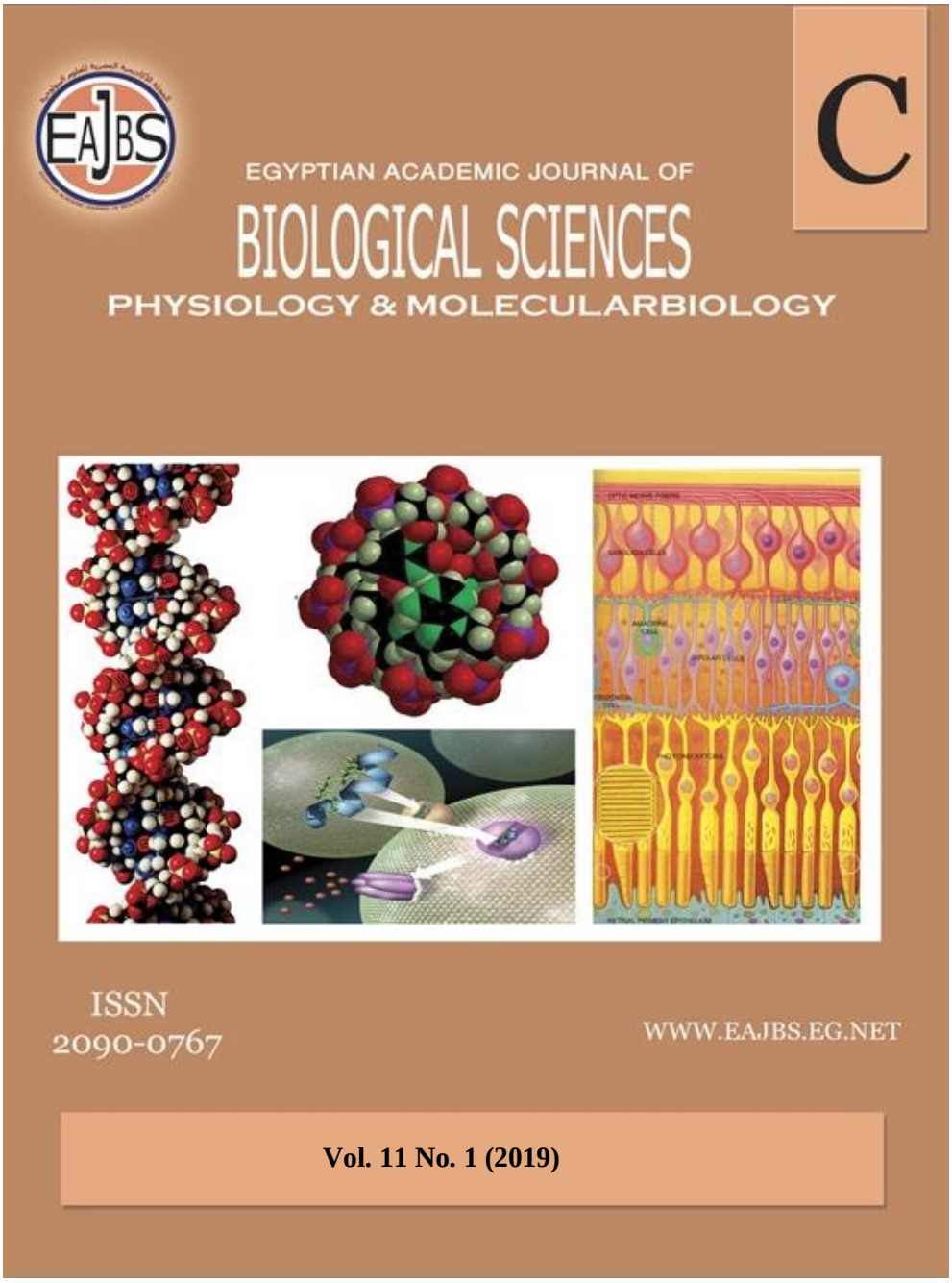


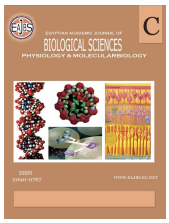
Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

C. Physiology & Molecular Biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidate important biological, chemical, or physical mechanisms of broad physiological significance.

http://eajbsc.journals.ekb.eg/

Egypt. Acad. J. Biolog. Sci., 11(1): 93- 104 (2019)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
http://eajbsc.journals.ekb.eg/



Physiological Studies of the Effect of *Moringa olifera* and Vitamin (C) on Hepatotoxicity and Oxidative Stress Induced by Lead Acetate in Male Albino Rats

Abd-El Reheem A. El Shater, Rana A. Ali and Eman M. RephaaI3
Department of Zoology, Faculty of Science, South Valley University
E.Mail : rana.ahmed@svu.edu.eg

ARTICLE INFO
Article History
Received:9/2/2019
Accepted:29/2/2019

***Keywords*:**
Lead acetate, *Moringa Olifera*, Vitamin C, hepatotoxicity, Albino rats

ABSTRACT

Lead is highly toxic naturally occurring element that affects numerous organ systems in humans, the purpose of this study was to investigate the role of Moringa olifera leaves extract and vitamin C in male albino rats against lead acetate toxicity. forty (40) male albino rats were used for the experiment. Group I was administered distilled water, Group II was administered lead acetate only (20mg/kg b.w.), Group III was administered lead acetate (20mg/kg b w) and *Moringa olifera* leaves extract (400mg/kg b.w.) and Group IV was administered lead acetate (20mg/kg b.w.) and Vitamin C (50 mg/kg b.w.). Animals were exposed to treatment once daily for 8 weeks orally. After the last day of treated animals were slaughtered and blood samples collected and serum separated for analyzing serum liver enzymes. Results obtained in this present study revealed a significant increased at ($p<0.05$) in aspartate aminotransferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP),bilirubin and Malondialdehyde (MAD), while showed a significant decreased at ($p<0.05$) in Total protein, Albumin, Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) in group treated with lead acetate only when compared with normal group. However, Effects of *Moringa oleifera* (MO) and Vitamin C on hepatic injury due to lead induced oxidative stress revealed a significant decreased at ($p<0.05$) in aspartate aminotransferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), bilirubin and Malondialdehyde (MAD), while showed a significant increased at ($p<0.05$) in Total protein, Albumin, Glutathione (GSH), Superoxide dismutase (SOD) and Catalase (CAT) when compared with the control group. It is concluded that *Moringa olifera* leaves extract and vitamin C have antioxidant properties.

INTRODUCTION

Lead is the metal that has been associated with past human activities and is considered one of the major pollutants to the environment (Ghorbe *et al.*, 2001). The ingested and absorbed lead is mainly stored in bone and soft tissue, but the highest concentration of lead is found in bone, brain, kidney, liver, spleen, teeth and lung (Mudipalli, 2007). The absorbed lead is conjugated in the liver and passed to the kidney, where a small amount is excreted in the urine and the rest accumulate and interfere with their function in different body organs. (Jarrar *et al.*, 2006).

Plants have played a substantial role in the maintenance of human health, from time immemorial. *Moringa oleifera* is one such multipurpose tree, commonly used in spices and cosmetic oils, and has various medicinal and therapeutic applications.

Moringa oleifera belongs to the Moringacaea family, a fast - growing, drought - resistant tree now distributed throughout the world in the tropics and sub - tropics, with extensive cultivation in Central and South America, Africa, Indonesia, Mexico, Malaysia, the Philippines and India.

Moringa oleifera is considered one of the most useful trees in the world, as nearly every part of the tree can be used for food or has other beneficial properties., Extracts from all parts of the plant also show pharmacological properties, which are recognized by popular use and confirmed by the scientific community. (Oliveira *et al.*, 1999).

In addition, Moringa oleifera's active antioxidant properties, known to include specific plant pigments, include carotenoids such as lutein, chlorophyll, xanthins, alpha-carotene and beta carotene. Quercetin, rutin, kaempferol and caffeoylqunic acids are other isolated phytochemicals of Moringa oleifera with antioxidant potential. Powerful vitamins antioxidant- A, C, E (Aslam *et al.*, 2005).

Ascorbic acid is well known for its antioxidant activity, which acts as a reduction in liquid oxidation reversal agent. When there are more free radicals (reactive oxygen species, ROS) in the human body than antioxidants the condition is called oxidative stress. (McGregor and Biesalski, 2006) .

The aim of this work was to assess the ameliorative effect of Moringa oleifera leaf extract (MO) and vitamin C in order to reduce the toxicity of lead acetate in the liver.

MATERIALS AND METHODS

Materials :

a) Chemicals

1-Lead(II) acetate ($Pb(CH_3COO)_2$), is a white crystalline chemical compound with a sweetish taste. It was obtained from El-Nasr Pharmaceutical Chemical Co.,A.R.E. Laboratory chemical division.

2-Vitamin C , ascorbic acid powdered pure was obtained from the local market

b) Plant Material (*Moringa oleifera*):

Moringa oleifera leaves were purchased from local market.

c) Preparation of Extract of *Moringa oleifera* Leaves:

Moringa oleifera leaves were powdered and 5g of the powder was mixed with 50 ml of 80% ethanol. The mixture was stirred using magnetic stirrer in an air-tight container for about 1 hour and filtered afterward. Resulting Filtrate was evaporated to remove alcohol in a rotary evaporator free residue of sample was weight (500mg) and dissolved in 100ml distilled water to constitute the final extract solution (5mg /ml) (Babu *et al.*, 2003).

d) Animals:

40 adult male Albino rats weighing about (150-200g) were divided into four groups (10 rats/cage) in room temperature, for 2 weeks before starting the experiment, under natural day and night periods and supplied with a balanced stable commercial diet and water.

Methods

1.Experimental Design:

The experimental animals were divided into 4 groups, 10 rats for each group.

Group 1 (Normal group): Normal received orally 1 ml distilled water, daily for 8 weeks.

Group 2 (control group): rats were received Lead acetate (20mg/kg body weight) (Nehru and Kanwar, 2004), daily for 8 weeks**.**

Group 3_(treated group): rats were received lead acetate (20mg/kg body weight) orally, following with oral administration of *Moringa Olifera* (400mg/kg body weight) (Sharida *et al.,* 2012) daily for 8 weeks**.**

Group 4_ (treated group):rats were received lead acetate (20mg/kg body weight) orally, following with oral administration of Vitamin C (50mg/kg body weight) (Mariam *et al.,* 2007) daily, for 8 weeks**.**

At the end of the experiment, all animals were sacrificed and the blood from each animal was transported into clean tubes., then serum was separated by centrifugation and stored at – 80^{o} c for biochemical analysis, liver tissue was collected from animals for biochemical analysis.

Liver Tissue Was Homogenized as Following:

a) Prior to dissection, perfuse tissue with phosphate buffer saline (PBS) solution pH=7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots.

b) Homogenize the tissue in 5-10 ml cold buffer (i.e., 50 mM potassium phosphate, pH7.5 1mM EDTA and 1gm⁄100ml of polyphenyl pyridine) per gram tissue in pastel homogenizer.

C) Centrifuge at 4000 rpm for 15min at 4^{o}c.

d) Remove for assay the supernatant and store it on ice. Freeze the supernatant at - 80oc if not tested on the same day, the sample will be stable for at least one month.

Assessment of Biochemical Parameters and Antioxidants:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to Reitman and Frankel, (1957), The level of Serum Alkaline Phosphates (ALP) was determined by the colorimetric described by (Bolfield and Goldberg, 1971). while albumin was determined according to Doumas *et al.,* (1971), but total protein according to Gornal *et al*., (1949). According to Walter and Gerade (1970), direct bilirubin concentration was also determined in serum, while total bilirubin was determined by Schmidt and Eisenburg (1975). According to Beutler et al. (1963), reduced glutathione (GSH) was determined, while super oxidismutase (SOD) was determined by Nishikimi et al. (1972), but Catalase (CAT) was determined according to Aebi, (1984) and Malondialdehyde (MDA) was determined according to Ohkawa *et al.,* (1979). All mentioned kits were bought from biodiagnostic co. Giza, Egypt.

Statistical Analysis:

All quantitative measurements were expressed as means ± S.D. of control and experimental animals. The data were analyzed using one-way analysis of variance (ANOVA) on SPSS (statistical package for social sciences). Statistical significance was set up $P < (0.05)$.

RESULTS

1- Effect of Oral Administration of Lead Acetate (20mg/kg b.w., daily) on Serum Level of Alanine Transaminase (ALT) or (GPT), Aspartate Transaminase (AST) or (GOT), Alkaline Phosphatase (ALP) , Total Protein , Albumin , Direct Bilirubin and Total Bilirubin Activities in Blood of Males Albino Rats:

As recorded in Table 1, there were a significant increase at ($p<0.05$) in serum Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP),Total bilirubin and Direct bilirubin ,while there was a significant decrease at ($p<0.05$) in serum Total protein and Albumin level when compared with normal animals.

2- Effect of Oral Administration of Daily Doses of *Moringa olifera* (400 mg/kg body weight) on Serum Level of Alanine Transaminase (ALT), Aspartate Transaminase

(AST), Alkaline Phosphatase (ALP), Total Protein, Albumin, Direct Bilirubin and Total Bilirubin Activities in Blood of Male's Albino Rats, Post Administration of Lead Acetate (20mg/kg b.w., daily) for 8 Weeks:

As recorded in table 1, there were a significant decrease at ($p<0.05$) in serum Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP), Total bilirubin and Direct bilirubin, while there was a significant increase at ($p<0.05$) in serum Total protein and Albumin level when compared with control animals but it was not reach to normal animals.

3- Effect of Oral Administration of Daily Doses of Vitamin C (50mg/kg body weight) on Serum Level of Alanine Transaminase (ALT), Aspartate Transaminse (AST), Alkaline Phosphatase (ALP), Total Protein, Albumin, Direct Bilirubin and Total Bilirubin Activities in Blood Of Male's Albino Rats, Post Administration of Lead Acetate (20mg/kg b.w., daily) for 8 Weeks.

As recorded in table 1, there were a significant decrease at ($p<0.05$) in serum Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP), Total bilirubin and Direct bilirubin, while there was a significant increase at ($p<0.05$) in serum Albumin when compared with control animals but it did not reach to normal animals.

4- Effect of Oral Administration of Lead Acetate (20mg/kg b.w., daily) on Malondialdehyde (MAD), Reduced Glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD) Activities in Liver Tissue of Male Albino Rats:

As recorded in table 2, there were a significant decrease at ($p<0.05$) in glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities in liver tissue of rats while there was a significant increase at ($p<0.05$) in Malondialdehyde (MDA) activities in liver tissue of the same rats compared with normal animals.

5-Effect of Oral Administration of Daily Doses of *Moringa olifera* (400 mg/kg body weight) on Malondialdehyde (MAD), Reduced Glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD) Activities in Liver Tissue of Male Albino Rats Post Administration of Lead Acetate (20mg/kg b.w., daily) for 8 Weeks:

As recorded in table 2, there were a significant increase at ($p<0.05$) in glutathione (GSH) and superoxide dismutase (SOD) activities but Catalase (CAT) activities showed a not significant increase at ($p<0.05$) in liver tissue of rats while there were a significant decrease at ($p<0.05$) in Malondialdehyde (MDA) activities in liver tissue of same rats compared with control animal but it not reach to normal animals.

6- Effect of Oral Administration of Daily Doses of Vitamin C (50mg/kg body weight) on Malondialdehyde, Reduced Glutathione, Catalase and Superoxide Dismutase Activities in Liver Tissue of Male Albino Rats Post Administration of Lead Acetate (20mg/kg b.w., daily) for 8 Weeks:

As recorded in table 2, there were a significant increase at ($p<0.05$) in glutathione (GSH) and superoxide dismutase (SOD) activities but Catalase (CAT) activities showed a not significant change at ($p<0.05$) in liver tissue of rats while there was a significant decrease at ($p<0.05$) in Malondialdehyde (MDA) activities in liver tissue of same rats compared with control animal but it did not reach to normal animals.

Table (1): Effect of lead acetate (20mg/kg b.w., orally daily), Moringa olifera (400 mg/kg b.w., orally daily) and vitamin C (50mg/kg b.w., orally daily) on serum level of ALT, AST, ALP, Total protein, Albumin, Direct bilirubin and Total bilirubin activities in blood of males albino rats post administration of lead acetate for 8 weeks.

Groups / Parameters	Group (1) Normal Group Mean±S.D	Group (2) Control Group Mean±S.D	Group 3 (Lead acetate+MO) Mean±S.D	Group 4 (Lead acetate+VC) Mean±S.D
ALT(u/ml)	45 ± 4.2	$63\pm6.9^{+a}$	$58.4\pm47^{+a-b}$	$58.6\pm4.1^{+a-b}$
AST(u/ml)	144.93± 10.6	$189.42\pm12.6^{+a}$	$166.50\pm6.4^{+a-b}$	$176\pm7.4^{+a-b}$
ALP(u/L)	233.86± 20	$33962\pm15.3^{+a}$	$262.11\pm24.5^{+a-b}$	$281.35\pm23^{+a-b}$
Protein(g/dL)	6.67±0.40	5.14 ± 0.37^{-a}	$5.74\pm0.68^{-a+b}$	5.26 ± 0.29^{-a}
Albumin(g/dL)	3.55±0.14	2.70 ± 0.40^{-a}	$3.27\pm0.51^{+b}$	$3.37\pm0.14^{+b}$
Total Bilirubin (g/dL)	0.09±0.063	$0.53\pm0.18^{+a}$	$0.28\pm0.09^{+a-b}$	$0.36\pm0.08^{+a-b}$
Direct Bilirubin (g/dL)	0.09±0.06	$0.29\pm0.03^{+a}$	0.007 ± 0.04^{-b}	0.12 ± 0.15^{-b}

The result presented the mean ± S.D. of 10 rats
+ significant increase at($p<0.05$). a → significantly different from normal rats.
– significant decrease at ($p<0.05$). b → significantly different from a control animal.

Table (2): Effect of lead acetate (20mg/kg b.w., orally daily), Moringa olifera (400 mg/kg b.w., orally daily) and vitamin C (50mg/kg b.w., orally daily) on Malondialdehyde (MAD), reduced glutathione (GSH), Catalase (CAT) and Superoxide Dismutase (SOD) in liver tissue of male albino rats for 8 weeks

Groups / Parameters	Group (1) Normal Group Mean±S.D	Group (1) Control Group Mean±S.D	Group 3 (Lead acetate+MO) Mean±S.D	Group 4 (Lead acetate+VC) Mean±S.D
MDA (nmol/g.tissue)	0.36±0.10	$1.00\pm0.23^{+a}$	$0.50\pm0.006^{+a-b}$	$0.73\pm0.05^{+a-b}$
SOD (U/g.tissue)	0.80±0.10	0.22 ± 0.07^{-a}	$0.34\pm0.100^{-a+b}$	$0.46\pm0.15^{-a+b}$
GSH (nmol/g.tissue)	13.71±1.26	2.80 ± 0.59^{-a}	$7.57\pm0.40^{-a+b}$	$9.11\pm0.79^{-a+b}$
CAT (U/g.tissue)	0.10±0.009	0.094 ± 0.001^{-a}	0.097 ± 0.00^{-a}	0.097 ± 0.00^{-a}

Results are expressed as mean ± S.D. of 10 animals.
+ significant increase at ($p<0.05$).
_ significant decrease at ($p<0.05$).
a ⟶ significantly different from normal.
b ⟶ significantly different from a control

DISCUSSION

In the current results, the elevation in the levels of ALT, AST, ALP, Total bilirubin and Direct bilirubin was noted, while the level of serum albumin and Total protein decreased in rat serum oral administration of lead acetate (20mg / kg b.w. daily). These results agree with the recorded data of Abdel Aal and Abeer, (2008) they reported that lead administration (30 mg/kg body weight of 1/20 th of LD_{50}) orally resulted in increased serum ALP, ALT and AST. This is because lead is known to cause oxidative injury in the liver tissue by producing membrane lipid peroxidation and causing derangement of several biochemical hepatic pathways and energy metabolism (Taki *et al.*, 1985).

Increasing AST and ALT serum activity was most likely a result of the hepatotoxic effect of lead i.e. toxic hepatitis. By ingestion, the lead entering the body is delivered to the liver through the blood circulation portal and smaller portions of the lead "break the liver barrier" and enter the circulation of the body. The accumulated lead in the liver can act by directly damaging the hepatocytes primarily by destroying the cell membrane's permeability, resulting in the release of cellular enzymes increasing their serum values. (Todorvic, *et al.*, 2005). Durgut, *et al.*, (2008) found that, the increased serum activities of AST and ALT are associated with liver damage and or cardiac or skeletal muscle damage.

Scholz *et al.*, (1989) explained the increasing of ALP level in plasma. The increasing of ALP activity in plasma by toxins could be due to mechanical obstruction of bile ducts, failure to excrete the enzyme through relatively narrower bile passages leading to its accumulation and enzyme - level increase in plasma.

From obtained results, there was the elevation of plasma bilirubin, with the administration of lead acetate (20mg/kg body weight). This might be due to the induction of hemeoxygenase, the catabolism of heme from all heme proteins appears to be carried out in the microsomal fraction of cells by a complex enzyme system, hemeoxygenase, which converted heme to bilirubin. This suggestion agrees with (Seddik *et al.*, 2010).

From obtained results, there was a reduction in plasma total soluble protein and albumin levels with administration of lead acetate (20mg/kg body weight). This might be due to the ingestion of lead acetate. These results show that the variation in total protein of plasma was correlated with the changes in albumin value. Murrey *et al.*, (2006) explained the reduction in plasma total soluble protein and albumin levels May be caused by protein biosynthesis inhibition through the specific enzymes in cell processes and low significant excretion of hormones such as triiodothyronine (T3) and (T4) which regulated protein biosynthesis. The significant decrease in serum total protein and albumin can also be recognized to injury of hepatocyte functions resulting in decreased cytochrome P-450 activity and protein metabolism in the liver (Asagba, 2010 and Ibiam *et al.*, 2013).

In the present study, A significant decrease in ALT, AST, ALP, direct bilirubin and total bilirubin was observed during oral administration of daily doses of Moringa olifera (400 mg / kg body weight) while total protein and albumin increased significantly, these results agreed with Verma *et al.*, (2009) Who reported the strong antioxidant properties of Moringa oleifera extracts due to the different

bioactive components found in different solvent extracts. These bioactive antioxidant compounds effectively prevent toxicity induced by hepatotoxin. The reversal of high serum enzyme levels by Moringa oleifera leaf extract after administration of lead can be attributed to the Moringa oleifera's stabilizing ability on the cell membrane, preventing leakage of enzymes (Saalu *et al.,* 2012). The stabilization of the serum total bilirubin level by the administration of Moringa oleifera extract is also a clear sign that the functional status of the hepatic cells is improved (Shah *et al.*, 2013).

Moringa oleifera leaf has to repair effect on the liver due to their nutritional properties such as the presence of essential amino acid like methionine and cysteine and thus boosting the total proteins and albumin level Ekam *et al.,* (2012).

In this study, oral administration of daily doses of vitamin C (50 mg / kg body weight) revealed a significant decrease in ALT, AST, ALP, direct bilirubin and total bilirubin, whereas total protein and albumin increased significantly. These results showed an improvement in the rats treated with vitamin C compared to the control group. The reduction of AST and ALT by vitamin C is in accordance with Rekka *et al.,* (1992), Who found that serum transaminases returned to normal activity with parenchyma tissue healing and hepatocyte regeneration Vitamin C significantly reduced serum transaminases, alkaline phosphatase, bilirubin and hepatic weight, and increased overall protein, albumin and body weight (Santhrani *et al.,* 2012).

The present investigation revealed decrease of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels while there were a significant elevate in Malondialdehyde (MDA) levels in the liver with the administration of lead acetate orally (20mg/kg body weight).

A number of recent studies have confirmed that reactive oxygen species (ROS) may be involved in lead - induced toxicity (Gurer *et al., 2000*). Several antioxidant enzymes and molecules were used in animal and human studies to assess lead - induced oxidative damage. The most commonly used markers in tissues or blood are reduced glutathione (GSH) and catalase (CAT) and modifications in superoxide dismutase (SOD) activity (Khaki *et al.,* 2010). Based on the observation that free radical was induced by lead exposure during pathogenesis processes.

Sivaprasad *et al.,* (2004) suggested that the decrease in (glutathione) GSH concentration May be due to the binding capacity of the lead with the SH group which decreases the GSH levels, thereby interfering with the antioxidant activity. Catalase is another major antioxidant enzyme with heme as the group of prostheses. Lead is known to reduce iron absorption and inhibit heme biosynthesis in the gastrointestinal tract. (Dresel *et al.,* 1954). Decreased catalase activity observed in lead-exposed animals was attributed to the interference of lead by both processes (Sandhir *et al.,* 1995).

Inhibition of SOD activity by lead was also demonstrated in an in vitro study in which the authors indicated that this effect of lead can lead to reduced ROS scavenging and oxidative stress. (Adler *et al.,* 1993).

Gurer *et al.,* (2000) Suggested that elevation of liver MDA after lead acetate which caused prooxidant / antioxidant balance disruption resulting in tissue injury through oxidative damage to critical biomolecules with malondialdehyde accumulation as a lipid peroxidation product.

In this study, oral administration of daily doses of

Moringa olifera (400 mg / kg body weight) showed significant increases in the activity of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in rat liver tissue and decreases the activity of Malondialdehyde (MDA) in same rat liver tissue.

Prasanna and Sreelatha, (2014) stated that *Moringa olifera* leaves extract treatment can act as effective modulators in reducing the toxicity in cells under oxidative stress by enhancing the stimulation of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) that are capable of removing oxygen radicals and their products and/or repairing the damage caused by oxidation stress. *Moringa olifera* leaves extract contains a rich amount of antoxidants (Chumark *et al.,* 2008). A possible explanation for the protective or therapeutic effect revealed in the present investigation of its antoxidant and scavenging properties.

The decreased levels of MDA in rats treated with Moringa oleifera may be due to the high levels of vitamin C and/or iron in the leaves (Aattar., 2006).

Moringa leaves extracts have a powerful antioxidant action against free radicals, prevent oxidative damage to major biomolecules and provide substantial protection against oxidative damage (Sreelatha and Padma, 2009). Bajpai *et al.,* (2005) reported antioxidant activity from the leaves of *Moringa oleifera* because of the founding of kaempferol in the leaves of Moringa

The present study showed a significant increase in glutathione (GSH) in daily doses of vitamin C (50 mg / kg body weight) by oral administration. Superoxide dismutase (SOD) and catalase (CAT) activity in rat liver tissue and reduction of malondialdehyde (MDA) activity in same rat liver tissue. vitamin C has been reported to efficiently scavenge free radicals and contribute to the stability of cell and basal membranes, before initiating lipid peroxidation, (Sies *et al.,* 1992).

The result of increased SOD and CAT activities suggests that vitamin C contains a free radical scavenging activity that may have a beneficial effect on pathological changes caused by O2•− and OH•. The increased activity of SOD accelerates dismutation of $O^{2\bullet-}$ to hydrogen peroxide (H_2O_2) which is removed by CAT (Aebi, 1984). Vitamin C increased the glutathione content, reduced lipid peroxidation in liver samples (Santhrani *et al.,* 2012).

Another mechanism of vitamin C mentioned by (Kadrabova *et al.,*1992) who reported that the vitamin C has been used to support cellular level of GSH because it acts as reducing agent preventing the oxidation of SH-group to non-functional disulphide groups, so the administration of vitamin C would enhance the antioxidant defense system.

Conclusion and Recommendation:

The results demonstrated that *Moringa olifera* leaves extract and Vitamin C showed a good ameliorating role against hepatotoxicity induced by lead exposure, this protection is mediated either by preventing the lead exposure -induced decline of liver antioxidant defense system or by their direct free radical scavenging activity. So the supplementation of Moringa olifera leaves and vitamin C containing several antioxidants may be important in occupationally exhibited workers to low doses of lead in undertakings, for instance, in paints, glass, aesthetic, batteries, shades, and welding. It is urged to be given with treatment traditions for lead lethality as it may have positive therapeutic repercussions.

REFERENCES

Aattar, A.A. (2006): Comparative physiological study on the effect of rosemary, tarragon and bay leaves extract on serum lipid Profile of quail, *Coturnix coturnix*. Saudi j boil. Sci., 13:1-98.

Abdel Aal, K.M.; Abeer, M.R. and Hussein, (2008): Therapeutic efficacy of alpha lipoic acid in combination with succimer against lead-induced oxidative stress, hepatotoxicity and nephrotoxicity in rats. Ass. Univ. Bull. Environ. Res., 11(2): 87-99.

Adler, A. J.; Barth, R. H. and Berlyne, G. M. (1993): Effect of lead on oxygen free radical metabolism: inhibition of superoxide dismutase activity. Trace elem. Med., 10:93-96.

Aebi, H. (1984): Catalase in vitro. In: *colowick sp.* methods in enzymology kaplan no (eds.), Academic press, new york, vol. 105 pp.121-126.

Asagba, S. (2010): Alteration in the activity of oxidative enzymes in the tissues of male Wister albino rats exposed to cadmium. Int. J. Occup. Med. Environ. Health, 23: 55-62.

Aslam, M. F.; Anwar, R.; Nadeem, U.; Rashid, T.G.; Kazi, A. and Nadeem, M. (2005): Mineral composition of *Moringa oleifera* leaves and pods from different regions of punjab pakistan. Asian j. Plant sci., 4: 417–421.

Babu R. C.; Nguyen B. D.; Chamarerk V.; Shanmugasundaram P.; Chezhian P.; Jeyaprakash P.; Ganesh S. K.; Palchamy A.; Sadasivam S.; Sarkarung S.; Wade L. J.and Nguyen H. T. (2003): Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Sci., 43: 1457–1469.

Bajpai, M.; Pande, A.; Tewari, S.K. and Prakash, D. (2005): Phenolic contents and antioxidant activity of some food and medicinal plants. Int. j. food sci. nutr., 56(4): 287-291.

Beutler, E.; Duron, O. and Kelly, B. M. (1963): Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bolfield, A. and Goldberg D.M. (1971): Colourimetric determination of alkaline phosphatase activity. Enzyme, 12: 561-568.

Chumark, P.; Khunawat, P.; Sanvarinda, Y.; Phornchirasilp, S. and Morales, N.P. (2008) : The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *moringa oleifera lam* leaves. J. ethnopharmacol., 116: 439-446.

Doumas, B. T.; Watson, W. A. and Biggs, H. G. (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta., 31: 87-96.

Dresel, E. I. and Falk, J. E. (1954): Studies on the biosynthesis of blood pigment. Haem synthesis in hemolysed erythrocytes of chicken blood. Biochem. J., 56:156-163.

Durgut, R. ; Koc, A.; Gonenci, R. ; Bal, R. ; Celik, S.; Guzaf, M.; Altug, M.E. and Atesoglu, O. (2008): Effect of high dose lead toxication on liver, kidney, heart, brain and blood rabbits: an experimental study. J. Appl. Biol. Sci., 2(2): 11-18.

Ekam, V.S.; Johnson, J. T.; Dasofunjo, K.; Odey, M.O. and Anyahara, S.E. (2012) : Total protein, albumin and globulin levels following the

administration of activity directed fractions of Vernonia amygdalina during acetaminophen induced hepatotoxicity in wistar rats. Ann. of Biol. Res., 3 (12) 5590-5594.

Ghorbe, F.; Boujelbene, M.; Makni-ayadi, F.; Guermazi, F.; Kammoum, A. and Murat, J. (2001): Effect of chronic lead exposure on kidney function in male and female rats: determination of a lead exposure biomarker. Arch. Physiol. Biochem., 109: 457-463.

Gornall, A.G.; Bardawill, C. J. and David, M. M. (1949): Determination of serum proteins by means of the biuret reagent. J. Biol. Chem., 177: 751-756.

Gurer, H. and Ercal, N. (2000): Can antioxidants be beneficial in the treatment of lead poisoning?. Free radic. Boil. Med., 29: 927-945.

Ibiam, A.U.; Ugwuja, E.I.; Ejeogo, C. and Ugwu, O.(2013): Cadmium-induced toxicity and the hepatoprotective potentials of aqueous extract of jessiaea nervosa leaf. Adv. Pharm. Bull., 3: 309-313.

Jarrar, B.M.; Mahmoud, Z.; Al-Enazi, M. and Taib, N. (2006): Histochemical alterations in the activities of hepatic dehydrogenases induced by lead in male albino rats. Egypt. J. Zool., 46: 217-225.

Kadrabova, J.; madaric, A. and Ginter, E. (1992): The effect of ascorbic acid on cadimum accumulation in guinea pig tissues. Experientia, 48: 989-991.

Khaki, A.; Fathiazad, F.; Nouri, M.; Khaki, A.A.; Abassi, M. N.; Ahmadi, P. and Jabarikh, H. (2010): Beneficial effects of quercetin on sperm parameters in streptozotocin -induced diabetic male rats. Phytother. Res. J., (24): 1285-1281.

Mariam, A.; Amr, A. and Ernest, A. (2007): The Effect of Vitamin C on the Metabolic

Parameters of Experimental Diabetes Mellitus. Am. J. Pharmacol. Toxicol., 2(1): 4-9.

Mcgregor, G. and Biesalski, H. (2006): Rationale and impact of vitamin C in clinical nutrition. Curr. Opin. Clin. Nutr. Metab. Care, 9: 697-703.

Mudipalli, M. (2007): Lead hepatotoxicity and potential health effects. Indian j. Med. Res., 126: 518-527.

Murrey, R.K.; Granner, D.K. and Rodwell V. W (2006): Micronutrients: Vitamins & Minerals in: Harper's Illusteated Biochemistry. 27th ed. McGraw-Hill. Boston. New York ,San Francisco, London, Singapore, (44): 489-492.

Nehru, b. and Kanwar, S.S. (2004): N-acetylcysteine Exposure on lead-induced lipid peroxidative damage and oxidative defense system in brain Regions of Rats. Biol. Trace. Elem. Res., 101: 257-264.

Nishikimi, M.; Roa, N. A. and Yagi, K. (1972): The occurrence of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.

Ohkawa, H.; Ohishi, W. and Yagi, K. A. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Oliveira, J. T.; Silveira, S. B.; Vasconcelos, K. M.; Cavada, B. S. and Moreira, R. A (1999): Compositional and nutritional attributes of seeds from the multiple purpose tree *moringa oleifera lamarck*. J sci food agric., 79(6): 815-20.

Prasanna, V. and Sreelatha, S. (2014): Synergistic effect of *Moringa oleifera* attenuates oxidative stress induced apoptosis in saccharomyces cerevisiae cells: evidence for anticancer potential, int j. Pharm. bio. sci.,5(2): 167-177.

Reitman, S. and Frankel, S. (1957): Colorimetric method for estimation of got/ast and gpt/alt transaminases. Am. J. Clin. Pathol, 28: 56-63.

Rekka, E.; Kourounakas, P.; Shahidi, F.; Janitha, P.K. and Anasundara, P.D. (1992): Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32: 67-103.

Saalu, L.C.; Ogunlade, B.; Ajayi, G.O.; Oyewopo, A.O.; Akunna, G.G. and Ogunmodede, O.S. (2012): The hepato- protective potentials of moringa oleifera leaf extract on alcohol-induced hepato-toxicity in wistar rat. Am. J. Biotechnol. Mol. Sci., .2(1): 6-14.

Sandhir, R. and Gill, K. D. (1995): Effect of lead on lipid peroxidation in liver of rats. Biol. trace elem. Res., 48:91–97.

Santhrani, T and maheswari, E. (2012): Carbamazepine provoked hepatotoxicity: attenuation by vitamin c. 2nd international conference on medical, biological and pharmaceutical sciences, 100-102.

Schmidt, M.and Eisenburg, J. (1975): Serum bilirubin determination in newborn infants. A new micro-method for the determination of serum of plasma bilirubin in newborn infants. Fortschr. Med., 93: 1461-1466.

Scholz, R.; Graham, K.; Gumpricht, E. and Readely, C. (1989): Mechanism of interaction of vit. E and glutathione in the protection against membrane lipid peroxidation. Ann. N. Y. A. Cad. Sci., 570: 514-517.

Seddik, I.; Bah, T.M.; Aoues, A.; Brnderdour, M. and Silmani, M. (2010): Dried leaf extract protects against lead induced neurotoxicity in Wistar rats. Eur. J. sci. res., 42(1): 139–151.

Shah, N. A.; Khan, M. R.; Ahmad, B.; Noureen, F.; Rashid, U.and Khan, R.A. (2013): Investigation on flavonoid composition and anti-free radical potential of sida cordata. BMC Comp. Altern. Med., 13: 276–288.

Sharida F.; Syazana A. S. and Palanisamy A. (2012): *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen- induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules, 17: 8334-8350

Sies, H.; Stahl, W. and Sundquist, A.R. (1992): Antioxidant functions of vitamin E and C, β-carotene and carotenoids. Ann. N. Y. Acad. Sci., (669): 7-20.

Sivaprasad, R.; Nagaraj, M. and Varalakshmi, P. (2004): Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead- induced lipid peroxidation in rat liver. J. Nutr. Biochem., 15: 18-23.

Sreelatha, S. and Padma, P.R. (2009): Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant. Food. Hum. Nutr., 64(4): 303-311.

Taki, Y.; Shimahara, Y. and Isselhard, W. (1985): Derangement of hepatic energy metabolism in lead- sensitized endotoxicosis. Eur Surg. Res., 17: 140–149.

Todorovic, T.; Dozic, I.; Vujanovic Dragana, J.; Pejovic and Marjanovic, M. (2005) : The influence of chronic lead poisoning on the activity of some serum enzymes in rats

Acta. Vet.-Beograd, 55(5-6): 471-482.

Verma, A.R.; Vijayakumar, M.; Mathela, C.S. and Rao, C.V. (2009): In vitro and in vivo antioxidant properties of different fractions of *moringa oleifera* leaves. Food chem. Toxicol., 47: 2196–2201.

Walter, M.and Gerade. H. (1970): Colorimetric method for determination bilirubin in serum and plasma. Micro. Chem, J., 15: 231-236.

ARABIC SUMMARY

" دراسات فسيولوجية لتأثير نبات المورينغا أوليفرا وفيتامين ج على سمية الكبد والاجهاد التأكسدي الناجم عن خلات الرصاص في ذكور الجرذان البيضاء."

أ.د/عبد الرحيم على الشاطر[1], د/ رانا عبد الستار على[2], ايمان مروان رفاعى [3]
قسم علم الحيوان - كلية العلوم - جامعة جنوب الوادى.

الرصاص هو عنصر شديد السمية وهو من العناصر الموجودة في الطبيعه ويؤثر على العديد من اعضاء الجسم البشرى، وكان الغرض من هذه الدراسة هو دراسة دور أوراق نبات Moringa olifera ، وفيتامين C في ذكور الجرذان البيضاء ضد سمية خلات الرصاص. وقد ضمت الدراسه أربعين (40) من ذكور الجرذان البيضاء استخدمت للتجربة وقد قسمت الى اربعه مجموعات. كانت المجموعة الأولى تُعطى ماءً مقطرًا لمدة 8 اسابيع ، المجموعة الثانية جرعت فمويا بواسطة خلات الرصاص فقط (20 ملغ / كغ وزن الجسم) لمدة 8 اسابيع، المجموعة الثالثة جرعت فمويا بواسطة خلات الرصاص (20 مغ / كغ وزن الجسم) ومستخلص أوراق نبات المورينغا (400 مغ / كغ وزن الجسم) لمدة 8 اسابيع, المجموعة الرابعة جرعت فمويا بواسطة خلات الرصاص (20 مغ / كغ وزن الجسم) وفيتامين ج (50 مغ / كغ وزن الجسم) لمدة 8 اسابيع. بعد أنتهاء التجربه تم ذبح الحيوانات المعالجة وتم جمع عينات الدم وفصل المصل لتحليل أنزيمات الكبد. أظهرت النتائج التي تم الحصول عليها في هذه الدراسة زيادة معنوية في ($p <0.05$) في الأسبارتات (AST aminotransferase) ، ألانين ترانسفيراز (ALT) ، الفوسفاتيز القلوي (ALP) ، البيليروبين و (MAD Malondialdehyde) ، في حين أظهرت انخفاض كبير في ($p <0.05$) في البروتين الكلي ، ألبيومين ، الجلوتاثيون (GSH) ، ديسموتاز الفائق (SOD) و الكاتلاز (CAT) في المجموعة المعاملة بخلات الرصاص فقط عند مقارنتها مع المجموعة العادية. ومع ذلك ، أظهرت آثار المورينغا وفيتامين C على الإصابة الكبدية بسبب الإجهاد التأكسدي الناجم عن الرصاص انخفاض كبير في ($P <0.05$) في الأسبارتات (AST aminotransferase) ، ألانين ترانسفيراز (ALT) ، الفوسفاتيز القلوية (ALP) ، بيليروبين و مالونديالدهيد (MAD) ، في حين أظهر زياده(CAT) بالمقارنة مع مجموعة التحكم. وخلص إلى أن أوراق المورينغا وفيتامين ج اوضحوا تحسن ملحوظ فى وظيفه الكبد نستخلص من هذه الدراسة إثبات فاعلية المعالجة بفيتامين ج و أوراق المورينغا كمضادات أكسدة حيث كان لهما تأثير تحسينى ضد الضرر التاكسدى الناجم عن سمية خلات الرصاص.