Scientific Journal for Damietta Faculty of Science 8 (1) 2018, 11-18 ISSN Print 2314-8594
ISSN Online 2314-8616

Protective and Therapeutic Effects of Moringa Oleifera Against Toxicity of Lead Chloride

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Received: 8 February 2018 /Accepted: 25 March 2018
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Abstract

The present study aimed to evaluate the protective and therapeutic effects of *Moringa oleifera* leaves against toxicity induced by lead chloride in albino rats male. Thirty-six albino rats weighing about 182.8 gm were divided into 6 groups each of 6 rats. Control group, left without treatment. The lead chloride group treated orally with 200 mg lead chloride daily for 30 days. The *Moringa oleifera* group treated orally with 200 mg *Moringa oleifera* daily for 30 days. Lead + *Moringa oliefera* group (200 mg lead chloride + 200 mg *Moringa oliefera*). Therapeutic group (200 mg lead chloride followed by 200 mg *Moringa oleifera*) and protective group (200 mg *Moringa oleifera* followed by 200 mg lead chloride). At the end of the experiment, both kidney and liver function tests were assayed as well as catalase and SOD enzymes activity and MDA level. The activity of ALT and the levels of bilirubin, urea, creatinine, cholesterol, ammonia, urea and triglycerides were elevated after lead administration compared with the control and protective groups ($p<0.05$) but the level of albumin of the same group was decreased compared with the control group. In protective group, the level of MDA was significantly decrease compared with that of the lead group. On the other hand, the activities of SOD and catalase were significantly increase in the protective group as well as other treated groups compared with the control group ($p<0.05$). In conclusion, treatment with MO leaves can protect liver and kidney against lead toxicity.
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Keywords: Moringa oleivera, Lead, Liver, Kidney, Rats.

Introduction

Lead is considered as one of the major environmental pollutants and is amongst four metals that have the most detrimental effects on human (Karrari etal, 2012). It is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans (Flora etal., 2016), including central and peripheral nervous systems (Bressler etal., 1999), haemopoietic system (Lanphear et al.,2000), cardiovascular system (Khalil et al.,1993) ,kidneys (Abdel Moneim et al., 2011), liver (Omotoso et al.,2015) and male (Lancranjan et al., 1975) and female reproductive systems (Ronis, etal., 1998). Lead interferes with bio-systems by alterations in their molecular interactions, signaling processes, and ultimately cellular function (Skerfving and Bergdahl, 2007).

Lead has been linked with high incidence of renal dysfunction with attendant glomerular and tubulointerstitial changes, resulting in chronic kidney disease, hypertension as well as hyperuricaemia (Rastogi, 2008).

Studies have shown that lead exposure to laboratory animals raised lipid peroxidation or lowered antioxidant defense mechanism (Bokara et al., 2008; Adegbesan and Adenuga, 2007). Some researchers further showed that the level of lipid peroxidation has a direct relationship with lead concentration in brain regions (Saxena and Flora, 2006) and in liver of lead exposed rats (Sandhir R, Gill KD, 1995).

Natural plants have drawn much attention for their pharmacological effects in the treatment and prevention of various diseases due to their high biocompatibility, low toxicity, and potential biological activity. Among them, *M. oleifera* is known to be important for the synthesis of useful drugs (Wadhwa, ET AL., 2013) that can serve medicinal purposes; they also have important agricultural, commercial and economical values.

Almost all the parts of *MO* are extensively used for the treatment of inflammation, (Mahajan et al., 2008) cardiovascular and liver disease (Omotoso et al., 2015) hematological and renal function (Gupta et al., 2005) and metal intoxications including cadmium (Toppo et al., 2015) and lead (Onah et al., 2016). The present study was conducted to investigate the effects of aqueous leaf extract of *Moringa oleifera* as hepato-renal protective agent against lead toxicity in rats.

Materials and Methods

All chemicals were purchased from the Scientific Office in Damietta, Egypt.

Plant Extraction

Moringa oleifera leaves were obtained from the Agricultural Research Center in Cairo, Egypt.

Preparation of extract

The leaves were dried then crushed into coarse powder. About 200 mg from the powder were soaked in 18 ml distilled water for about 24 hours, then filtered and the desired volume was administered orally.

Experimental animals

Thirty-six albino rats (weighed about 182.81 ± 6.6 gm) were obtained from Mansoura University Lab. The experimental rats were housed in the animal house in Zoology Department, Faculty of Science, Damietta University, New Damietta, Egypt. They were housed in plastic cages under controlled temperature and allowed to adapt for two weeks. Liscence for animal handling.

Experimental design

6 groups, six rats per each group assigned as followed:

Control group: left without treatment for 30 days.

Lead chloride group: treated with 200 mg orally lead chloride daily for 30 days.

***Moringa oliefera* group:** treated with 200 mg orally *Moringa oliefera* daily for 30 days.

Lead + *Moringa oliefera* group: treated with 200 mg orally lead chloride + 200 mg orally *Moringa oliefera* daily at the same time for 30 days.

Therapeutic group: was treated with 200 mg orally lead chloride daily for 30 days followed by 200 mg orally *Moringa oliefera* daily for another 30 days.

Protective group: was treated with 200 mg orally *Moringa oliefera* daily for 30 days followed by 200 mg orally lead chloride daily for another 30 days.

Haematological measurements

At the end of experiment, rats were sacrificed using chloroform and blood samples were collected directly form heart of each rat; one part of blood was collected into heparinized tubes for haematological determinations. The other part was collected without anticoagulant agent to prepare serum. The collected serum was used for liver and kidney functions parameters by using automated analyzer and available commercial kits.

Statistical analysis

The differences between the groups was estimated by the statistical analysis using one-way anova and Tukey tests. A p value less than 0.05 was considered significant.

Results

Physiological studies

Haematological Parameters

Table 1 shows the activity of haematological parameters of male albino rats. WBCs was significantly higher in Therapeutic group and

Protective group than groups control, *Moringa olievera*, Lead chloride and (M + Pb), (P<0.006). In addition, Monocytes count was significantly higher in Therapeutic group and Protective group than Lead chloride, (P <0.05). On the other hand, Lymphocyte was significantly lower in group *Moringa olievera* and *Moringa olievera* + Lead chloride than groups Therapeutic group and Protective group; (P<0.01).

Liver & Kidney Functions :

Table 2 shows the serum activity of liver and kidney enzymes of male albino rats.
ALT activity was significantly higher in Therapeutic group compared to all other groups (P <0.0004), While Albumin concentration was significantly higher in Therapeutic group than control group, *Moringa olievera* and (M + Pb), (P <0.02). Ammonia activity was significantly higher in Therapeutic group than all other groups except control group, (P<0.04).While Urea was significantly higher in (M + Pb) than all other groups, (P <0.04).On the other hand, Creatinine concentration value was significantly lower in group Protective group than *Moringa olievera* and Therapeutic group, (P <0.04).
While Cholesterol concentration value was significantly lower in (M + Pb) than all other groups, (P <0.04).

Antioxidant Enzymes Studies :

Table 4 showed antioxidant enzymes activity of male albino rats. MDA activity was significantly higher in Protective group than groups control, *Moringa olievera* and (M + Pb), (P <0.04).
Also both SOD and CAT activity was significantly higher in *Moringa olievera*, Lead chloride, (M + Pb), Therapeutic group and Protective group than control group, (P <0.002 and <0.004, respectively).

Table (1): Haematocrit (PCV), haemoglobin (Hb) and haematimetric indices of albino rats treated with lead chloride only and / or *Moringa oleifera* for a period of 30 days.

Groups / Parameters	Control (n=6)	*Moringa olievera* (n=6)	Lead chloride (n=6)	*Moringa olievera* + Lead chloride (n=6)	Therapeutic group (n=6)	Protective group (n=6)	P value
Hb (g/dl)	8.3±0.5	8.9±0.4	9.8±0.4	9.2±0.5	9.7±0.8	10±0.9	NS
RBCs (10^6/µl)	2.4±0.2	2.8±0.2	2.9±0.3	2.5±0.2	3.2±0.3	3.4±0.4	NS
WBCs (10^3/µl)	2.7±0.3 [a]	2.7±0.3 [a]	3.4±0.1 [a]	3.1±0.3 [a]	5.5±0.5 [b]	4.9±0.5 [b]	<0.006
Lymphocyte (%)	61±11.1 [ab]	50.8±7.5[b]	27.8±3.2[b]	51.4±10.6[ab]	73±8.5[a]	75.4±11.2[a]	<0.01
Monocytes (%)	6±0.7	4.7±0.6 [ab]	4.3±0.4[b]	4±0.4 [ab]	4.7±0.3 [a]	3.6±0.24 [a]	<0.05
Eosinophil (%)	2.7±0.3	2.7±0.4	2±0.5	2.7±0.4	4.2±0.5	3±0.8	NS

Values are means ± S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, P<0.05). NS, non-significant.

Table (2): Liver functions, Kidney functions and lipid profile of male albino rats treated with lead chloride only and / or *Moringa olievera* for a period of 30 days.

Groups / Parameters	Control (n=6)	Moringa olievera (n=6)	Lead chloride (n=6)	Moringa olievera + Lead chloride (n=6)	Therapeutic group (n=6)	Protective group (n=6)	*P value
ALT(U/ml)	41.3±2.2[a]	54.7±3.2 [a]	52.3±7.3 [a]	46.3±3.2 [a]	89.8±8.5[b]	58.8±1.7 [a]	<0.0004
AST(U/ml)	22.7±2.5	24±1.8	21±1.7	21.8±1.5	19.8±1.7	21.2±1.4	NS
TP(gm/dl)	5.3±1.7	10.3±0.7	4.73±0.55	7.7±0.6	8.4±0.9	8.9±0.3	NS
Albumin (gm/dl)	2.6±0.1 [ab]	1.6±0.4 [b]	2.34±0.19 [ac]	2.5±0.2 [ab]	3.8±0.2 [c]	3.1±0.3 [ac]	<0.02
Ammonia (gm/dl)	114±35.8[ab]	111.8±32.3[b]	99.2±19.3 [b]	67.5±15.8 [b]	209±57.9[a]	83.7±27.1 [b]	<0.04
Urea (mg/dl)	92.7±12.4 [a]	176.6±48.9[ab]	366.7±28.3[b]	546±60.4[c]	179.8±79.8[a]	79.1±3.4[a]	<0.04
Uric Acid (mg/dl)	4.5±1.2	3.9±0.5	4.3±0.9	4.6±0.7	4.3±0.5	4.5±0.7	NS
T. Bilirubin (mg/dl)	2.4±0.5	3.9±0.1	4.7±0.8	3.5±0.6	3.4±0.6	4.5±0.7	NS
Creatinine (gm/dl)	2.5±0.3 [ab]	2.9±0.3 [a]	2.8±0.3 [ab]	2.7±0.3 [ab]	3.2±0.3 [a]	2±0.2 [b]	<0.04

Values are means ± S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, P<0.05). NS, non-significant.

Table (3): lipid profile of male albino rats treated with lead chloride only and / or *Moringa olievera* for a period of 30 days.

Groups / Parameters	Control (n=6)	Moringa olievera (n=6)	Lead chloride (n=6)	Moringa olievera + Lead chloride (n=6)	Therapeutic group (n=6)	Protective group (n=6)	*P value
Cholesterol (mg/dl)	294.4±28 [a]	237.1±39.2[a]	214.1±15.1[a]	128.1±13.4[b]	224±44.6 [a]	254.9±44.3[a]	<0.04
Triglycerides (mg/dl)	91.2±9.8	110.4±15.6	65.9±7.4	84.5±15.3	103.8±25.7	83.3±14.3	NS
Glucose (gm/dl)	92.9±9.6	101.7±0.13	95.1±1.8	94.6±6.5	102.5±1.5	89.9±6.6	NS

Values are means ± S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, P<0.05). NS, non-significant.

Table (4): Lipid peroxidation, superoxide dismutase and catalase activity of male albino rats treated with lead chloride only and / or *Moringa olievera* for a period of 30 days

Groups / Parameters	Control (n=6)	Moringa olievera (n=6)	Lead chloride (n=6)	Moringa olievera + Lead chloride (n=6)	Therapeutic group (n=6)	Protective group (n=6)	*P value
MDA (nmol/ml)	17.2±5.8 [ab]	8.5±2.1[b]	40±7.7 [ac]	12.4±2.8 [b]	34.8±8.5 [ac]	31.08±9.85 [c]	<0.04
SOD (gm/dl)	92.1±33.8[a]	229.2±20.8[b]	260.4±10.4[b]	218.8±31.3[b]	239.6±25.1[b]	262.5±23.4[b]	<0.002
CAT (U/L)	563.1±54 [a]	988.4±3.6 [b]	1017.4±7.9[b]	1033±4.6 [b]	932.7±27.1[b]	938.7±28.8[b]	<0.004

Values are means ± S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, P<0.05).

DISCUSSION

The present study evaluates the protective and therapeutic effects of *M. oleifera* on Lead-induced oxidative stress in blood cells, liver and kidneys as the most prominent target organs of the toxicity of these metals.

Moringa oleifera is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Various parts of this plant, such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine (Anwar et al., 2007).

Lead is a common environmental toxic heavy metal that has no known biological function in the body and also it is known to induce a broad range of physiological, biochemical and behavioral dysfunctions in laboratory animals and humans, including affecting the central and peripheral nervous system, hematopoietic system, cardiovascular system, kidneys, liver and reproductive system (ATSDR, 2007).

The liver is considered the principal target organ for lead toxicity, the activity of ALT and AST are sensitive indicators of acute hepatic necrosis (Modesto et al., 2013).The liver is a vital organ with a wide range of functions such as detoxification, protein synthesis and production of biochemical necessary for digestion. It is actively involved in many metabolic functions and is the frequent target for a number of toxicants (Flora et al., 2003). Any hepatic damage is associated with distortion of these functions (Wolf, 1999). The absence of consistent hepatoprotective drugs in allopathic medicine, herbs demonstrate therapeutic functions in treatment of several liver damage and disorders (Buraimoh et al., 2011).

Pb hepatotoxicity has been related to the elevation in the levels of serum liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Omobowale et al., 2014) and alterations in hepatic cholesterol metabolism (Abdou and Hassan, 2014). This elevated may be due to hepatocellular necrosis, which caused increase in the permeability of the cell membrane resulting in the release of transaminases in the

blood stream (Naik, 2010).

Exposure to lead for a short period affects the liver (Sivaprasad et al., 2004). In the present study, there was a significant increase in ALT level in therapeutic group compared to all other groups. Our findings are parallel with those obtained by Omobowale et al., (2014) and Onah et al., (2016) who reported increased liver enzymes after lead chlorides administration, which is an indication of impaired liver function, hepatocyte or biliary epithelial necrosis, compromise of hepatocyte membrane integrity, and cholestasis (Adaramoye et al., 2008). Also, there is non-significant decrease in AST level of lead injected rats then treated with *Moringa oleifera.* In addition, no significant elevation observed in total protein level, but Alain et al., (2016) found that total serum protein level decreased significantly in the lead-exposed group compared to control and rats treated with MO or the combination of lead and MO showed no significant variation in total protein level compared to control group.

Albumin decreased significantly in Mo group and the combination of lead and MO group compared to all other groups, which accordance with (Alain et al., (2016)) who found that the administration of lead chloride to Wistar rats induce a decrease in albumin levels.

The present study showed non-significant increase in total bilirubin and blood glucose level of lead injected rat group and MO group or association of both lead and MO when compared to control, which accordance with (Alain et al., 2016) who found similar to our findings. But Onah et al., (2016) who stated that total bilirubin levels increased significantly after 6 weeks of lead administration compared to their therapeutic group and other groups.

Cholesterol and triglyceride are the two major blood lipids. Cholesterol show significant decrease in *Moringa olievera* + Lead chloride group compared to all other groups, this result is in contrast with (Alain et al., (2016)) and (Hassan and Jassimm, 2010) who reported that no significant variation in the serum cholesterol level was noted in the experimental groups compared to the control. On the other hand, Blood triglyceride levels non-significant decrease in protective group, lead chloride group and lead chlorid& *MO* group compared to control group, also it shows that non-significant increase in therapeutic group and *MO* group compared to control group. These results in contrast with (Hassan and Jassimm, 2010) who showed that the triglyceride levels significantly decreased in lead group.

Kidney is a target organ for lead toxicity. The toxic effects of Pb on the kidney appear to be primarily localized in the kidney tubule and are manifested as excessive urinary excretion of amino acids, glucose and phosphate, natriuresis, kaliuresis and intranuclear bodies inclusion (Jadhav et al., 2007). Lead affects the excretion function of nephrons, the structural and functional unit of the kidneys (Sivaprasad, et al., 2004a).

The present study showed that ammonia level was significant increase in therapeutic group compared to all other groups except control group. Also, urea was significantly increased in *MO* &lead chloride group than all other groups. On the other hand, creatinine was significantly decrease in Protective group compared to *Moringa olievera* and Therapeutic group. These findings were in agreement with Onah et al., (2016); Alain et al., (2016) and Lakshmana et al. (2013) who reported that *MO* was found to be an effective herbal medicine in animal models of renal failure.

Also the uric acid level show non-significant change was found between treated groups and control. The current results clearly indicated that treatment with *MO* shows protective role against renal cytotoxicity-induced by lead toxicity.

The present study, MDA shows significantly increase in Protective group compared to control, *Moringa olievera* and *MO* & Lead chloride group, these findings were in agreement with (Onah et al., 2016) who found that significant increase in lipid peroxidation index (MDA) of liver after the administration of lead when compared to the control group.

The most important consequence of Pb-induced oxidative stress in liver is lipid peroxidation (Flora et al., 2003; Omobowale et al., 2014) that causes the alteration of membrane integrity and fatty acid composition (Lawton and Donaldson, 1991) and is associated with the increase in malondialdehyde (MDA) level in liver (Liu et al., 2012; Xu et al., 2008). Lead has the ability to bind to enzymes such as superox-ide dismutase (SOD) and catalase (CAT) (Patra et al., 2011).

The present study found a significant increase in SOD and CAT levels in all treated groups compared to control group which may be due to the presence of flavonoids such as quercetin and kaempferol, vitamin A, ascorbic acid, which is a potent antioxidant. Ouedraogo et al (2013); Bharali et al., (2003) and Sreelatha et al., (2009) suggesting therapeutic effect (antioxidant effect) of *MO*.

In the blood, haematimetric indices (MCH), and Thrombocytes (TC) and Leucocytes (WBCs)

showed significant increase on lead exposure, but the administration of *MO* restored all the parameters back to controls also RBCS, hemoglobin and hematocrit showed no significant changes, in the contrast to Velaga et al., (2014) who observed significant decrease in lead group, this may be due to Acute bleeding and blood loss, Allergic reactions, Cancer, Chronic kidney failure or another kidney disorder , Exercise, Heart attack, Coronary artery bypass ,Infections, including tuberculosis, Iron deficiency, Vitamin deficiency, Removal of your spleen, Trauma, Burns, Exercise, Hemolytic anemia and Inflammation.

In conclusion, the present study revealed hepato-renal protective activities of *MO* against lead toxicity and throw light on the effects of *Moringa oleifera* leaves powder have antitoxic properties. It has prevented some lead toxicities effect on many biochemical oxidative stress parameters of blood, kidney, liver and brain in albino rats so the *MO* extract is recommended to pharmaceutical industries for further research and possible use in the manufacture of drugs.

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الملخص العربي

عنوان البحث: التأثير الوقائي والعلاجي لنبات المورينجا ضد سمية كلوريد الرصاص

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تهدف الدراسة الحالية الي تقييم التأثير الوقائي والعلاجي لأوراق نبات المورينجا أوليفيرا ضد السمية الناتجة عن كلوريد الرصاص في ذكور الفئران البيضاء. تم تقسيم ٣٦ فأر ألباني وزنها حوالي ١٨٢,٨ جم الي ٦ مجموعات كل منها ٦ فئران. المجموعة الضابطة أعطيت النظام الغذائي العادي والماء لمدة شهر. مجموعة كلوريد الرصاص تمت معالجتها ب ٢٠٠ملغم كلوريد رصاص يوميا لمدة شهر عن طريق الفم. مجموعة المورينجا أوليفيرا تمت معالجتها ب٢٠٠مل جم مورينجا اوليفيرا يوميا عن طريق الفم لمدة شهر. كلوريد الرصاص مع المورينجا أوليفيرا تمت معالجتها ب (٢٠٠ملجم كلوريد الرصاص + ٢٠٠ملجم مورينجا أوليفيرا) يوميا عن طريق الفم لمدة شهر. المجموعة العلاجية (٢٠٠ملجم كلوري الرصاص تليها ٢٠٠ملجم مورينجا أوليفيرا). المجموعة الواقية (٢٠٠ملجم مورينجا أوليفيرا تليها ٢٠٠ملجم كلوريد الرصاص). في نهاية التجربة تم اختبار كل من اختبارات الكلى والكبد وكذلك نشاط أنزيم الكتاليز وSOD وMDA. تمت زيادة نشاط ALT ومستوي البليروبين واليوريا والكريتيانين والكوليسترول والامونيا والدهون الثلاثية بعد حقن الرصاص مقارنة بالمجموعة الضابطة والواقية ($P<0.05$) ولكن تم تقليل مستوى الالبيومين لنفس المجموعة مقارنة مع المجموعة الضابطة. في المجموعة الواقية كان مستوي MDA انخفض مقارنة مع المجموعة الضابطة. أظهرت أنشطة الكتاليز وSOD زيادة معنوية في المجموعة الوقائية وكذلك المجموعة المعالجة مقارنة مع المجموعة الضابطة ($P<0.05$). في الختام تبين أن العلاج بأوراق نبات المورينجا أوليفيرا يمكن ان تحمي الكبد والكلي ضد سمية كلوريد الرصاص.