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PROTECTIVE ACTIVITY OF *MORINGA OLEIFERA* SEED EXTRACT AGAINST PBNP-INDUCED HISTOPATHOLOGICAL CHANGES IN RATS

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ABSTRACT : This study investigated the possibility of using *Moringa oleifera* seed extract to prevent poisoning caused by commercial lead nanoparticles (CLNP) and induced by pulsed laser ablation (LLNP) in white Wistar rats. The rats were divided into six groups with seven rats in each group: (1) 160 mg/kg *Moringa* seed extract (M.), (2) 5 mg/kg commercial lead nanoparticles, (3) 5.8 mg/kg nanoscale lead, (4) *Moringa* seed extract + commercial lead nanoparticles (CLNP. M.), (5) *Moringa* seed extract + prepared lead nanoparticles (LLNP. M.) and (6) control. The experimental period was 45 days. At the end of the exposure period, the rats were dissected. Their liver and kidneys were removed and preserved in 10% formalin solution as a fixative solution to prepare them for histological sections. Histological imaging results showed that the CLNP group had prevascular cuffing of inflammatory cells with binucleate hepatocyte and hydronic degeneration. In the LLNP group, blood vessels were dilated and the central vein and hepatocytes had vascular degeneration. The changes in CLNP. M. and LLNP. M. groups were less severe. In the kidneys, the changes in the LLNP group were indicated by congestion in the blood vessels and inflammatory cell infiltration. In the CLNP group, kidney tissue imaging revealed congested blood vessels with the massive dissolution of red blood cells and the proliferation of inflammatory cells.

Key words : Lead nanoparticles, *Moringa* seed, histological, liver, kidneys.

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INTRODUCTION

Heavy metals are natural components of Earth's crust, whose biochemical balance is easily altered by humans (Lazarus *et al*, 2018). One of the common toxic heavy metals is lead because it can be easily mined and refined. It is also used in the construction of buildings and the manufacture of water pipes, batteries and alloys (Duah *et al*, 2012). The widespread use of lead increases environmental and health challenge (Ali *et al*, 2018). A major exposure to lead affects the central and peripheral nervous systems, leading to neuronal degeneration (Assi *et al*, 2016). It also causes alterations in the structure of liver tissue, including congestion, central vein dilation, hepatic emptying and inflammatory cell infiltration with necrosis and enlargement of liver cells (Ali *et al*, 2018). Lead also affects the heart, testes, eyes, skeletal muscles, bones, blood and kidneys (Owolabi *et al*, 2017).

Nanotechnology is a modern technology that has many applications, including the fabrication of particles in the nanoscale range. However, secondary particles have migrated into the surrounding environment as a result of the unlimited use of nanotechnology products and secondary materials. As such, the sources of secondary particles, their behaviour and impacts on the environment have been explored. Different techniques for assessing the prevalence, fate, behaviour and potential hazards of secondary materials in different environments have been developed (Ahmeda *et al*, 2017). During manufacturing, nanoscale lead particles (PbO-NPs) may be released into the surrounding air, posing possible exposure through inhalation; therefore, exposure to lead is a public health concern (WHO, 2013). Several studies have shown an increased toxicity of nanoparticles compared with that of microparticles with the same composition; this phenomenon has raised concerns about the impact of nanoscale particles on human and animal health (Amiri *et al*, 2016).

Moringa oleifera is a medium-sized tree that grows

in northwestern and central Indian plains with hot and dry climates and in arid regions in the Indian peninsula (Padayachee and Baijnath, 2012). It can also be grown as a crop on lands with high temperature and low water availability (Leone *et al*, 2015). The different parts of this plant contain minerals and serve as a good source of proteins, vitamins, amino acids and various phenols (Coz-Bolaños *et al*, 2018). These characteristics indicate that *M. oleifera* has medicinal and nutritional uses (Rani and Arumugam, 2017). Most of its parts, including leaves, flowers and seeds, are edible. Other parts, such as bark and pods, are used in biodiesel production and water purification (Hendrawati *et al*, 2016). *M. oleifera* has tremendous healing properties, including anticancer, anti-ulcer, antimicrobial and antioxidant properties. Therefore, *Moringa* can be used as a functional ingredient in food products (Sahay *et al*, 2017).

This research aimed to investigate the effect of the acetic-alcoholic extract of Iraqi *Moringa* seeds in preventing or reducing the toxicity of nanoparticles in the tissues of the liver and kidneys of rats.

MATERIALS AND METHODS

Plant material

M. oleifera seeds were obtained from the Iraqi National Herbarium (Baghdad) on 1-4-2019, picked from the trees, dried and crushed with a laboratory grinder to obtain their powder form.

M. oleifera seed extraction

Moringa seeds were extracted in solvent acetone/ethanol by using the Soxhlet system (1:1). In this procedure, 300 g of the sample was placed in a pure thimble cellulose jar. Extraction was conducted for 72 h, and the extract was concentrated using a rotary vacuum evaporator (Suleiman, 2007).

Stuck commercial nanoscale lead

Nanoscale powder (Nanoshel) was less than 100 nm in diameter. Lead oxide nanopowder was dissolved in distilled deionised water by using a sonicate system to prevent the nanoparticles from aggregating (Sarkozi *et al*, 2009).

Nanoparticle preparation by laser

After completing the preparation of the compressed lead sample (the target), the lead (Pb) was bombed in a medium of double distillation water . Its size was (15mL) with a height about (8mm) above the target disk surface. A condenser Nd-YAG laser pulse was used at the energy of (100 mJ) and the pulses were (100, 500, 1000, 1500, 2000, 2500) pulse. The colloidal solution becomes slightly cloudy (like smoke), indicating the formation of colloidal metallic nanoparticles, taking into account the container's size in which the disk is placed so that the sample is away from the laser source about (12cm). 500 pulse provided the lead concentration nanoparticles of 5.8 mg/l, which was used in this study.

Experimental animals

Adult white male Swiss Wistar rats (*Rattus norvegicus*), which were collected from the Animal House at the Faculty of Veterinary Medicine at Tikrit University, Iraq, were used in this experiment. Their age ranged from 12 weeks to 14 weeks, and their average weight was 250 g. They were reared under standard laboratory conditions (24 ± 1°C, a normal light cycle, 12 h light and 12 h dark, and fed freely).

Experimental design

The experimental animals were divided into six groups, with seven rats in each group. *Moringa* and commercial lead were given to the rats in accordance with previously described methods (Perret-Gentil, 2010). The first group or the control group was given distilled water. The second group was treated with 160 mg/kg *Moringa* extract (M) through the stomach (Aleksiichuk *et al*, 2018). The third group was injected with 5 mg/kg commercial nanoscale lead (CLNP) via periton (Oszlánczi *et al*, 2011). The fourth group was injected with the prepared nanoscale lead (laser; 5.8 mg/kg) by periton. The fifth group was administered with *Moringa* extract containing commercial lead nanoparticles (CLNP. M.). The sixth group was given *Moringa* extract and injected with lead nanoparticles (L. M.).

Animal dissection

After the expiration of the prescribed period for each group, the rats were left untreated for 2 days; on day 3, they were weighed and anesthetised with chloroform (McKenzie, 2011). Afterwards, they were dissected, and their liver and kidneys were removed and preserved with 10% formalin solution as a fixative solution for the preparation of tissue sections.

Histological study

The tissue sections were prepared in accordance with the methods described by Bancroft and Stevens (2012).

RESULTS AND DISCUSSION

Liver

Figs. 1 and 2 shows the liver tissue of the control rats and the group treated with *Moringa* extract. The tissue appeared normal, whereas the changes were evident in the rats' liver tissue in both groups (CLNP., LLNP). Where these changes were representative of the group CLNP. The prevascular cuffing of inflammatory

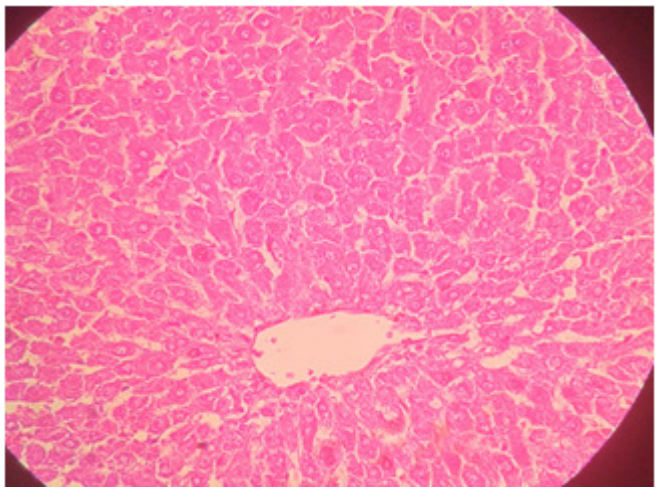

Fig. 1 : Section of a liver tissue of the control group with haematoxylin-eosin stain (40×).

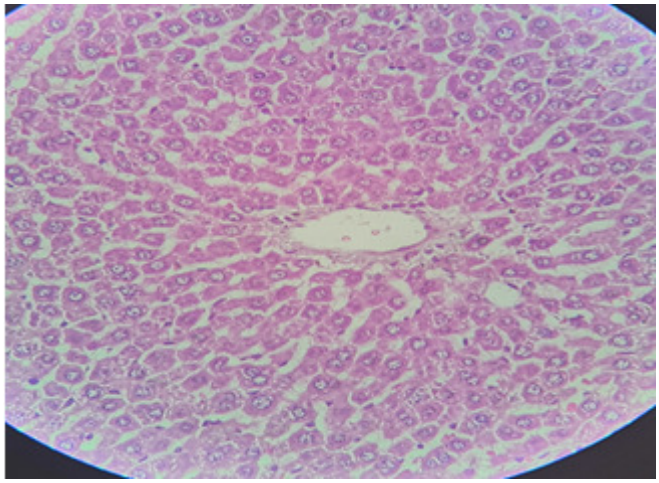

Fig. 2 : Section of a liver tissue of the M. group with haematoxylin-eosin stain (40×).

cells (arrow) with binucleate hepatocytes and hydronic degeneration (arrowhead). In the L group, histological imaging revealed the dilation of blood vessels and the vascular degeneration of the central vein (arrow) and hepatocytes (arrowhead). This finding may be attributed to the effect of lead on liver enzymes, which affect the mechanism of action of hepatocytes, as the liver is considered a target site of lead toxicity (Ali *et al*, 2018). Lead can interact with proteins and enzymes in the hepatic interstitial tissue that interferes with the antioxidant defense mechanism and results in the generation of reactive oxygen species (Johar *et al*, 2004). Figs. 5 and 6 illustrate that the changes in the CLNP. M. and LLNP. M. groups were less severe possibly because of the occurrence of less severe changes in the rats treated with lead and *Moringa* seed extract compared with that in the groups administered with only lead nanoparticles; this observation suggested that *M. oleifera* extract elicited protective effects against lead toxicity. Omotoso *et al* (2015) also demonstrated that *Moringa* provides

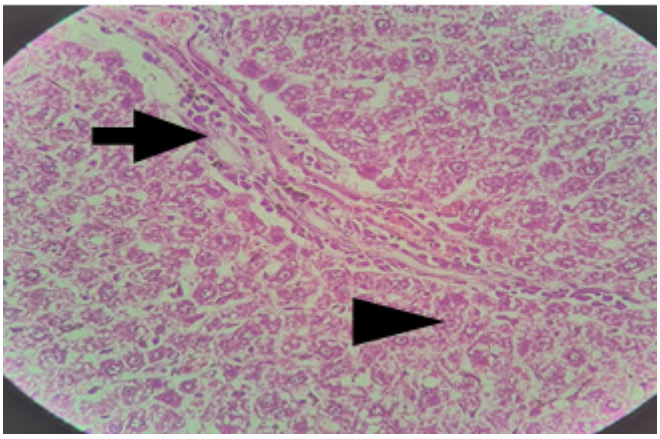

Fig. 3 : Section of a liver tissue of the CLNP group showing the prevascular cuffing of inflammatory cells (arrow) with binucleate hepatocyte and hydronic degeneration (arrowhead), with haematoxylin-eosin stain (40×).

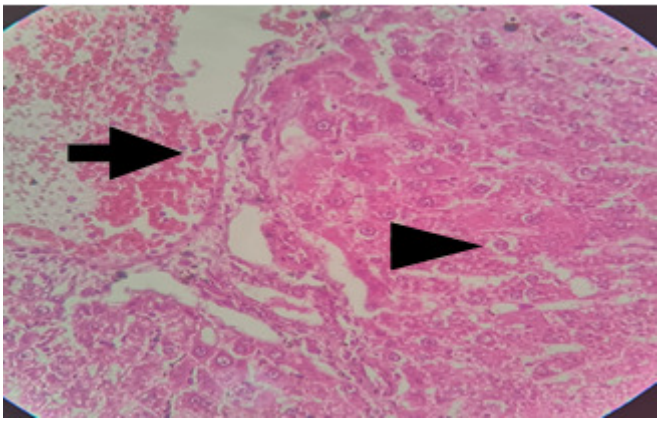

Fig. 4 : Section of a liver tissue exposed to laser treatment, with dilation of blood vessels and vascular degeneration of the central vein (arrow) and hepatocytes (arrowhead), with haematoxylin-eosin stain (40×).

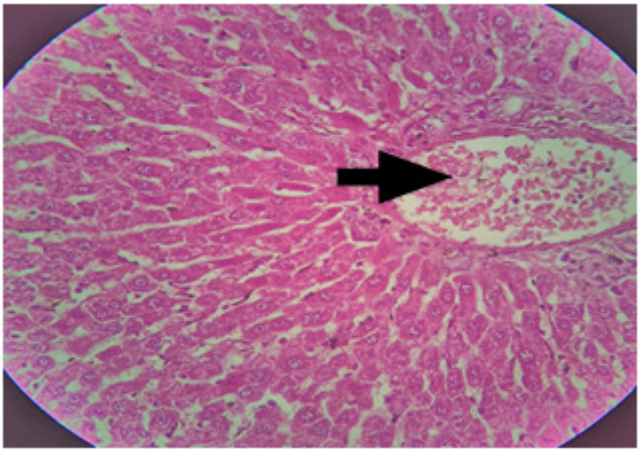

Fig. 5 : Section of a liver tissue of LLNP. M. with vascular congestion (arrow) with haematoxylin-eosin stain (40×).

protection for liver cells because of its chemical components that have protective properties against lead-induced hepatotoxicity.

Kidney

The histological results showed no clear changes in

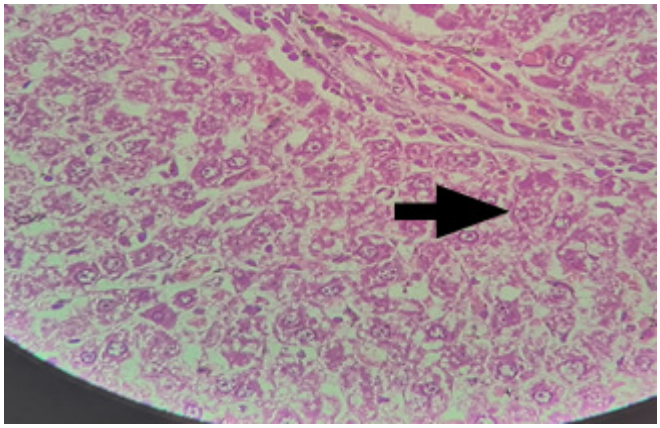

Fig. 6 : Section of a liver tissue of CLNP. M. with binucleate hepatocyte and hydronic degeneration (arrow) with haematoxylin-eosin stain (40×).

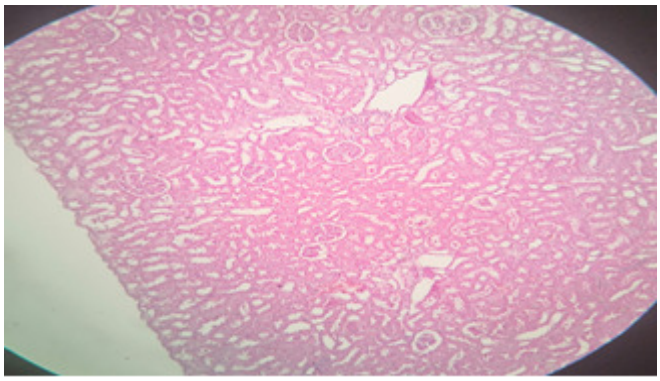

Fig. 8 : Section of a kidney tissue of the M group with haematoxylin-eosin stain (20×).

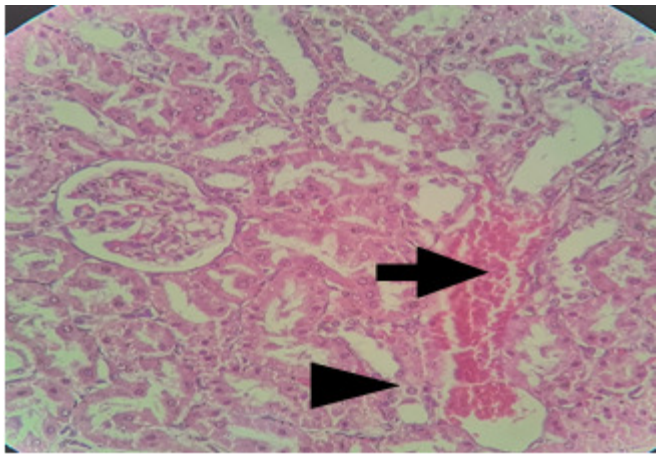

Fig. 10 : Section of a kidney tissue of the laser group showing vascular congestion (arrow) and inflammatory cell infiltration (arrowhead) with haematoxylin-eosin stain (40×).

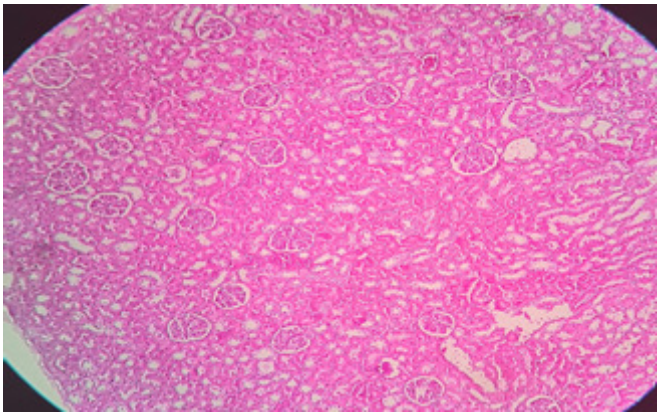

Fig. 7 : Section of a kidney tissue of the control group with haematoxylin-eosin stain (10×).

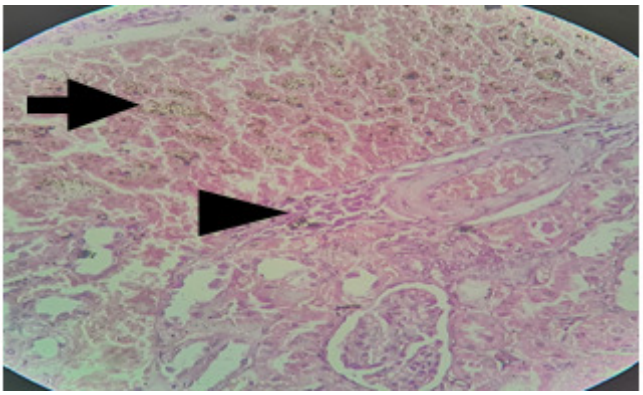

Fig. 9 : Section of a kidney tissue in the CLNP group with the appearance of congested blood vessels with RBC lysis (arrow) and the proliferation of inflammatory cells (arrowhead; 40×).

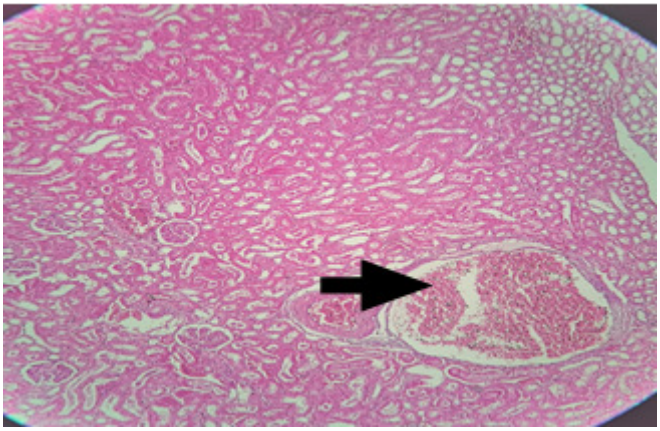

Fig. 11 : Section of a kidney tissue of the treated LLNP. M., with the appearance of congested blood vessels and the proliferation of melanocytes (arrow) with haematoxylin-eosin stain (40).

the control group (Fig. 7) and the group treated with M., as presented in Fig. 8. By comparison, the changes in the two treatments CLNP and LLNP were clear, as illustrated in Figs. 9 and 10. Slight changes were observed in CLNP. M. And LLNP. M., as shown in Figs. 11 and 12 compared with those in the groups treated with lead without *Moringa*. Changes in the laser group were indicated by congestion in blood vessels and infiltration of inflammatory cells (arrow). The kidney tissue imaging of the CLNP group revealed the emergence of congested blood vessels with massive decomposition of RBCs (arrow) and proliferation of inflammatory cells (arrowhead).

These changes might be the result of hydrological changes in renal tissues, as lead poisoning is a predisposing catalyst that leads to the partial failure of ion pump transfer

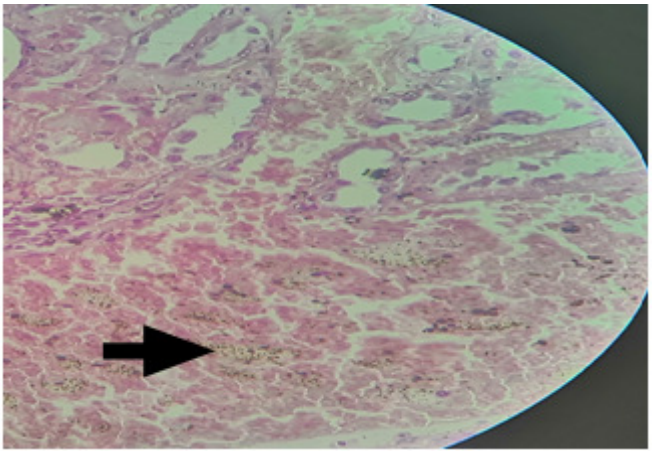

Fig. 12 : Section of a tissue from the kidney of the CLNP. M. RBC (arrow) with haematoxylin-eosin stain (40×).

to tubular cells, which in turn produce tubular swelling (Kirschbaum, 1973). No changes were observed in the groups that were given *Moringa* extract with commercial and prepared nanoparticles possibly because *Moringa* has a high content of antioxidants that suppress lead-induced damage (Al-Malki and El Rabey, 2015).

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