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Study the Physiological and Histological Effects of *Moringa oleifera* alcoholic Extract to Reduce the Harmful Effects of Red Lead Oxide in Male Albino Mice

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Abstract

This study included investigating red lead oxide effects on the bodies of laboratory animals (Albino mice) and how to remove or reduce the effect of this pollutant that was dosed orally using the ethanolic extract of the *Moringa oleifera* (MO) plant. In the study, 40 adult male mice were used. At an average age of 8-10 weeks and with 21-29 grams average weight, the animals were randomly distributed among four groups with 10 mice/group. The first group M1 was the control group, the second group M2 was treated with red lead oxide only [1.25 mg/kg], while the third group M3 was treated with red lead oxide + *Moringa oleifera* [C1 = 8.4 mg/kg], and the last group M4 was treated with red lead oxide + *Moringa oleifera* [C2 = 9 mg/kg]. The results showed a significant difference increase in the growth rates of laboratory mice ($p \leq 0.05$) for all studied groups, while the results of animals exposed to red lead oxide and treated with MO extract and those exposed to red lead oxide only showed a significant difference increase ($p \leq 0.01$) compared to the control group. In addition, the results of the FBS analysis showed that there was a highly significant increased ($p \leq 0.01$) among the four groups that were mentioned earlier and when compared to the control group. The histological examination sections showed different changes in the tissues of the liver, pancreas and kidneys of the mice. The results of this study indicated that an extract of MO, is important to reduce the adverse effects of exposure to red lead oxide toxicity. The administration of MO returned some parameters to control, especially histological sections. It showed that red lead oxide had a harmful effect on tissue sections, while MO plant extract reduced the toxicity of red lead oxide for the M3 and M4 groups.

Keywords: Red lead oxide, *Moringa oleifera*, Liver, Pancreas and Kidney.

دراسة التأثيرات الفسيولوجية والنسجية لمستخلص المورينكا أوليفيرا الكحولي لتقليل التأثير الضار لأكسيد الرصاص الأحمر في ذكور الفئران البيضاء

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الخلاصة

تضمنت هذه الدراسة التحري عن تأثير اوكسيد الرصاص الاحمر في أجسام الحيوانات المختبرية (الفئران البيضاء) وكيفية ازالة او تقليل تأثير الملوث الذي تم تجريعه فمويا باستخدام المستخلص الكحولي لنبات المورنكا اوليفيرا، استخدمت في الدراسة ٤٠من ذكور الفئران البيض البالغة وبمعدل عمر٨ – ١٠ أسابيع

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وبمعدل وزن ٢١ -٢٩ غرام ووزعت عشوائيا على اربعة مجاميع بعدد ١٠ فئران/ مجموعة ، قسمت المجاميع اربعة اقسام. المجموعة الاولى مجموعة السيطرة، المجموعة الثانية معالجة بأكسيد الرصاص الاحمر فقط [١،٢٥ مجم / كجم]، اما المجموعة الثالثة معالجة بأكسيد الرصاص الاحمر + المورنكا اوليفيرا [الجرعة الاولى = ٨ ، ٤ مجم / كجم]، المجموعة الرابعة معالجة بأكسيد الرصاص الاحمر + المورنكا اوليفيرا [التركيز الثاني = ٩ مجم / كجم]. اظهرت النتائج فرقاً معنوياً ($p \leq 0.05$) في معدلات النمو للفئران المختبرية لكافة المجاميع المدروسة، بينما نتائج الحيوانات المعرضة لأوكسيد الرصاص الاحمر والمعاملة بمستخلص نبات المورنكا اوليفيرا والاخرى المعرضةالى اكسيد الرصاص الاحمر اظهرت ارتفاعا معنويا عاليا ($p \leq 0.01$) مقارنة بمجموعة السيطرة. اما فيما يخص نتائج تحاليل السكر الصائم، فقد وجد ارتفاع معنويا عاليا ($p \leq 0.01$) بين المجاميع الاربعة التي تم ذكرها سابقا وعند مقارنتها بمجموع السيطرة. أظهرت نتائج المقاطع النسجية ان هنالك تغييرات مختلفة في أنسجة الكبد، البنكرياس والكلى لدى الفئران. تشير نتائج هذه الدراسة إلى أن مستخلص اوراق نبات المورنكا مهم لتقليل الآثار السلبية للتعرض لسمية أكسيد الرصاص الأحمر، حيث ان إعطاء مستخلص نبات المورنكا اعاد بعض المؤشرات الى وضعها الطبيعي، وخاصة الأنسجة، لوحظ أن أكسيد الرصاص الأحمر له تأثير ضار على الأنسجة، بالمقابل اعطاء مستخلص النبات قد قلل من سمية أكسيد الرصاص الأحمر للمجموعتين الثالثة والرابعة.

1. Introduction

Moringa oleifera (MO) is a fast-growing, drought-resistant tree that is native to the Indian subcontinent and to the Moringaceae family, commonly referred to as moringa [1]. It is frequently grown for its immature seed pods which are consumed as vegetables and for its leaves which are used in traditional herbal medicine. Additionally, it purifies water [2]. MO is a rapidly growing deciduous tree that can grow to a height of 10-12 meters (33-39 feet) and a stem 45 centimeters in diameter. The fragrant, hermaphroditic blooms have five uneven, thin-veined, yellowish-white petals surrounding them. The blossoms are around 2 cm in width and 1 to 1.5 cm in length [3]. However, according to laboratory research, supplementing with MO leaf extract is safe at doses under 1,000 mg/kg and may be harmful at doses over 3,000 mg/kg in humans. The plant leaves are the most nutrient-dense section since they are a good source of protein, beta-carotene, vitamin K, manganese, vitamin C and pro-vitamin A as well as B vitamins [4].

The leaves are distinguished by a high nutritional content. In addition to all essential amino acids, MO leaves also contain 27% protein. High levels of vitamins and beneficial plant-active ingredients are also present. Polyphenols and sugar-modified aromatic glucosinolate are among the ingredients [5]. Phytochemicals like alkaloids, anthraquinones, coumarins, flavones, phenols, quinones, saponins, tannins, terpenoids and steroids are found in the leaves that help protecting plants from predators [6].

The ethanol extract of MO leaves has been observed to have a wide safety index and low toxicity, with the median lethal dosage "LD50" ranging from 2800 to 5000 mg/kg body weight [7]. Human health is directly impacted by toxic metal contamination of soil and water sources, with a few trace amounts of heavy metals such as Cu, Fe, Zn, Ni, Mg, Mn, Mo, Cr, and Se. The physiology of humans plays a functional role in the risk assessment of heavy metals for human health in various soils and sediments. Numerous anthropogenic and natural processes emit enormous quantities of heavy metals which enter the air, water and soil and form part of the ecosystem. Higher concentrations of these metals can have harmful impacts on health. The increased exposure of people to heavy metals is caused by the many heavy metals entering our daily lives. [8].

Ecosystems contain large amounts of lead as well. The primary causes of lead exposure include drinking water, cigarettes, combustion oil and industrial waste, notably automotive waste (particularly iron and steel production) exhaust. Humans may be exposed to lead by drinking water and eating lead-rich plant products because lead is discharged into the atmosphere where it eventually finds its way into soil and water sources [9]. Following the ion process, lead can be harmful because it can displace other bivalent cations like Ca^{++}, Mg^{++} and Fe^{++} as well as monovalent cations like Na+ [10].

Red lead oxide with linseed oil is used as a coating to protect the external iron. It is important to follow safety precautions when working with paint containing lead, as when inhaling lead oxide, especially in high doses, leads to chest pain, abdominal pain, and also irritation of the lungs. After oral administration, it dissolves in stomach acid and is absorbed. Upon prolonged exposure to this pollutant, it may accumulate inside the body with symptoms of lead poisoning. Possible absorption through high doses through the skin, and the symptoms of lead poisoning are irritation, disturbances in vision and high blood pressure, while some studies have proved that lead oxide is a carcinogen for laboratory animals [11].

The aim of this study was to investigate the possibility of MO plant leaves extract to reduce and eliminate the harmful effects of red lead oxide.

2. Materials and Methods

2.1 Plant Sample Collection

Plant samples included *Moringa oleifera* and its seeds (Figure (1)A), while leaves of *Moringa oleifera* shown in Figure (1)B, were obtained from trees planted at the Botanical Garden of the Department of Biology, College of Science, University of Baghdad, in April to May months. The leaves of the plant were then collected air-dried and pulverized and stored at 4^{0}C for the purpose of extraction.

Figure 1:A depicts *Moringa oleifera* seeds while **(B)** shows *Moringa oleifera* leaves.

2.2 The Ethanolic *Moringa oleifera* Leaf Extract was Made as Follows

MO was extracted according to previous studies [12, 13]:

1 - M. *oleifera* leaves were carefully cleaned with distilled water and dried for three days at room temperature (25-35 ^{0}C). they should be kept out of direct sunlight to avoid any possible damage to their phyto-continuous.

2 - After that, a high-speed milling machine was used to smash the dried leaves.

3 - The 10 g dry powder was dissolved in 90 ml absolute ethanol (80%), stirred thoroughly for 10 minutes, and was then left for 72 hours.
4 - 90 ml of absolute ethanol (80%), where it is diluted with DDW as solvents.
5 - After 72 hours figure (2)A, it was filtered twice with sterile filter paper with 2 µm pore size.
6 - The mixture filtrate evaporated from ethanol in a water bath at 60^{0}C. Left it for at least three hours until its color became darker (Figure (2)B).

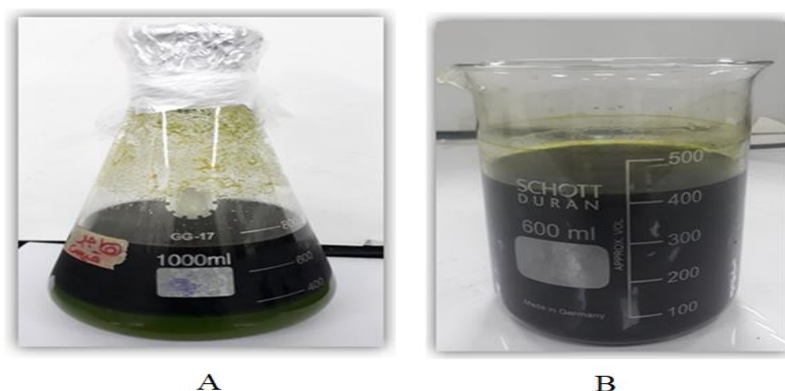

Figure :2 Figure A depicts *Moringa oleifera* and ethanol solution after 72 hours, while B the solution after it had evaporated in the water path.

7 - After that, it was poured into petri dish and left in the oven device at 50 ^{0}C for 24 to 48 hours to dry completely.
8 - Figure 3(A) shows the final result. The extract was placed in a vial coated with parafilm and kept in the refrigerator at a temperature of 4 ^{0}C until use (Figure 3(B)).

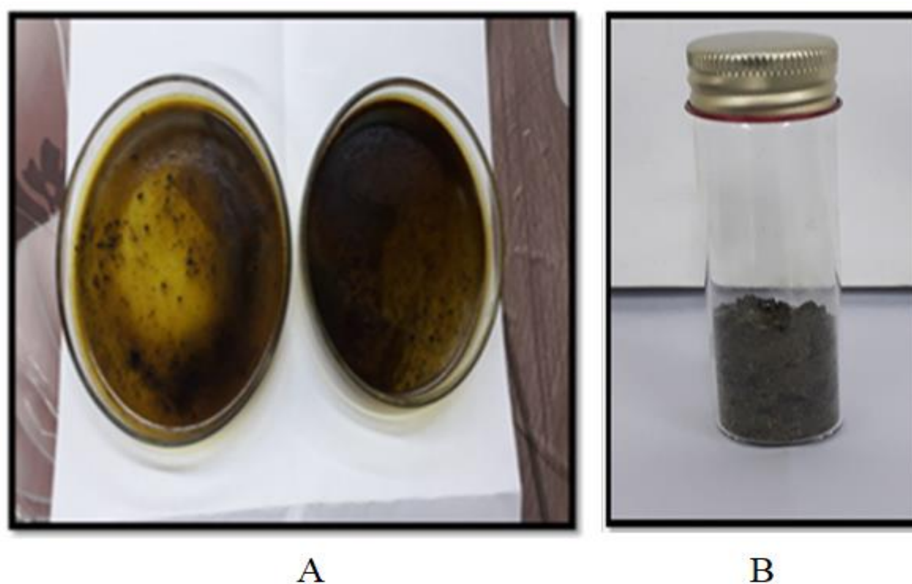

Figure 3: A depicts *Moringa oleifera* leaves extract after drying, while **(B)** shows the final form of the *Moringa oleifera* leaves extract.

2.3 Experimental Animals Design

The experimental animals were divided into four groups. This study was approved by the Department of Biology, College of Science, University of Baghdad Ethical Committee:
1 - **Group M1**: The healthy control group that was not exposed to any substance. It received only water and food *ad-libitum*.

2 - **Group M2**: The group received lead oxide red (Pb_3O_4) orally only at a dose of **1.25 mg/kg**.
3 - **Group M3**: This treated group was orally traeted with lead oxide [**1.25 mg/kg**] + MO [**C1= 4.8 mg/kg**].
4- **Group M4**: This treated group was orally administered with lead oxide [**1.25 mg/kg**] + MO [**C2= 9 mg/kg**].

2.4 Treatment of Experimental Animals

The study used MO to indicate the reduction of the negative effects of lead oxide. After extracting the MO plant leaves, it was tested on mice to check its effectiveness in removing and reducing its negative impacts after administrating mice with lead oxide orally. Forty male albino mice were divided randomly into M1, M2, M3 and M4, with 10 mice per each group.

The first group M1 was the control and not exposed to anything and was given only food and water. M2 group received orally lead oxide at a dose of 1.25 mg/g dissolved in 0.1 corn oil [15]. While group M3 was treated orally with 1.25 mg/g lead oxide. Three hours later, MOClwas administered orally at a dose of 4.8 mg/g dissolved in 1ml of DDW. M4, the last group, was treated with MO C2 at a adose of 9 mg/kg given orally, after being treated with a 1.25 mg/kg lead oxide dose. MO extract was administered orally for 40 days [14, 15]. The process of dosing the mice was done on alternative days. This system continued for a total of 40 days.

Used 0.1 ml (0.4 ml XYL-M2 and 0.6 ml ketamine 10%) to anesthetize the mice 24 hours after the last treatment. A 1ml heart punch was used to collect blood in an EDTA tube for hematological testing. An Eppendorf centrifuge was used to separate serum from another blood sample which was centrifuged at 3000 rpm for 10 minutes at 30^0C and kept at -20^0C in the freezer.

2.5 Housing the Animals

Forty mature male Albino mice (*Mus musculus*) were obtained from the National Center for Drug Control and Research of the Ministry of Health. The mice were healthy and aging between 8-10 weeks with an average weight of 21-29gm. All mice were housed in polypropylene cages (30cm x 15cm x 15 cm diameter) covered with a wire grid covers, the temperature was regulated at 25±5°Cwith 50-60 percent% humidity, and 12±2 hours light/dark cycle. Feeding consisted of standard food *ad-libitum* drinking water. Cages were cleaned using tap water and 70% alcohol on a regular basis and then bedded with sawdust, fine wood chips or carton paper which was kept dry and replaced on a regular basis.

The animals were acclimatized to laboratory conditions for seven days before beginning the experiments. In accordance with the ethical guidelines for the use of animals in researches and all animals were dealt with care, following the National Research Council 2011 guidelines for the use of laboratory animals.

2.6 Weight of Body

Each animal's weight was recorded daily until the experiment period was over. Following the collection of blood samples, the animals were sacrificed. Body weight of animals was measured at the beginning of the research to calculate growth rate/week using the following formula [16]:
Growth rate/week (%) = weight at last week - weight at first week/ weight at last week.

2.7 Samples Digestion Preparation

The serum samples were digested and measured by using atomic absorption spectroscopy (AAS). One mL blood was taken and then added to each tube that contained 9 ml of 68-72% nitric acid (HNO_3). Afterwards, 1 ml of 37% hydrochloric HCL acid and 2 mL of 30% hydrogen peroxide (H_2O_2) were added to the tubes. The tubes were heated to 180 ^{0}C [17]. After the tubes had cooled completely, the digestion solution was quantitatively transferred and diluted with DDW in a 25 mL volumetric flask. The analytical solution was then transferred to regular tube with a cover for storage.

2.8 Histopathological Investigation

2.8.1 Sampling of Tissues

Animals were dissected at the end of the dosing period to examine organs for histological alterations such as the liver, pancreas and kidney. Tissue slides were made following the conventional paraffin-based method for microscopic examination. Initially, the samples were preserved in a 10% formalin solution and then transferred to a 70% ethanol solution. [18].

2.8.2 Tissues Preparation Procedures

The tissue slices of the liver, pancreas and kidney were fixed in 10% formalin after being dehydrated in successive 70%, 80%, 90%, and 100% ethanol and xylene, and then were embedded in paraffin wax [19]. Using a rotary microtome, paraffin blocks of the tissues were sectioned at 5-6 nm in thickness. The tissue pieces were immersed in a water bath set at 57-58˚C. Later, droplets of substance Canada balsam were put on the slides to fix a cover slide which had been stained with hematoxylin and eosin (H&E). Each slide was photographed using a Canon camera.

3. Statistical Analysis

The Statistical Analysis System- SAS, (2018) program was used to analyze the effects of different factors on the study parameters. Least significant difference (LSD) test (Analysis of Variation-ANOVA) was used to compare between means within this study [20].

4. Results and Discussion

4.1 Consequences of the Growth Rate of all Experimental Animal Groups

Table 1 shows the growth rate of mice during this study of the four groups. M1 represents the control healthy group, while M2 represents the control group that received lead oxide red (Pb_3O_4) orally only with at a dose of 1.25 mg/kg. M3 treated group was given lead oxide [1.25 mg/kg] + MO [C1= 4.8 mg/kg] orally. Finally, the fourth treated group M4 received lead oxide [1.25 mg/kg] + MO [C2= 9 mg/kg] orally.

The highest mean value of 0.2397 ±0.089% of growth rate was recorded in M3 mice when compared with the M1 (0.0918 ±0.016%) control group that was not treated with anything, and the second group M2 that was exposed to lead oxide had a mean value of 0.1598 ±0.073%. The lowest mean value of 0.1288 ±0.064% was found in the M4 sample compared with M2 group, the highest value from the group M1 (Table 1). The results observed significant differences from the analysis of variance ($p \leq 0.05$) effects between the four groups, the growth rate of body weight significant value gave clear differences between mean values of body weight (Table 1).

Table 1: Comparison of growth rate among different laboratory animal groups (M1, M2, M3 and M4) .

Group	Growth Rate (%), Mean ± SE
M1	0.0918 ±0.016 b
M2	0.1598 ±0.073 ab
M3	0.2397 ±0.089 a
M4	0.1288 ±0.064 ab
LSD value	0.1239 *
Means with the different letters in the same column indicate significant difference. * ($p \leq 0.05$), NS: Non-Significant.	

The results mentioned in Table 1 show C1= 4.8 mg/kg and C2= 9 mg/kg of MO, increased the growth rate compared to the control group M1. As for the M3 group, the value was higher than M1 and M2 groups which were exposed to lead oxide only.

The current study agrees with a previous study that an increase in the body weight of laboratory animals exposed to lead was observed. Twenty male albino mice were selected and divided into groups, two groups received lead (25 mg/kg body weight) while the other group was given distilled water (1 ml/100-gram body weight) orally serving as the control group. The weight of the animals was measured daily. The results showed that the longer the period of exposure to lead, the greater the weight of laboratory animals compared to the control group [21]. Five mL/kg/BW of saline was administered to mice every day. The animals received the same treatment in addition to 10 and 20 mg/kg/BW of MO. According to the findings, MO might considerably lower BW, water, and food consumption while also reducing inflammation, improving carbohydrate and lipid metabolism, and reducing the risk of non-alcoholic fatty liver. Furthermore, previous research has showed that MO, an edible plant product, may be beneficial in the management of metabolic syndrome [22].

Due to reticular cell hyperplasia, which is the body's response to lead exposure, group M2 liver weight in the current study dramatically increased compared to group M1. These outcomes come from [23]. This observed increase in organ weight was believed to be caused by necrosis and apoptosis, both of which might be linked to the buildup of lipids in the organ. Researchers that looked into the effects of lead have discovered that rat kidney cells exposed to Pb^{2+} treatments accumulated lipids significantly. The increase in liver weight and other organs is attributable [24].

4.2 The Blood Tests

4.2.1 The Results of Laboratory Animals to Clarify the Portability of the MO Extract to Remove Pb_3O_4

The highest value was read in M2 that was exposed to lead oxide where the mean value was 0.982 ±0.16 mg/L compared to the control group M1 which had a mean value of 0.091 ±0.018 mg/L. As for groups M3 and M4, the mean values were 0.415 ±0.06 mg/L and 0.439 ±0.06 mg/L respectively. The results showed that the dose of C1=M3 and C2=M4, the dose of lead oxide was less valuable when compared with the M2 group that was exposed to lead oxide only in the serum of laboratory animals, based on the results shown in the Table 2.

Table 2: Comparison of lead oxide red (Pb3O4) removal between the treated and untreated laboratory animal groups (M1, M2, M3 and M4) to remove.

Group	Con. of Blood Lead Oxide (ppm) Mean ± SE
M1	0.091 ±0.018 c
M2	0.982 ±0.16 a
M3	0.415 ±0.06 b
M4	0.439 ±0.06 b
LSD value	0.267 **

Means with the different letters in the same column indicate significant difference. ** ($p \leq 0.01$). NS: Non-significant

MO has been shown to have antioxidant activity both *in vitro* and *in vivo* against the hydroxyl radicals produced by the Fenton reaction [25]. In metabolism, ROS is essential for processes including energy synthesis, phagocytosis, development, controlling a cell and intracellular signaling [26]. MO seed powder can lower ROS levels by directly scavenging free radicals generated by metal toxicity. Some studies have indicated that the MO plant reduces lipid peroxidation which has important properties as it works on antioxidants [27].

Previous research on the blood of lead-exposed laboratory mice came to the conclusion that administering M. *oleifera* to mice boosts their levels of red blood cells, white blood cells and hemoglobin. This is a result of M. *olivera* protective function in returning blood parameters to normal. These findings support the idea that M. *olivera* can shield certain mouse blood types against lead poisoning [28].

The reduced sulfhydryl groups of DMSA take part in antioxidant processes that lessen the mineral's oxidative stress, and they can also encourage the manufacture of glutathione which is required for mineral extraction during the chelation process. However, using natural chelates over synthetic ones is always preferable. Although there may be a few negative consequences, a similar mechanism may be hypothesized for this inquiry since MO seed and leaf powder includes several amino acids such as cysteine and methionine, as well as many polypeptides with various cation functional groups. Using infrared spectra for methionine/metal interactions, it can generate protein/amino acid-metal complexes that result in the elimination of lead. Another potential mechanism is that the 20,000 MW = Megawatt) glycoprotein found in MO seed powder might bind to lead and produce a secret-able complex [29].

4.2.2 Level of Fasting Blood Glucose (FBG)

The results of this study showed that the groups treated orally with MO leaf extract, M3 and M4 (treated orally with red lead oxide), had lower blood sugar levels compared with the M2 group that was administrated with red lead oxide only (Table 3).

Adipose tissue from people with T2DM had higher cytokine levels. Their findings revealed that extra body fat, particularly in the belly, resulted in low-level aberrant inflammation that persists over time, affected insulin function, and was a factor in illness because distinct cytokines were controlled by a variety of intricate signal transduction pathways, the MO extract's level of impact varied for various cytokines. The differing effects of MO extract on various cytokines have been challenging in explaining any detail. Pro-inflammatory cytokine suppression caused by MO extract may be inhibited by oxidative stress which may explain why different cytokines are affected by oxidative stress or glycosylation of proteins caused by high glucose or diabetes to varying degrees [30].

Table 3: Comparing various groups in terms of their fasting blood sugar levels..

FBS (mg/dl)	Mean ± SE				LSD Value
	M1	M2	M3	M4	
Week 1	99.10 ±5.10 b	245.60 ±17.11 a	220.50 ±6.04 a	227.80 ±6.79 a	28.74 **
Week 2	106.30 ±5.92 c	224.80 ±12.51 a	168.50 ±11.98 b	186.20 ±11.63 b	31.11 **
Week 3	121.00 ±7.12 b	185.80 ±7.39 a	114.50 ±2.55 b	117.60 ±4.23 b	16.33 **
Week 4	113.80 ±6.04 b	239.70 ±7.67 a	103.90 ±3.20 b	120.00 ±5.79 b	16.92 **
Means with different letters in same row indicate significant difference. ** ($p \leq 0.01$). NS: Non-significant					

According to the results of the present research, MO supplementation may have the ability to lower blood levels of pro-inflammatory cytokines and thereby prevent the development of arterial inflammation in T2DM. Cytokines are a defense system to function once they are released. All blood cells and other cells that support the body's immunological and inflammatory reactions are affected by cytokines. By transmitting signals that can cause abnormal cells to perish and normal cells to survive longer, they also support anti-cancer action. To determine whether MO can lower levels of pro-inflammatory cytokines in the T2DM patient group, the clinical proof that MO can suppress markers of vascular inflammation must be examined. If so, MO supplementation might be used as an adjunct treatment to treat T2DM-related cardiovascular illness and vascular inflammation [31].

4.3 Histological Sections

Throughout the investigation period of 40 days, every mouse was closely watched for its behavior, appearance, histological changes, and infections noted. After the experiment, the liver, pancreas and kidneys were all inspected.

4.3.1 Liver

A. Control Group (M1)

A study of the liver's histopathology in the control group revealed that the liver's normal structure was encircled by the capsule's collagenous connective tissue. Figure 4(A shows portal system is normal. The capsule releases trabeculae that divide the liver parenchyma into lobules. Hepatic cells surround the central vein which is located in the middle of the lobule (hepatocytes) (Figure 4(B)).

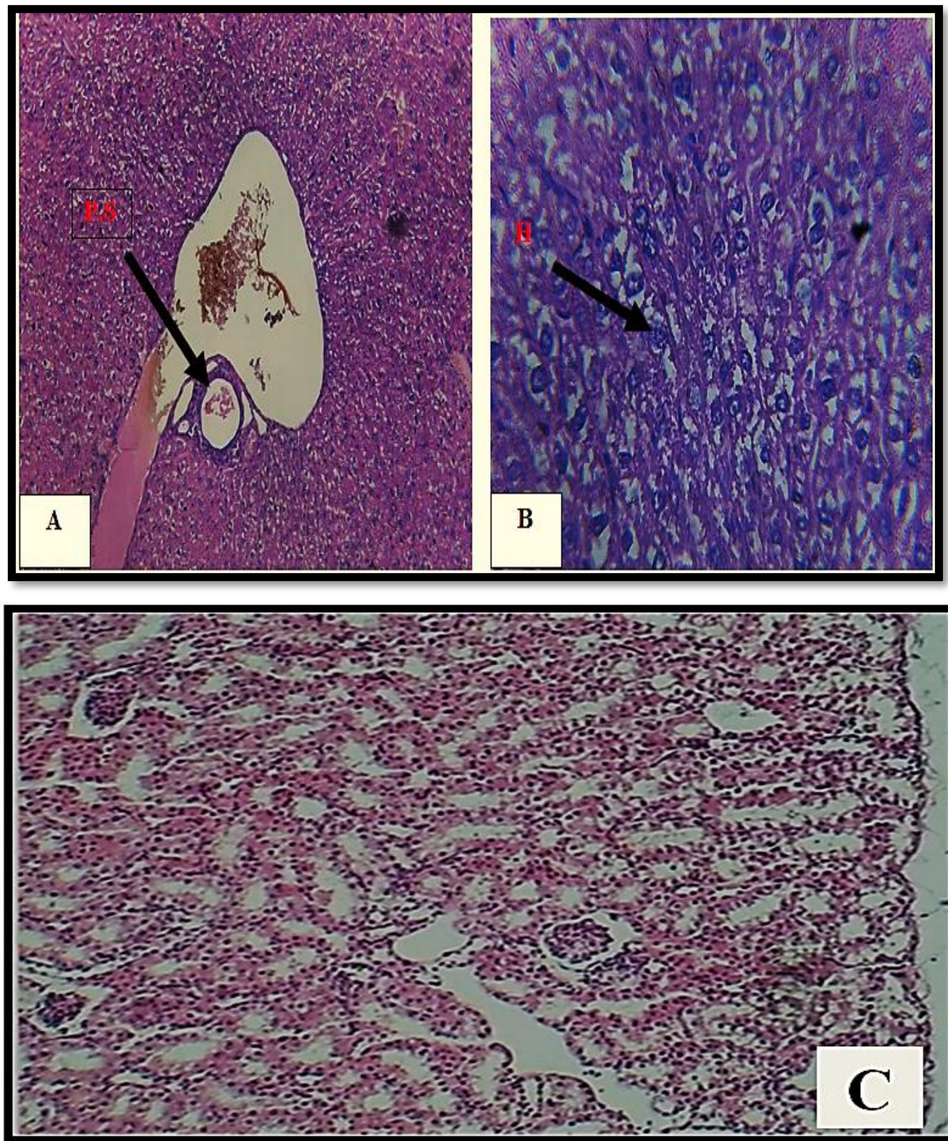

Figure 4: (A) Cross section in liver showing normal tissue, (P.S): portal system is normal, (10x) magnification; (B) Showing normal hepatocyte (H) (40x) higher magnification; (C) Section of a kidney control (10x).

B. Control Group with Pb_3O_4 Only (M2)

When compared with the M1 group that was not given anything but food and water, it was observed that liver section vacuolation in the hepatocyte, (pyknosis and binucleated cell are high, and binucleated hepatocyte, diluted vessels with inflammatory cells, and some blood vessels the damage of endothelial layer, necrosis, and degeneration was highly found in sections (Figure 5).

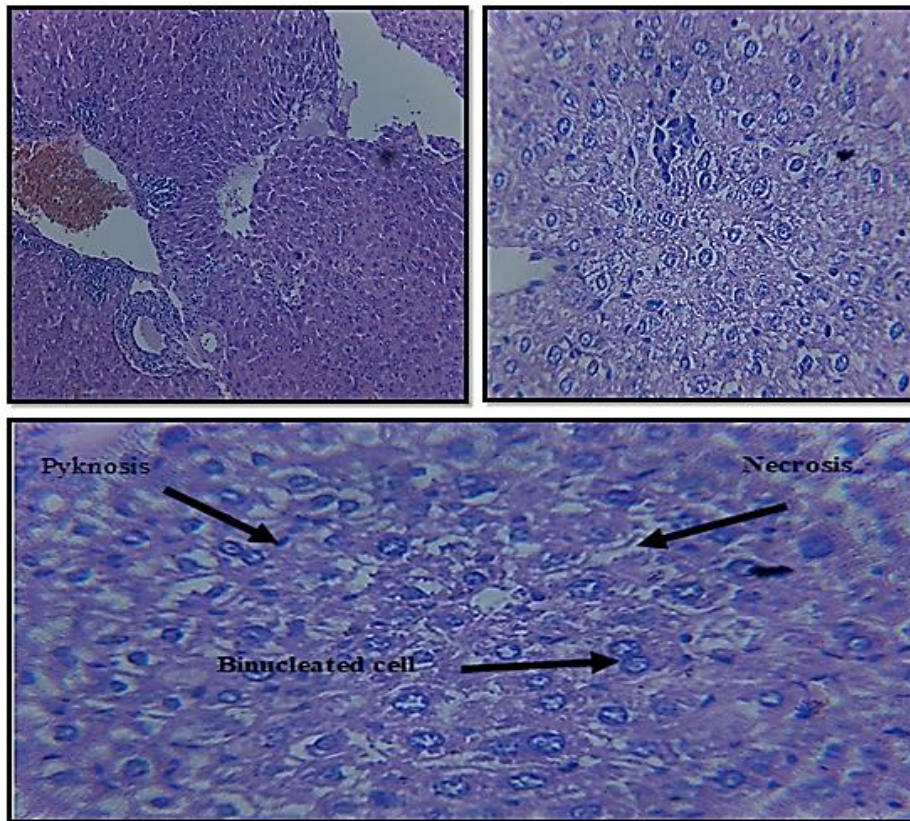

Figure 5: Liver sections exhibit vacuolation in hepatocyte, (major pyknosis and binucleated cell existence) diluted vessels with inflammatory cell and some blood vessels and damage to the endothelial layer, necrosis and degeneration was highly found in sections (40x)

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C. Group Treated with Pb_3O_4 and MO-C1 (M3)

According to the tissue sections shown in figures below the liver the sections show necrosis in the parenchyma of tissue, loss of nuclei of the hepatocyte, degeneration of cells pyknosis of some cells but the portal system is less effective, and semi-luck vacuolation in hepatocyte cells.

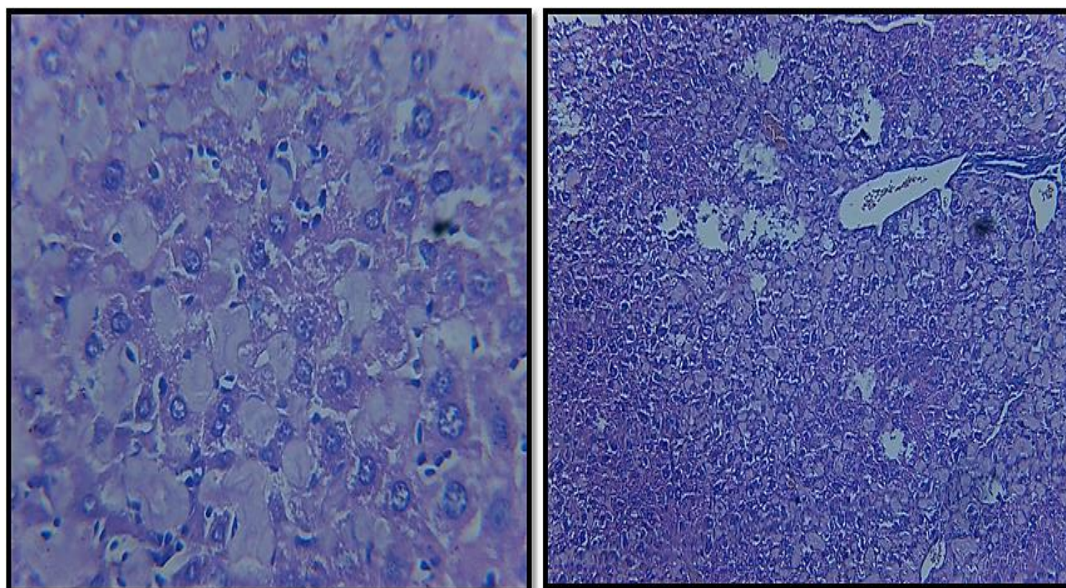

Figure 6: Normal liver section (40x).

D. Group Treated with(Pb_3O_4 and MO-C2 (M4)

Liver section was the same as in group M3, but differed in the absence of vacuolation in the appearance of hepatocytes, while some blood vessels were congested (Figure 7).

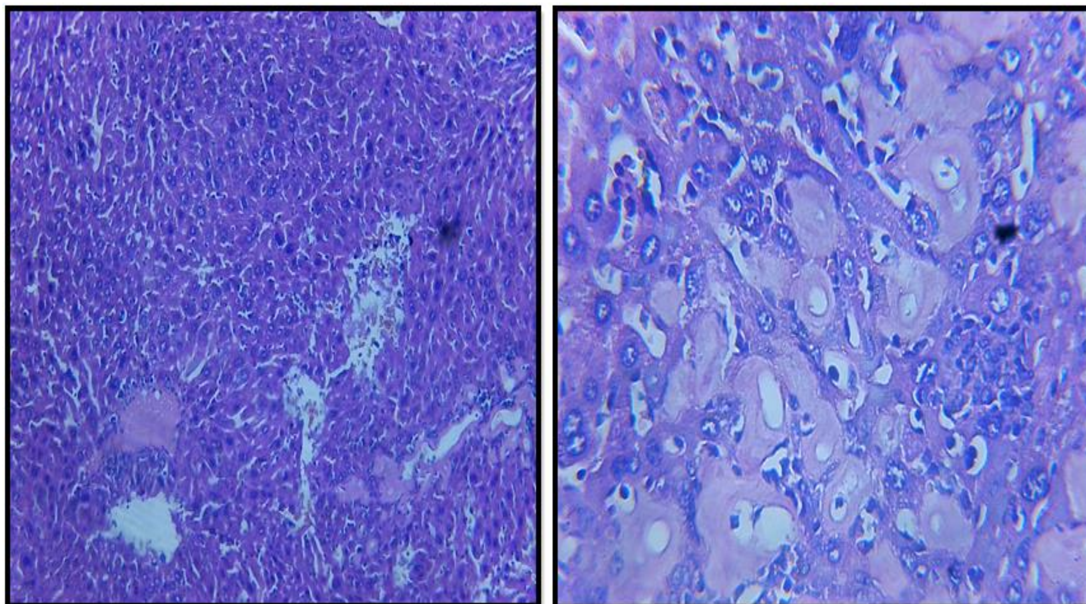

Figure 7: Liver section is normal (40x).

Lead built up in bone up until a point when it could cross a threshold at which point it got deposited in other tissues, particularly the kidneys. Eighty two percent of the lead particles that were consumed or breathed were eliminated in urine and feces. Only 0.5% of the milk was expelled, while the remaining 17.95% was retained in tissues and organs [32]. The liver functions as the body's primary detoxifying organ, had the greatest system of phagocytic reticuloendothelial cells, making it susceptible to various xenobiotic-intercede insults that could result in unfavorable histopathological changes [33].

In the current investigation, liver tissues from the experimental groups were examined for degrees of histopathological alterations than biochemical changes and compared with control group M1. These alterations were time-dependent; the longer the exposure time, the more harm was done. The first organ to be seen was the liver via the portal vein. Nutrients and other foreign substances were absorbed inside. The liver is composed of extremely metabolically active tissues and has a wide variety of detoxifying machinery systems. The earlier results are consistent with the results of this study. Noticeable changes were recorded in the histological sections of the liver, including necrosis. The necrosis of the hepatocytes caused by persistent lead exposure could be an indication of oxidative stress brought on by glutathione depletion on these cells [34].

As cellular defense mechanism against harmful compounds, cell vacuolation is one way that hepatic toxicity manifests itself. These compounds were separated in vacuoles, preventing interference with cellular processes. According to earlier research, liver damage was indicated by lymphocytic infiltration and sinusoidal blood congestion following lead therapy. Investigations like this have also been reported by some other researchers. This study observation of lymphocytic infiltration after lead treatment provided evidence of cell irritability, inflammation, and lead sensitivity [35].

Hepatotoxins cause Kupffer cells to quickly produce proinflammatory cytokines and chemokines including TNF- and IL-1, the cytokine and chemokine synthesis to entice

circulating immune cells, further enhancing an inflammatory response. As a modulator of leukocyte maturation and activation, chemokine (released by Kupfer cells after liver injury) played a significant role in inflammatory reactions. It observed that lead injection dramatically reduced the content of zinc and copper in the liver and heart tissue of rats which reduced the absorption of micronutrients from the gastrointestinal tract [36].

Previous studies, like the results of this study, showed that rats were treated with an extract of MO seeds which successfully effected on ROS levels, lipid peroxidation products (LPP) and total protein carbonyl content (TPCC) in MO returned to normal. This could be attributed to *Moringa oleifera* seed powder antioxidant characteristics which included direct free radical scavenging and an increase in antioxidant enzymes which lowered lead toxicity. Flavonoids and antioxidants the MO plant products own can inhibit the actions of enzymes that produce ROS, quench free radicals and chelate transition metals [37].

A congested liver that has trouble in secreting plasma proteins could be the result of pathological changes brought on by toxicant metallic elements that create congestion. This caused the blood osmotic pressure to drop which in turn causes the tissue outflow to drop [38]. I was found that attributable infiltration in the liver tissue increase the permeability of blood vessels which happens when the endothelial cells of blood vessels contract in response to specific chemicals or when desmosomes, which are particles that lie between the endothelial cells and allow the passage of blood vessels, are lost. According to other research, the surge of inflammatory cells from the vessel's core to its periphery causes the endothelial lining to alter resulting in the enlargement of blood vessels [39].

4.3.2 Pancreas

A. Control Group (M1)

In the control group, pancreatic tissue was found to have normal structures for the acini and islets of Langerhans the interlobular connective tissue on the islets was dense and spherical (Figure 8).

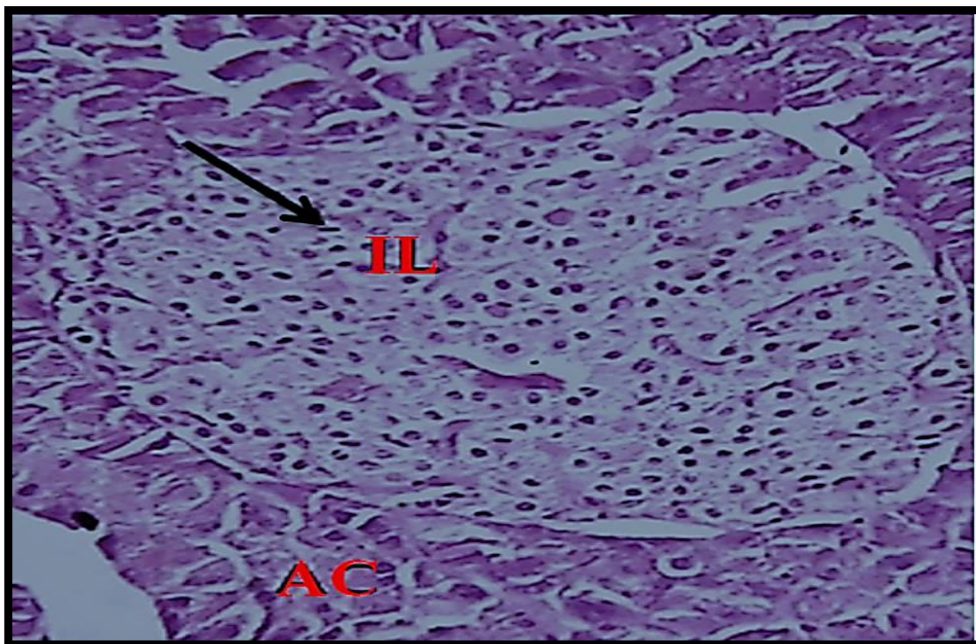

Figure 8: Pancreas section showing normal (IL): Islets of Langerhans and (AC): Pancreatic acini (40x).

B. Control Group with Pb_3O_4 Only (M2)

The pancreas section revealed poorly ordered pancreatic acini, necrosis, and vacuolization in the islets of Langerhans. There were fewer and smaller islets which also indicating deterioration in intralobular channel islets of Langerhans enlargement and acini, a decrease in islet size and number. There was no evidence of auto-restoration (Figure 9).

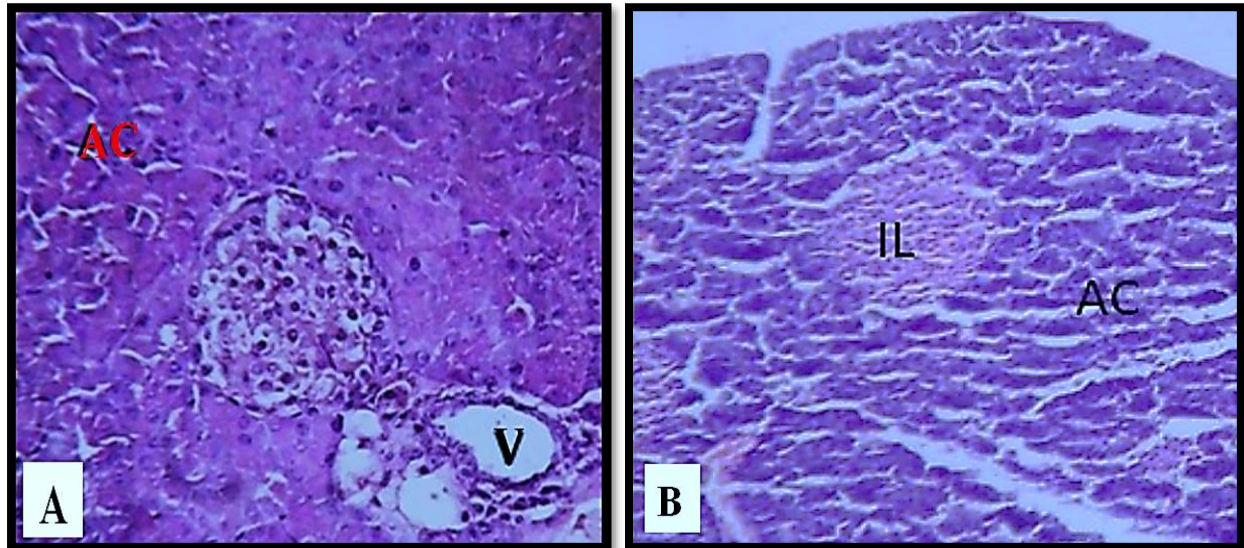

Figure 9: (A) Pancreas section showing (AC): Pancreatic acini have necrosis and vacuolations while (V): Dilated intralobular duct; (B) AC: vacuolations in acini and islets shrinkage and (IL): Islets of Langerhans (40X).

C. Group Treated with Pb_3O_4 and MO-C1 (M3)

Figure 10 below shows regeneration in the Langerhans islets which were more and bigger. Normal acini and intralobular duct. The figure displays the regrowth of islets with β-cells.

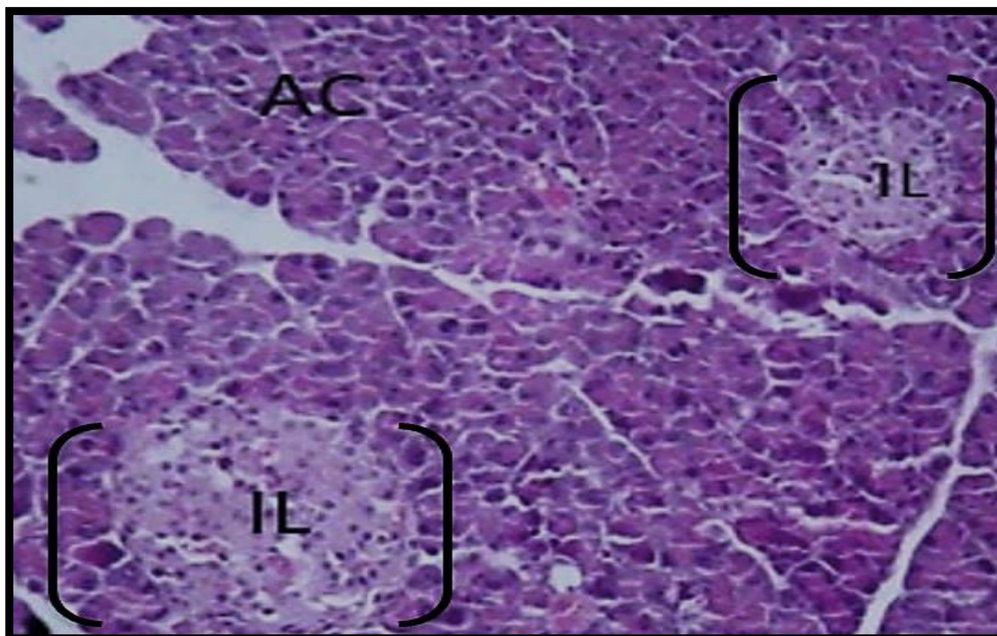

Figure 1:0 Cross section in pancreas treated with ethanol MO showing no changes in the tissue of pancreas, (IL): Islets of Langerhans and (AC): Pancreatic acini (40x).

A toxic heavy element known as lead has been linked to the pathology of numerous persistent human illnesses. The significant impact of lead on glucose homeostasis and metabolic processes in the liver and pancreas has been well highlighted in previous research. In another study, rodents were given the lead for 32 days, and after the rats' livers were examined for signs of oxidative stress and inflammation, and diabetes-related factors, the activity of the gluconeogenesis and glycogenolysis enzymes was also assessed. Lead interfered with the islets' ability to secrete insulin and raised the activity of the gluconeogenic enzymes responsible for glucose sensitivity in the liver. Through effects on the pancreas and liver, particularly through the development of insulin resistance, chronic lead exposure can disturb glucose balance [40].

The MO leaves are rich in active compounds that have both therapeutic and dietary benefits. It is frequently employed in folk medicine as a treatment for several illnesses. As it brought elevated serum levels of glucose, triglycerides, cholesterol and malondialdehyde back to normal and brought back normal mRNA expression of the enzyme glucose pyruvate carboxylase in liver tissue, M. *oleifera* leaf extract countered the effects of alloxan-induced glucocorticoids in rats. Additionally, it boosted *in vivo* weight growth and restored the livers of diabetic rats' decreased fatty acid synthase mRNA expression. Additionally, it repaired the liver and the pancreas natural histological structure after alloxan injury in diabetic mice. According to this study, the aqueous extract of M. *oleifera* leaves lowered blood sugar levels by lowering increased levels of the enzyme hepatic peroxylase and by repairing injured pancreatic and liver cells thanks to its antioxidant characteristics [41].

In previous studies, laboratory rats were dosed with two types. The lower dose was given orally "200 mg/kg/day for 30 days". The higher dose was "400 mg/kg/day for 30 days" from the MO plant. High doses of *Moringa oleifera* extract were administered to diabetic rats, and both blood glucose and insulin levels significantly decreased and increased. Diabetic rats treated with a low dosage of MO saw a minor drop in plasma glucose and a little rise in insulin levels. Histologically, diabetic mice treated with a low dose of MO did not exhibit any improvement in their pancreatic islets and acinar cells, whereas diabetic mice treated with a high dose of MO displayed an apparent recovery of their pancreatic islets and acinar cells to an almost normal state. In diabetic mice, there was a reduction in beta cells expression which produce insulin [42].

4.3.3 Kidney

A. Control Group (M1)

The capsule, a thick connective tissue structure with some smooth muscle fibers, makes up the kidney's histological structure in the M1. Along with blood vessels and related connective tissue, the cortex also includes portions of nephrons and collecting tubules. The medulla is made up of renal pyramids which include vasa recta, descending thick limbs of the Henle loop, ascending thick limbs of the Henle loop and thin limbs of the Henle loop. Collecting tubules are lined with simple cuboidal epithelium. In Figure 11: (A) Cross section in kidney shows normal glomeruli and tubules, while (B) shows kidney tubules, and (C) shows that glomeruli and Bowman's capsules were normal.

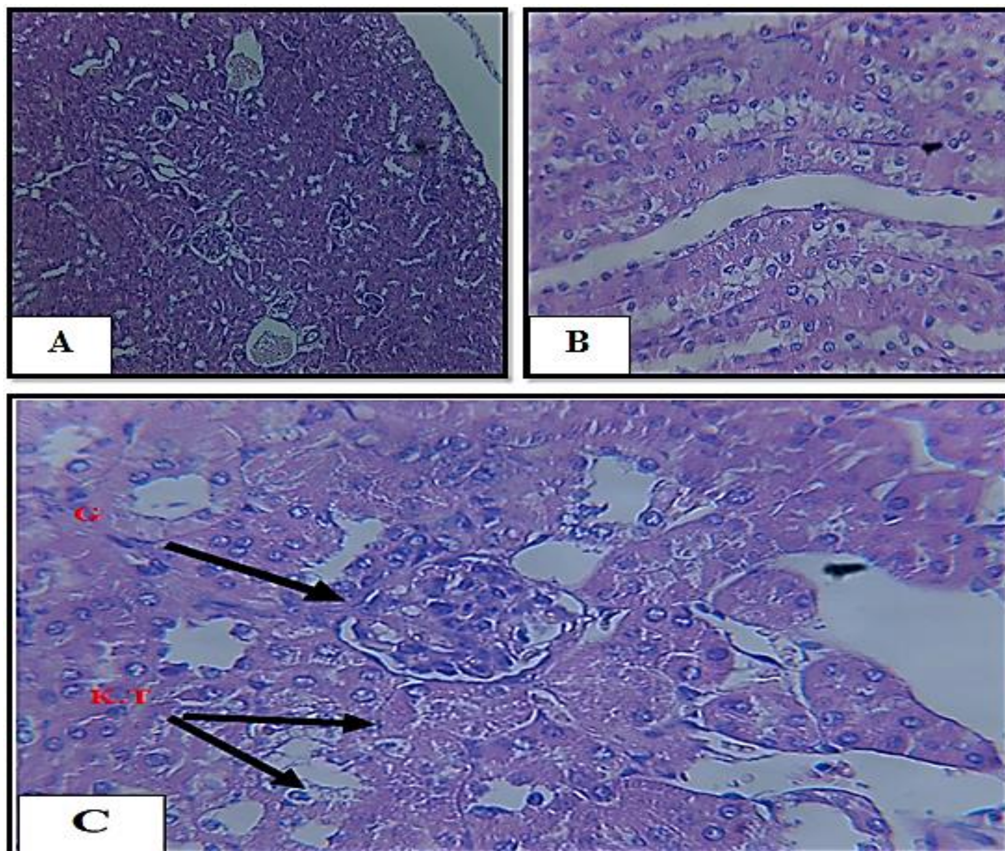

Figure-:11 (A) cross section in Kidney showing normal glomeruli (G) and tubules (10x); (B) showing kidney tubules (K.T) in higher magnification (40x); (C) showing glomeruli and Bowman's capsules is normal (40x).

B. Control Group with Pb_3O_4 Only (M2)

Based on the Figure 12 below, the kidney section shows increases in Bowman's capsules, diluted kidney tubules, flattenedd cell increased in the collecting duct, and fibrosis in the collecting duct (atrophy).

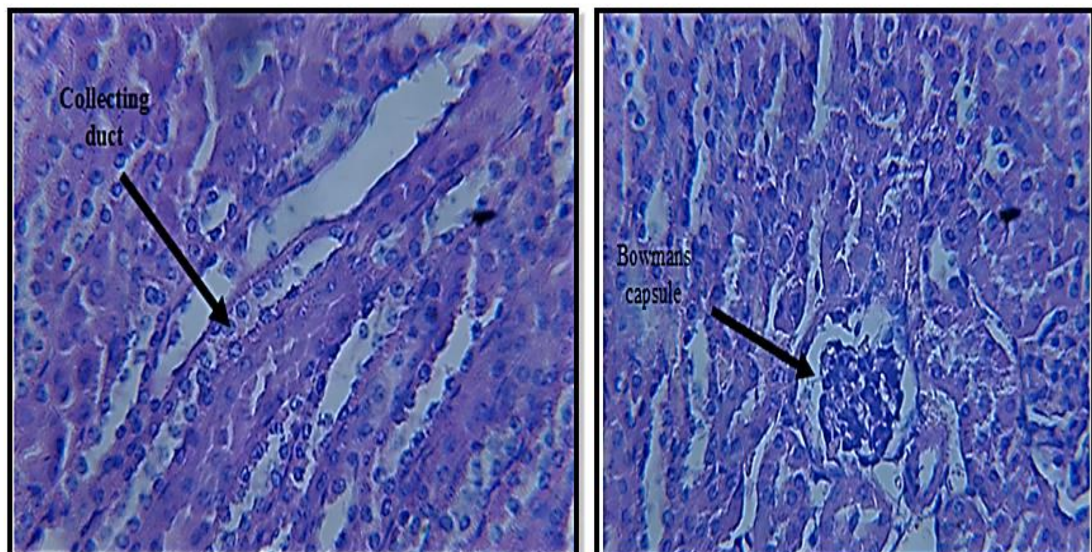

Figure 1:2 Kidney section showing atrophy in some glomeruli, increase of Bowman's capsules, necrosis in kidney tubules, flattened cells increase in the collecting duct {degeneration of collecting duct} (40x).

C. Group Treated with Pb_3O_4 and MO-C1 (M3)

Kidney section had some tubules that had normal histology, but the glomeruli were congested with capillaries, and some collecting ducts were normal (Figure 13).

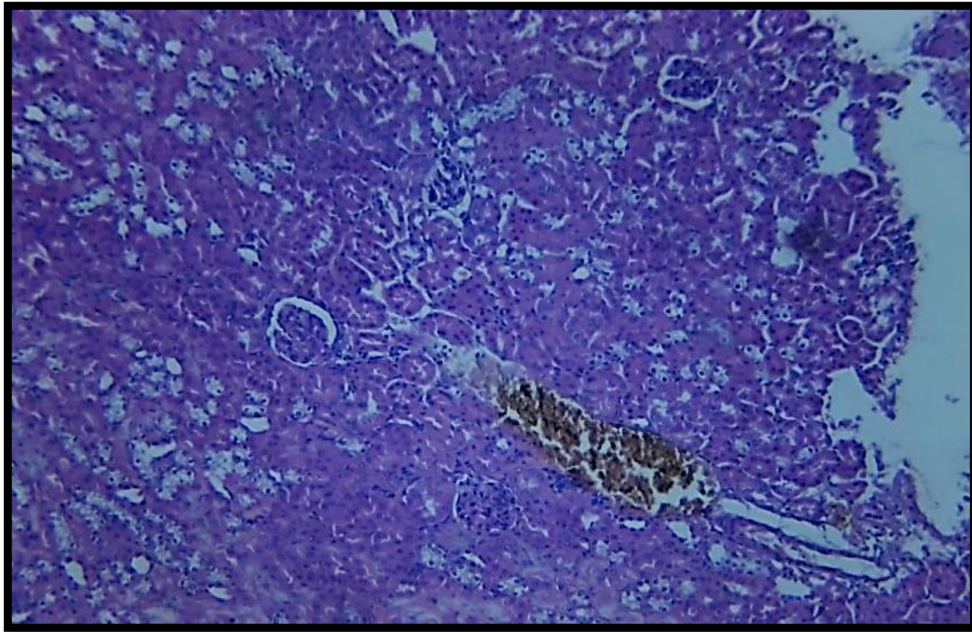

Figure 13 : Normal kidney section (10x).

D. Group Treated with Pb_3O_4 and MO-C2 (M4)

The kidney section showed that glomeruli had highly eosinophilic chromatin and dilated capillaries, most of the tubules were normal, the collecting duct showed flattened cells, the majority of the duct appeared normal. (Figure 14) shows these results in contrast to the control groups which that showed no significant lesions. But in all areas of the kidneys that were investigated, mice pre-treated with either M. *oleifera* showed a considerably lower severity score, indicating a considerable reduction of lead oxide-induced damage.

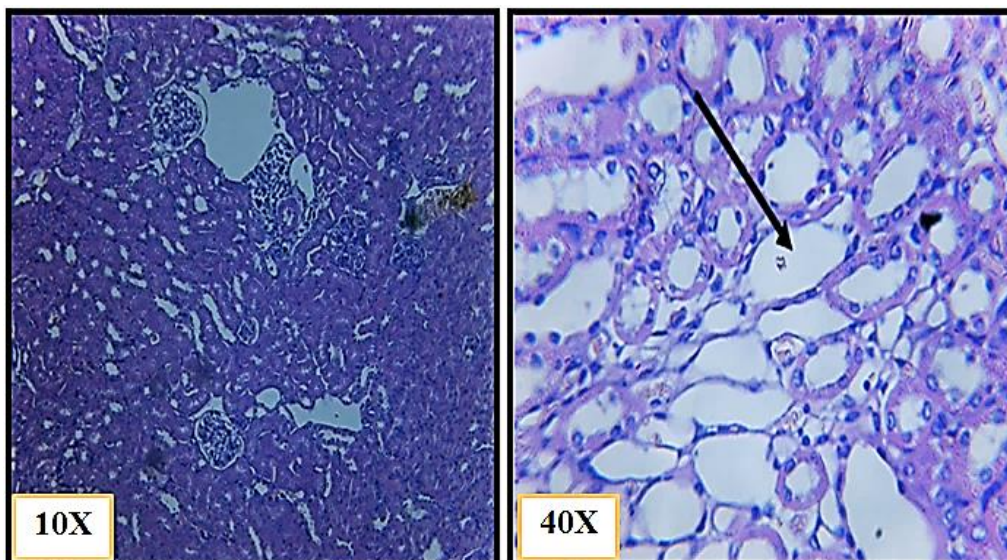

Figure 14: The kidney section shows spaces increase within the kidney, flattened cell in the collecting duct and and an increase in number of ducts with vacuolation.

Interestingly, the current research revealed that pre-treatment with the *Moringa oleifera* fraction significantly improved the kidney histology in mice. The extract had a less negative impact on mice at both doses. The dose-dependent decrease in serum Pb_3O_4 levels in mice pre-treated with either dose of the extract also pointed to the extract's capacity to protect tissue. Oxidative stress and inflammation were brought on by renal apoptosis and finally resulted in kidney damage. Pb exposure increased kidney apoptosis. Additionally, lead acetate could replace the divalent ion Ca, disrupting the delicate balance between proteins that prevent and promote apoptosis [43]. Chlorophylls, beta-carotene, tocopherol, polyphenols, tannins, saponins and other chemicals were the renewable resources found in MO leaves. These components could be in charge of the anti-apoptotic actions on renal cell death induction. The extract displayed anti-inflammatory activity in renal tissue when administrated as an aqueous extract solution from the MO leaves, which dramatically decreased the inflammatory mediators in the kidney tissue of lead acetate-treated rats [44].

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