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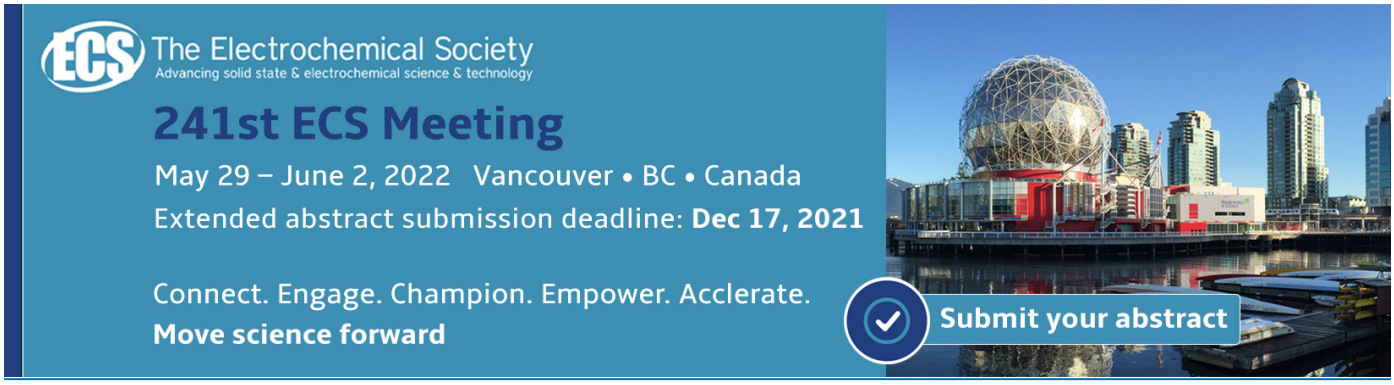

Effects of *Moringa oleifera* seed extract on PbNP- Toxicity induced in rats

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Abstract. This work was conducted to study the protective role of Moringa oliefera seeds extract (MSE) against the toxic effect of commercial lead nanoparticles (CPbNP) in rats. Twenty eight albino male rats were divided into four groups (seven animals in each one) as follow: (A) control group, (B) 160mg/kg body weight per day (b. wt./d.) of M. oliefera (MSE)seed extract, (C): 5mg CPbNP /kg b. wt./d. and (D): MSE + CPbNP . The experimental period was 45 days. Blood was drawn for protein and enzyme estimation at the end of the period of exposure. The rats were dissected and the liver, kidney, spleen and muscle were isolated for Pb estimation. Results showed that the levels of bioaccumulated lead, serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, malondialdehyde were significantly increased in exposed animals to CPbNP ($P < 0.05$). In contrast, there was a significant reduction of total proteins, albumin, glutathione, superoxide dismutase enzyme and catalase enzyme activities ($P < 0.05$) in comparison to control. But the administration of the CPbNP with MSE, all the above biochemical changes were improved significantly. It is concluded that CPbNP has a significant toxic effect on rats, and that seed extract of M. Oleifera can reduce it.

Keywords. *Moringa oliefera*, Toxicity, Lead Nanoparticles, Antioxidants, liver functions.

1.Introduction

Medical plants are traditionally used in treatments of different healthy cases because it has active biological materials. A significant number of researches have been done to using extracts from medicinal plants as preventive agents against specific pathogens or against xenobiotic toxicity [1, 2, 3, 4]. *Moringa* belongs to the Moringaceae family and spread in Africa, especially in Ethiopia, Kenya, and Sudan. It grows in tropical areas, originally in India, but known in Africa [5]. Each part of the Moringa has a lot of essential components. For example, the leaves contain minerals [6], vitamins [7, 8]. In addition to anti-cancer agents (such as glucosinolates, isothiocyanates), phytochemicals such as terpenoids, flavonoids, alkaloids and sugar reduction [9]. According to [10], *Moringa* leaves have significantly higher antioxidant contents. Other authors have reported the leaves' antioxidant potential [11, 12]. The study of [13] observed that folate's bioavailability from *Moringa* leaves using the rat model was 82%, suggesting that the *Moringa* leaves can be a potential dietary folate source. *Moringa* seeds are also found to have significant biological activity. Powder of seeds was investigated for its

antidiabetic activity in induced diabetic rats due to the presence of glucomoringin, phenols and flavonoids [14]. Ethanol extract of *Moringa* seed showed antifungal activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton xoccosum* and *Microsporum canis* [15]; while the water extract of Moringa seed kernel possessed potent antioxidant activity [16].
Several studies were found that the seeds of *Moringa* have a protective effect against toxicants, prevented the elevation of triglycerides, glucose, and urea in rats exposed to arsenic [17], worked as a prevention against the toxic effect of mercury on rat testicular function [18], can act against liver damage caused by CCl4 and fibrosis in rats, hepatoprotective action against hepatocellular harm caused by DMBA in mice [19], alleviates (TiO2-NPs)-induced cerebral oxidative damage, and increases cerebral mitochondrial [20]. Lead is a toxic metal to the environment and organisms. Mining, smelting, refining, recycling, lead paint, and lead gasoline are significant sources. Children are sensitive to lead's poisonous effects and can suffer different health effects, particularly the brain and nervous system's development. Lead also causes harm in adults, including blood pressure, liver and kidney damage, miscarriage and stillbirth [21]. Lead nanoscale particles (PbO-NPS) may be released into the surrounding air and water during different activities, increasing the possibility of exposure to it. The high toxicity of nanoparticle raised concerns about particles impact with their nanoscale on human and animal health [22]. The study of [23] reported that in the exposed rats to lead sulfide nanoparticles caused changes in the fatty acid of lipids of the liver by increasing arachidonic fatty acid and reduction of the stearic fatty acid content. Long exposure of rats to lead sulfide nanoparticles had a harmful effect. They led to significant body and liver weight changes, total protein, albumin, glucose, lipids, triglycerides in blood serum, and dystrophic changes in the liver [24]. The exposure of mice to lead oxide nanoparticles (PbO-NPS) caused a formation of clusters inside the cytoplasmic vesicles in the lung. It was demonstrated that the liver, kidney, spleen, and brain were as tissue-specific subcellular processing [25]. This research was undertaken to determine the protective role of *Moringa oleifera* seed extract's against commercial lead nanoparticle toxicity in rats.

2.Materials and Methods

2.1. Plant material

Moringa oleifera seed was obtained from the Iraqi National herbarium (Baghdad) on 1-April-2019, after being picked from the trees, dried and crushed to be powder by using a Laboratory Grinder.

2.2. Moringa oliefera seed Extraction

Moringa seed was extracted by using the Soxhlet device in solvent acetone/ethanol (1:1). A 300 grams of the sample was used. The extraction process continued for 72 hours, and then the extract concentrated by using a vacuum rotary evaporator [26].

2.3. Commercial lead Nanoparticles

The nanoscale powder was obtained from NANOSHEL company and was less than 100 nm in diameter. Lead Oxide Nanopowder was dissolved in distilled deionized water using a sonicate device to prevent nanoparticles' aggregation [27].

2.4. Experimental animals

The adult white male Swiss Wistar Rats (*Rattus norvegicus*) were used in this experiment. It's were brought from the College of Veterinary Medicine, University of Tikrit, Iraq-with ages ranging between 12-14 weeks and with an average weight of 250 grams. The animals were reared under standard laboratory conditions (24 ± 1° C, 12h light and 12h dark, fed freely).

2.5. Design of the experiments

Article I. Four groups of animals were used with seven rats in each group. *Moringa* and commercial lead were given to the rats following [28] method. Group A or the control group was given distilled water. Group B was treated with 160 mg/kg body weight per day (b. wt./d.) *Moringa* seed extract (MSE) through the stomach (24Aleksiichuk et al., 2018). Group C was injected with 5 mg/kg commercial nanoscale lead (CPbNP.) via peritoneum [29]. Group D was administered with *Moringa* extract and commercial lead nanoparticles (CPbNP).

2.6. Animal dissection

After the end of the exposure period, the rats were left untreated for two days; on day 3, they were anesthetized with chloroform. Afterward, they were dissected, and their liver, kidneys, spleen and muscles were removed and digested for lead estimation. The blood was drawn from the animals by stabbing their hearts. The serum was separated by a centrifuge (3500 rpm) for 10 minutes to obtain blood serum, keeping in the freezer at -20° C until conducting chemical tests.

2.7. Determination of lead concentrations in tissues

The accumulated lead in rats' organs was determined using a Flameless Atomic Absorption spectrophotometer (Analytik Jena-Germany), following the method described by [30]. One gram was digested by 2.5 ml of HNO_3+0.5 ml $HCLO_4$ in a conical flask (100ml). The flasks were left for an hour at room temperature, then placed on Hotplate at 100° C until the appearance of red fumes, then the temperature was raised to 200 °C, until the formation of white vapor. The remaining yellow color liquid was dissolved in nitric acid and used for lead detection after suitable dilution.

2.8. Biochemical tests

Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) activities, total protein and albumin concentrations were determined by using the Spanish origin Biosystem A15 automated device. GSH was measured according to [31]. SOD and CAT activities were determined by Using the Elab science Analysis Kits. MDA was measured according to the method described by [32].

2.9. Statistical analysis

Statistical analysis of the results by using the statistical program SPSS (V.21), as the statistical analysis included calculating the mean and the standard error. The complete random design was used, and the mean of the coefficients was compared using a modified Least Significant Difference test at a 0.05 significant level.

3.Results

Rats were treated with 160 mg/kg b.wt./d. of seed extract of *M. oliefera* for 45 days. All rats in this group remain healthy. They did not show any significant changes in their behaviour or all studied parameters (accumulated lead, the activity of enzymes and concentration of proteins). The bioaccumulated lead was estimated in the liver, kidney, spleen and muscle of rats (Table 1). The lead concentrations in the control animals (group A) and animals, which given *Moringa* seed extract MSE (Group B) were between 5.1-8.4mg/kg in all tested organs. Its concentrations in animals exposed to commercial lead nanoparticles CPbNP (group C) in all tested organs were between 25.3 -30.8 mg/kg, which revealed a highly significant difference in comparing with groups A and B ($P \leq 0.05$). whereas in rats treated with MSE+CPbNP (group D) was 12.5 and 16.5 mg/kg, which differs significantly

(P≤0.05) in comparison with other groups. The liver's accumulated lead was the highest among other organs in different groups except for the animals' liver treated with *Moringa*.

Table 1. Lead bioaccumulation in the organs of rats. A (control), B, MSE (*Moringa* Seed Extract), C, CPbNP (commercial lead nanoparticles).

Organs / **Groups**	Liver (mg/kg)	Kidney (mg/kg)	Spleen (mg/kg)	Muscle (mg/kg)
A (Control)	8.4043±0.46570^{b}	6.8893±0.49005^{a}	6.3002±0.52075^{ab}	6.4960±0.55923^{ab}
B (MSE)	5.3960±0.36341^{a}	6.4565±0.37649^{a}	5.5837±0.10814^{a}	5.1599±0.18794^{a}
C (CPbNP)	30.8166±1.12046^{e}	25.3794±1.21682^{c}	25.7768±0.24178^{d}	25.8280±0.33186^{d}
D (CPbNP+MSE)	16.8850±0.89757^{c}	13.7378±0.76439^{b}	13.2402±0.45598^{c}	12.5153±0.24273^{c}

Data are represented as mean ±SE n=7, P≤0.05. Different letters refer to significant differences among the treatments.

Table (2) showed the concentrations of total serum proteins, albumin and the enzymes serum ALT, AST and ALP. By comparison, to control (group A), the CPbNP group (C) displayed significantly elevated levels of serum ALT, AST and ALP, (P≤0.05) but significantly reduced levels of total serum proteins and albumin (P≤0.05). There is an improvement in hepatic function parameters observed in the PbNP +MSE (group D).

Table 2. The result of commercial lead nanoparticles and seed extract of *Moringa* on serum markers of liver damage. A(control), B, MSE (*Moringa* seed extract), C,CPbNP (commercial lead nanoparticles), D, (MSE+CPbNP).

Parameters / **Groups**	AST (U/L)	ALT (U/L)	ALP (K.A.U./100ml)	Total protein (g/dl)	Albumin (g/dl)
A (Control)	47.2857±1.44279^{a}	50.7143±1.42619^{a}	563.0000±35.6517^{ab}	52.4286±2.31822^{c}	36.9286±2.65570^{c}
B (MSE)	50.0000±2.27826^{a}	48.2857±2.02031^{a}	497.2857±40.0896^{a}	49.8571±3.69960^{c}	32.7857±3.57547^{bc}
C (CPbNP)	64.5714±2.53412^{d}	61.3571±2.57902^{c}	690.1429±20.0674^{c}	38.5714±1.58651^{a}	21.6143±1.00058^{a}
D (CPbNP+MSE)	56.0714±1.32030^{bc}	52.2143±1.21429^{ab}	596.1429±5.70893^{b}	50.7143±2.17906^{c}	25.4286±1.14657^{ab}

Data are represented as mean ±SE n=7, P≤0.05. Various letters refer to significant differences among the treatments.

The levels of the antioxidants GSH, SOD, CAT and MDA were estimated (Figure 3). A significantly elevated level of MDA was found in the PbNP exposed animals (group C) in comparison with control animals (group A) (P≤0.05), but significantly reduced levels of GSH, SOD and catalase (P≤0.05). *Moringa* revealed improvement changes in oxidative stress markers in the MSE+PbNP (D group).

Table 3. The results of commercial lead nanoparticles and seed extract of *Moringa* on oxidants parameters. A(control), B, MSE (*Moringa* seed extract), C,CPbNP (commercial lead nanoparticles), D, (MSE+CPbNP).

Parameters / **Groups**	CAT (U/ml)	GSH (µmol/L)	SOD (U/ml)	MDA (µmol/L)
A (Control)	2.3583±0.16137^{a}	20.4239±1.19882^{c}	208.8857±23.1697^{b}	7.0765±0.33562^{cd}
B (MSE)	2.4417±0.20276^{a}	21.1656±0.47940^{c}	211.0000±4.54475^{b}	7.2921±0.30198^{d}
C (CPbNP)	6.2333±0.83479^{c}	11.7614±2.59223^{a}	90.7286±2.93474^{a}	6.1765±0.03419^{a}
D (CPbNP+MSE)	2.5166±0.33530^{a}	16.8125±2.61772^{bc}	142.7857±48.8149^{ab}	6.7312±0.04879^{bc}

Data are represented as mean ±SE n=7, P≤0.05. Different letters refer to significant differences among the treatments.

4.Discussion

This study aimed to investigate the protective role of *Moringa* seed extract (MSE) against the toxicity of commercial lead nanoparticles (CPbNP). The lead was found to be accumulated in different organs of rat [33]. The accumulation was mainly in the liver, led to toxic effects, enzymes inhibition, oxidative stress [34]. In this investigation, rats were treated with lead nanoparticles (CPbNP). The organs: liver, kidney, spleen and muscles accumulated significant concentrations of lead. The liver got a high concentration of lead (CPbNP group), but *Moringa* seed extract in combination with lead (CPBNP+MSE- group D) reduced accumulated metal to about a half. This reduction may be due to the *Moringa* ability to bind with toxic metal ions and form complex structures out of the body, as in chelating agents [35]. The present study revealed that exposed rats to CPbNP exhibited higher activities of serum ALT, AST and ALP and reduced levels in total serum proteins and serum albumin in comparison to control, which may indicate the cellular leakage and hepatic cell membranes functional disability. These results and conclusions were in agreement with those obtained by the effects of MSG [36, 37, 38], 4-tet-acetyphenol [39] and acetaminophen [40]. The ameliorative alterations observed in the accumulated lead, hepatic enzymes ALT, AST, ALP, total protein and albumin in rats treated with PbNP in combination with seed extract of *Moringa,* corroborating other studies' results [41, 42]. The cause for cellular toxicity may be due to lipid peroxidation in the presence of PbNP. This study reported that the PbNP group had significantly higher serum MDA levels ($P<0.05$) and considerably lower levels of GSH, SOD and catalase ($P< 0.05$). Thus, high levels of lipid peroxidation may be deduced to damage the hepatic tissue and inhibit the capacity of antioxidants to remove excess ROS output [43]. The obtained results were in agreement with [41], where that *M. oleifera* increased antioxidant enzymes' activity, improving the condition of liver antioxidants, while neutralizing the hepatotoxic effect triggered by paracetamol. Our study is also consistent with [44], who found that *Moringa* seed extract can neutralize acetaminophen's hepatotoxic effects. As a result, the current study presents evidence that the acetonic-alcoholic *Moringa oliefera* seeds extract provides effectively, protection against the changes caused by commercial lead nanoparticles in rats.

5.References

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