and metal compounds that can impact human health. Many authors discussed eight common heavy metals, these are: arsenic, barium, cadmium, chromium, lead, mercury, selenium, and silver. They all are naturally occurring substances that are often present in the environment at low levels. In larger amounts, they can be dangerous. Generally, humans are exposed to these metals by ingestion

(drinking or eating) or inhalation (breathing). Working in or living near an industrial site that utilizes these metals and their compounds increases ones risk of exposure, as does living near a site where these metals have been improperly disposed. Subsistence lifestyles can also impose higher risks of exposure and health impacts because of hunting and gathering activities (Martin and Griswold, 2009).

B. Role of Cadmium (Cd) on Health:

Cadmium exposure encountered in industries dealing with pigment, metal plating, some plastics, and batteries. Cadmium pollution (e.g., the emissions of a cadmium smelter or industry and the introduction of cadmium into sewage sludge, fertilizers, and groundwater) can result in significant human exposure to cadmium through the ingestion of contaminated foodstuffs, especially grains, cereals, and leafy vegetables (Hu, 2002).

The health implications of cadmium exposure are exacerbated by the relative inability of human beings to excrete cadmium (it is excreted but then re-absorbed by the kidney). Acute high-dose exposures can cause severe respiratory irritation. Occupational levels of cadmium exposure are a risk factor for chronic lung disease (through airborne exposure) and testicular degeneration (Benoff *et al.*, 2000) and are still under investigation as a risk factor for prostate cancer (Ye *et al.*, 2000).

Cadmium produces toxic lesions in various tissues (Friberg *et al.*, 1986) the largest amount of this metal is deposited in the liver and kidney tissues (Marafante, 1976). It causes morphological and functional changes in the liver (Kopp *et al.*, 1983; Bomhard *et al.*, 1984).

Kowalczyk *et al.* (2003) studied the effects of anthocyanins from *Aronia melanocarpa* to reduce the harmful results caused in animals receiving cadmium chloride, on selected liver and renal function biochemical tests (ALT and AST activities, bilirubin, urea and creatinine serum concentration) and the content of cadmium. They found that, administering cadmium chloride to rats resulted in a statistically significant decrease of hemoglobin concentration in blood, increased AST and ALT activity, concentration of bilirubin and urea in blood serum. Adding anthocyanins to cadmium chloride did not normalize the hemoglobin concentration, while it significantly decreased AST and ALT activity, concentration of bilirubin and urea in blood serum. The anthocyanins also statistically significantly decreased the accumulation of cadmium in liver and kidneys. (Rikans and Yamano, 2000).

Mantur *et al.*, (2014) concluded that cadmium is one among the most environmental pollutants that affects many organs like kidney, liver and testis. Cadmium chloride induced alterations in lipid profile and liver histology.

C. Role of *Moringa oleifera* (*M. oleifera*) on Heavy metals:

Moringa oleifera, a miracle tree, grown widely in various parts of the world, is known to possess various medicinal properties like anti-pyretic, anti-inflammatory, anti- cancer, anti-ulcer, etc. The antioxidant and/or free radical scavenging

property of *M. oleifera* may be due to the presence of phenolic compounds (Paliwal *et al.*, 2011).

The effect of ethanolic extract of *M. oleifera* root extract on liver and kidney functions and hematological parameters in mice were studied by Mazumder *et al.* (1999). They reported no alteration in hematological and biochemical parameters at low and moderate dose level of daily and low dose level of weekly treatment of *M. oleifera* extract. They added that, the extract at moderate dose level in weekly treatment significantly changed serum AST, ALT and plasma cholesterol levels.

Sharifudinet *al.* (2013) investigated the therapeutic effect of *M. oleifera* hydroethanol extract against acute liver injury using a hepatotoxin, acetaminophen-induced characteristic features similar to those of acute hepatitis in humans. As it is well known, the liver appears to be, due to its unique metabolic function, the most common target organ of any toxicity.

III. MATERIALS AND METHODS

Materials:

a. Experimental Animals:

Adult male albino mice of the Wistar strain (*Rattus norvegicus*), weighing 72.40 ± 3.4 g were used in the present study. The experimental animals were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the laboratory conditions for 10 days prior to the initiation of experimental treatments. The experimental animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature ($20 \pm 1^\circ\text{C}$) and 12:12 h light: dark cycle. Rats were fed *ad libitum* on normal commercial chow and had free access to water. The experimental treatments were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

b. Moringa oleifera Leaves Extraction:

Fine qualities of *Moringa oleifera* leaves were purchased from a local commercial market, Jeddah, Saudi Arabia. The leaves were scientifically defined by the herbarium

of Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The leaves were thoroughly washed and dried at room temperature. The dried leaves were powdered and kept in a dry plastic container used

for extract preparation. The methods of Al-Attar and Abu Zeid (2013) was used to prepare the extracts with some modifications. Fifty grams of the dried *M. oleifera* leaves powder were added to 1.5 liter of hot water in a flask. After 6 hours, the mixture was slowly boiled for 45 minutes. After boiling period, the mixture was cooled at room temperature and it was gently subjected to an electric mixer for 10 minutes. Thereafter the solution of *M. oleifera* was filtered using 250 mm filter papers (Whatman, England), and the filtrate was evaporated in an oven at 40°C to produce dried residues (active principles). With reference to

the powdered sample, the yield of the *M. oleifera* extract was 16.4%. The leaves extract was stored in a refrigerator for subsequent experiments.

c. Heavy metals:

Samples of cadmium chloride (CdCl_2), and other chemicals were purchased from one of the chemical companies in Jeddah market.

Methods:

Experimental groups:

The animals were divided into four main groups:

1- Control group :

Rats received distilled water through gastric intubations daily for six weeks.

2- Moringa oleifera Leave extract treated group :

Rats were orally administered 300 mg/kg b.w. of *Moringa oleifera* leaves extract dose by using the stomach tube day after day for six weeks.

3- Cadmium treated group :

Rats were orally administered 1/10 LD50 of cadmium chloride (6 mg/kg b.w.) by using the stomach tube day after day for six weeks.

4- Moringa oleifera Leave extract treated group + cadmium:

Rats orally administered 300 mg/kg b.w. of *M.oleifera* leaves extract then given 6 mg/kg b.w. cadmium chloride by using the stomach tube day after day for six weeks.

At the end of the experiment, six weeks of treatment, samples of blood were taken for biochemical analysis. Then rats of all groups were sacrificed and dissected and samples of liver and kidney were collected for histological studies.

Determination of LD50:

In this study, the toxicity of cadmium and lead in mice (MF1 strain) was calculated to determine lethal and sub lethal doses LD50. For cadmium chloride, the LD50 was 60 mg/kg b.w.

Blood Serum Analyses

At the end of the experiment, all animals were fasted for 12 hours, water was not restricted, and then anaesthetized with diethyl ether. Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2500 rpm for 15 minutes. The blood sera were then collected and stored at 4°C prior immediate determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).

Biochemical Results:

Results of the activities of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)

Table (1): Mean values ± SE of the activities of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in control group, *Moringa oleifera* leaves extract treated group, Cadmium treated group, and *Moringa oleifera* leaves extract + cadmium treated group.

Parameters	AST (U/L)	ALT (U/L)	ALP (U/L)
Control Group	103.33±6.13	24.00±3.47	70.00±2.12
<i>M. oleifera</i> Group	187.40±33.30a	36.25±1.84	75.20±12.02
Cadmium Group	127.57±11.75	32.00±5.79	114.71±17.48a
<i>M. oleifera</i> + Cadmium Group	110.63±9.99b	21.67±2.348b	65.88±8.12b

Values are given as means±SE for 6 rats in each group.
 a: Significantly increased when compared with control group
 b: Significantly decreased when compared with *M. oleifera* group.

HISTOLOGICAL RESULTS:

- d. Histological results of Liver:
- e. Light microscopy observations in control group :

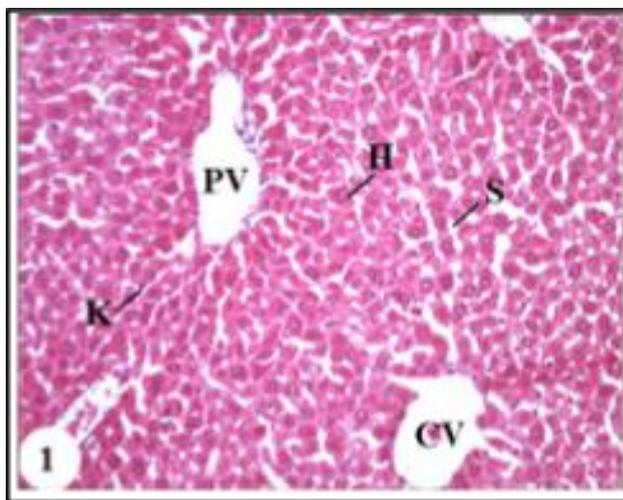


Fig. (1): Histological section of liver in control group showing the hepatic lobules with its central vein (CV), notice the arrangement of hepatocytes as cords (H) separated by liver sinusoids (S), also appears Kupffer cells (K). Notice also a branch of the portal vein (PV) in the portal area.
 (H & E stain X = 200)

Light microscopy observations in *Moringa oleifera* group :

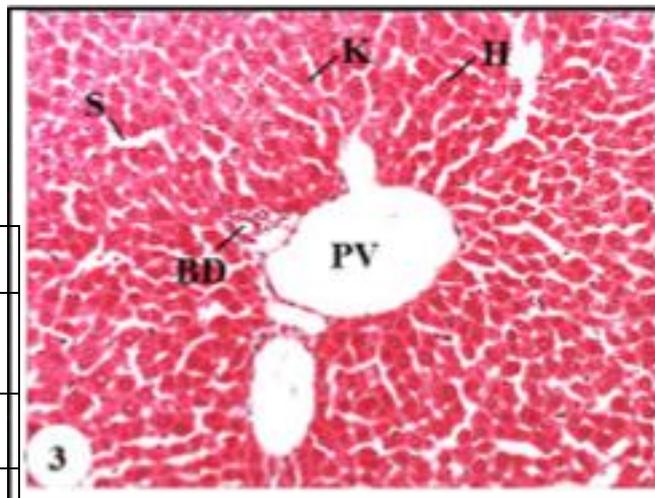


Fig. (2): Histological section of liver in *M. oleifera* group showing normal arrangement of hepatocytes in cords (H) with rounded nuclei found in the middle of the cytoplasm (N). In addition, blood sinusoids appeared between hepatic cords (S), branch of the portal vein (PV). Also, branch of the bile duct (BD) and Kupffer cells (K) are seen.
 (H & E stain X = 200)

Light microscopy observations in cadmium group :

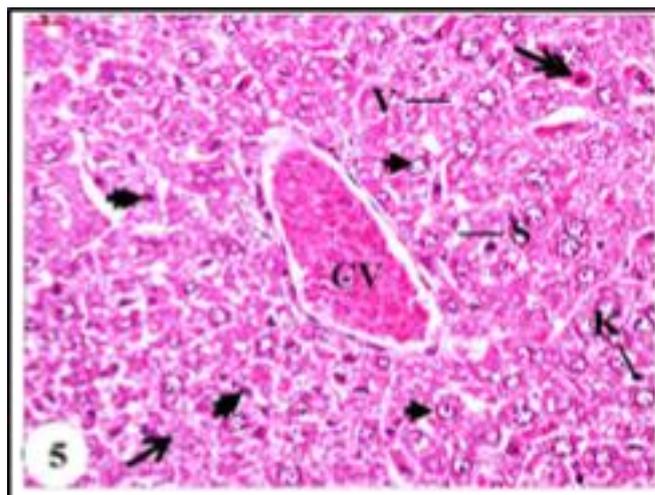


Fig. (3): Histological section of liver in cadmium group showing central vein filled with blood (CV), some hepatocytes with cytoplasmic vacuolation (V), pyknotic nuclei (double arrows). Also, blood sinusoids appeared between hepatic cords (S) and also seen Kupffer cells (K).
 (H & E stain X = 400)

Light microscopy observations of liver in cadmium + *Moringa oleifera* group :

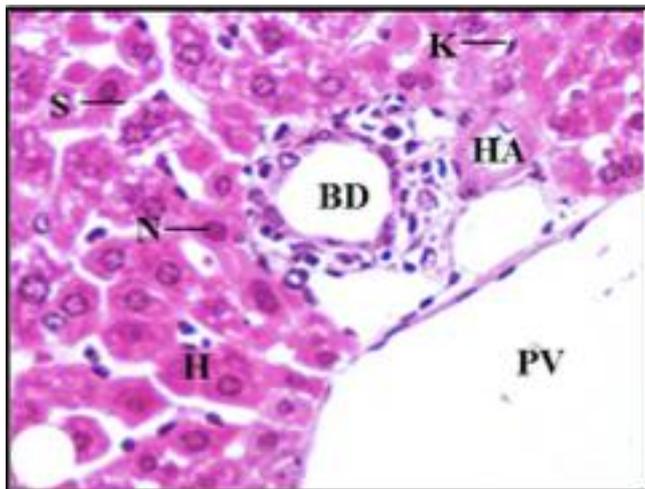


Fig. (4): Histological section of liver in cadmium + *Moringa oleifera* group showing hepatic cords and hepatic cells (H), with rounded centrally located nuclei (N), the portal vein (PV), branch of bile duct (BD) and branch of hepatic artery (HA). Also Kupffer cells (K) and blood sinusoids (S) were seen.

(H & E stain X = 400)

Histological observations of Kidney: Control group :

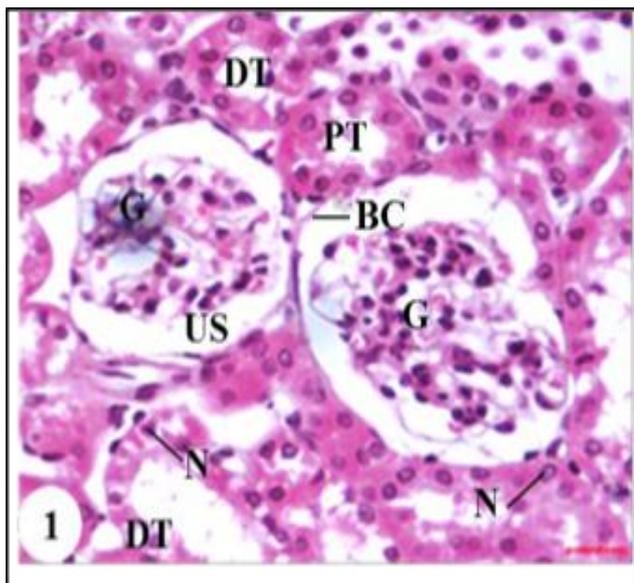


Fig. (5): Histological section of kidney of rat of the control group showing the cortex with renal corpuscles (glomerulus) (G), inside the Bowman's capsule (BC) and the urinary space (US), proximal (PT), and distal (DT) convoluted tubules, which appeared normal with normal nuclei (N).

(H & E stain X = 600)

***Moringa oleifera* leaves extract treated group :**

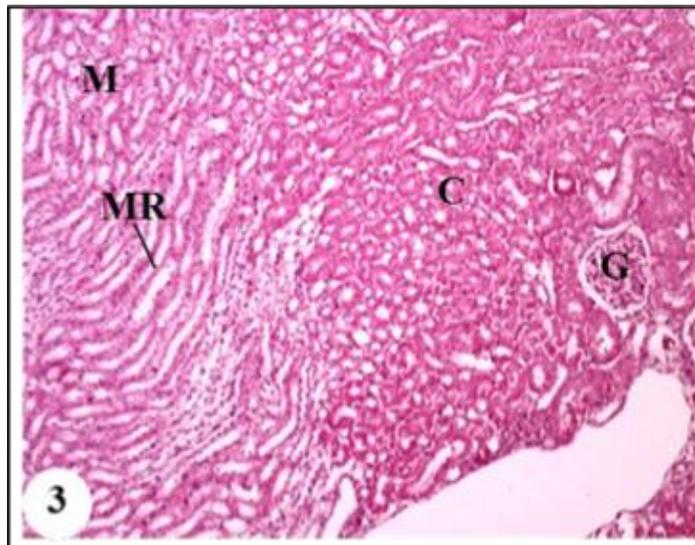


Fig. (6): Histological section of kidney of rat treated with *M. oleifera* leaves extract showing normal structures in cortex (C), medulla (M), glomerulus (G) and medullary rays (MR).

(H & E stain X = 200)

Cadmium treated group :

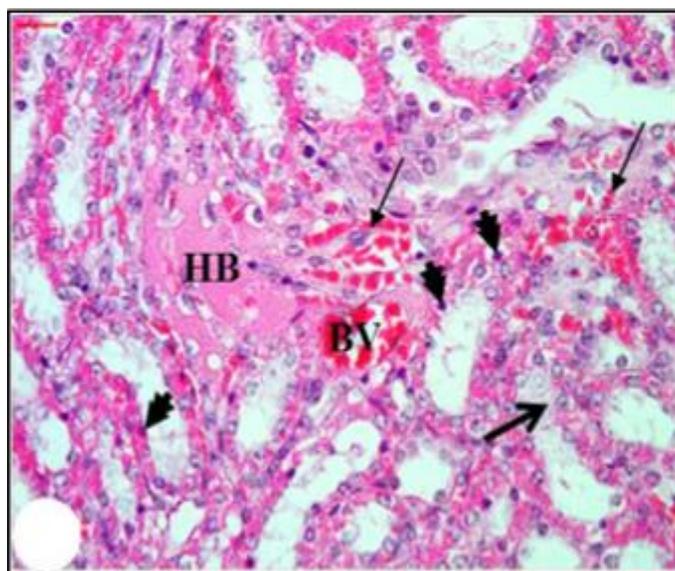


Fig. (7): Histological section of kidney of rat of the cadmium treated group showing normal blood vessel (BV) engorged with blood cells, aggregations of hemolytic blood cells (HB) in between the tubules, some damaged cells (thick arrow) and nuclear pyknosis (double headed arrow), also leaked blood cells from blood vessels (thin arrow).

(H & E stain X = 400)

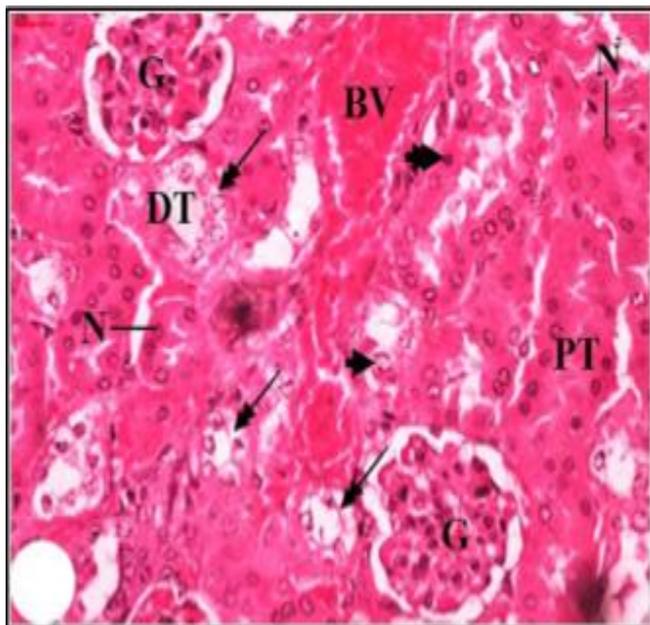
***Moringa oleifera* and Cadmium treated group :**

Fig. (8): Histological section of kidney of rat of *Moringa oleifera* leaves extract and cadmium treated group, showing normal glomeruli (G), proximal convoluted tubules (PT) and some affected distal convoluted tubules (DT). Also, normal intact nuclei (N) and blood vessels filled with blood (BV) are found.

(H & E stain X = 400)

IV. DISCUSSION

An individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache. Chronic exposure to metals at a high enough level to cause chronic toxicity effects (such as hypertension in individuals exposed to lead and renal toxicity in individuals exposed to cadmium) can also occur in individuals who have no symptoms. It is possible that low-level metals exposure contributes much more towards the causation of chronic disease and impaired functioning than previously thought (Hu, 2002).

The results of the present study showed that cadmium causes toxicities and elevation in the biochemical parameters and in the histological sections of both liver and kidney.

In the present study, the levels of the enzymes AST, ALT and ALP activities were increased after administration of cadmium and then decreased approximately to the control level when administering leaves extract of *M. oleifera* to rats administered cadmium. These results could be due to the protective effect of *M. oleifera*.

Vinodini *et al.* (2014) reported that, pre-treatment with *M. oleifera* leaf extract in cadmium exposed rats showed a significant decrease in the levels of AST and ALT as compared with the cadmium alone treated rats. Therefore, they suggested that pretreatment with *M. oleifera* leaf extract alters the levels of the liver enzymes and hence can improve the liver functions in cadmium chloride-induced rats.

Cadmium produces toxic lesions in various tissues (Friberg *et al.*, 1986) the largest amount of this metal is deposited in the liver and kidney tissues (Marafante, 1976). It causes morphological and functional changes in the liver (Kopp *et al.*, 1983; Bomhard *et al.*, 1984). The liver is one of the most susceptible organs after acute or chronic exposure to cadmium. Cadmium-induced damage of the liver is manifested by an

increase in AST and ALT in the blood (Guilhermino *et al.*, 1998; Kowalczyk *et al.*, 2003).

Horiguchi *et al.* (2006) reported that chronic cadmium exposure causes irreversible kidney damage and renal tubular dysfunction.

In the present study, there was a significant improvement in the histopathology of the liver in rats treated with *M. oleifera* leaves extract and cadmium chloride. Hepatocytes appear to be little swollen, mild distortion of 'Lobular' architecture of liver parenchyma, mild widening of sinusoidal spaces. Mild distortion of lobular architecture of liver parenchyma, with widening of sinusoidal spaces was observed in *M. oleifera* alone treated group. Hence it may be concluded that cadmium chloride is a toxic heavy metal, that-affects the serum lipid profile and causes degenerative histopathological changes in rat liver. Simultaneous treatment with *M. oleifera* partially improved cadmium chloride induced hepatocellular damage.

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