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Effect of *Moringaoleifera* (Moringa) on tand lead mixture in rats body

Hamida A. Helal, Nehad R. El- Tahan Fatma El-SayedAbd El-rasheed

Department of Nutrition and Food Science, Faculty of Home Economics, MenoufiaUniversity

Abstract

The main target of the present investigation was to study the effect of Moringaoleifera leaves to get rid of toxic heavy metals (Cadmium and lead mixture) in albino rats . Thirty healthy adult male albino rats "Sprague Dawley strain" weighing 150±5g, were used and divided into 5 equal groups, one was kept as a negative control group, while the second Group(6rats) fed on basal diet with cadmium and lead at the level of 0.2% (control positive group). Groups (3,4 and 5) were fed on diet with toxin mixture and moringa leaves at different levels 2.5, 5 and 10 %. At the end of the experiment, feemd intake (FI), body weight gain (BWG), feed efficiency ratio (FER) and relative weights of liver, kidneys and spleen were calculated. Also, Serum liver functions (GOT, GPT, ALK), kidney functions (Urea, CR, ALb), lipid profile (T.Ch, Tri, LDL, HDL,) were determined in serum. Histopathological changes of liver samples were examined. The results indicated that rats infected toxins mixture which fed on diet with tested leaves at 10% significantly increased in body weight gain, organs weight ,serum glucose, cholesterol, triglycerides, LDL, liver enzymes kidney functions and decreased HDL. Treating rats which were fed on diet with this level of tested leaves improved all other parameters and internal organs histopathological confirmed weights. The examination the improvements in biological parameters and cell structure.

*Key Words :*Sprague Dawley Strain, toxin mixture, moringa leaves, liver enzymes ,kidney functions.

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1. Introduction

Human activity in the last few decades has led to global contamination by organic and inorganic compounds (Chaerunet al., 2011) and (Sahuet al., 2007). The presence of the pollutants generated from industrial and agriculture activities in the waterways has been identified to produce potential harmful effect on the aquatic living organisms and the food webs (Katnoriaet al., 2011) and (Oliveira et al., 2004). Nowadays, heavy metal contamination is considered to be among the most serious environmental problems. Heavy metals are any inorganic metallic compounds that can exert their toxicity via binding to the thiol group and disulfide bond that contribute to the stability of the enzyme (Frasco et al., 2005). The metals have high affinity to the disulfide bridge between two cysteine residues in any protein compound. Heavy metals are very dangerous to living organisms especially for humans since they can cause DNA damage and exert carcinogenic effects. Medicinal plants play important role in individuals and communities health. The medicinal value of these plants depends on some chemical compounds that produce a definite physiological action in the human body. The most important of these bioactive constituents of plants are alkaloids tannins flavonoids and phenolic compounds (Hill, 1952). The state of medicinal plants research has been emphasized in many developing countries (Edeogaet al., 2005). The appropriate utilization of local resources to cover drugs needs is dependent on the preliminary scientific study to determine the efficacy and safety of any preparation (Burkill, 1984). The awareness of the role of medicinal plants in health care delivery of developing countries has resulted in researches into traditional medicine with a view to integrating it with modern orthodox medicine (Sofowara, 1993). Metal poisoning is a global problem with humans being exposed to a wide range of metals in varying doses and varying time frames. Traditionally, treatment involves removal of the toxic source or chelation therapy. An intermediate approach is needed. This study reported that the use of essential metal supplementation was very important as a strategy to induce metallothionein expression and displace the toxic metal from important biological systems, improving the metal burden of the patient. Specific recommendations are given for supplementation with calcium, zinc and

vitamin E as a broad strategy to improve the status of those exposed to toxic metals (Wayne,2014).

Pure cadmium is a soft, silver-white metal .The level of this toxic hazardous metal in the environment have increased dramatically in the last few years as it is naturally emitted into the environment through volcanic activities, forest fires and generation of sea salt aerosols . Cadmium is mostly used in the production of batteries, pigments, coatings and plantings, stabilizers for plastics, nonferrous alloys and photovoltaic devices. Studies have also shown that tobacco leaves accumulate high levels of cadmium from the soil. Cadmium is a major concern for people living near cadmium-emitting industries. Highest risk of occupational exposure occurs from processes involving heating cadmium containing materials such as smelting and electroplating. Exposure to this environmental pollutant can be prevented through personal protective equipment, though cigarette smoking is known to double the toxic effects (**ATSDR,2008**).

The injurious effect of cadmium is related with diverse clinical manifestations like renal and hepatic dysfunction, bone diseases, anaemia, immune toxic effects along with the alterations of the lipid profile, pulmonary oedema and testicular damage (Vinodinietal. (2013).

lead poisoning" or "lead intoxication" has been defined as exposure to high levels of lead typically associated with severe health effects. Poisoning is a pattern of symptoms that occur with toxic effects from mid to high levels of exposure; toxicity is a wider spectrum of effects, including subclinical ones (those that do not cause symptoms). However, professionals often use "lead poisoning" and "lead toxicity" interchangeably, and official sources do not always restrict the use of "lead poisoning" to refer only to symptomatic effects of lead (**Guidotti and Ragain, 2007**).

The amount of lead in the blood and tissues, as well as the time course of exposure, determine toxicity. Lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeat low-level exposure over a prolonged period), but the latter is much more common. Diagnosis and treatment of lead exposure are based on blood lead level (the amount of lead in the blood), measured in micrograms of lead per deciliter of blood (μ g/dL). Urine lead levels may be used as well, though less commonly. In cases of chronic exposure lead often

sequesters in the highest concentrations first in the bones, then in the kidneys. If a provider is performing a provocative excretion test, or "chelation challenge", a measurement obtained from urine rather than blood is likely to provide a more accurate representation of total lead burden to a skilled interpreter (Lowry, 2010).

The US Centers for Disease Control and Prevention and the World Health Organization state that a blood lead level of $10 \mu g/dL$ or above is a cause for concern; however, lead may impair development and have harmful health effects even at lower levels, and there is no known safe exposure level. Authorities such as the American Academy of Pediatrics define lead poisoning as blood lead levels higher than $10 \mu g/dL$.

Numerous plant in nature possess medicinal properties. One among many is *Moringaoleifera*. This *Moringaoleifera* plant is one of the naturalized species of *Moringaceae* family. The tree thrives best under the tropical insular climate. Moringa , originally from India , is now distributed throughout the world. In some parts, it is often referred to as the drumstick or the kelor tree while in other places it also known as Shagara al Rauwaq(Anwar et al., 2007).

The leaves of this miraculous plant are a source of protein, Bcarotene, vitamins (A, B, C, E, riboflavin), nicotinic acid ,folic acid ,pyridoxine, amino acids, minerals, various phenolic compound s. Leaves of Moringaoleifera are known to have hypolipidemic, anti atherosclerotic, antioxidant, hypotensive, tumour suppressive and immune boosting effect and also for its role in the prevention of cardiovascular diseases (**Khalafalla***et al.*(2010).

Best known as miracle tree, Moringa is an important tropical crop that is used as human food, medicine and in oil production (**Anwar** *et al.*, **2007**). It has a wide range of health benefits and hence extracts from different parts of Moringa could be used to combat various metal intoxications like cadmium, arsenic ,lead etc., (**Sirimongkolvorakul***et al.*,**2012**).

It not only has a positive effect in lowering the lipid levels but also alters the levels of the liver enzymes and hence can also improve the liver functions (**Vinodini***et al.*, **2014**).

So, This study aimed to investigate the effect of different levels of *Moringaoleifera* on the biological prameters of rats toxic with cadmium and lead mixture.

4-Materials and Methods

4-1.Materials:

Moringa leave and Casein were obtained from Morgan Company, Cairo, Egypt. Vitamins mixture, salt mixture and biological kits were purchased from El – Gomhoria Company., Cairo, Egypt. Thirty healthy adult male albino rats "Sprague Dawley strain" weighing 150 ± 5 gwere used in the study. The rats were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. They were housed in galvanized iron cages measuring $40 \times 24 \times 20$ cm (5 rats to each cage).

Methods:

A . Preparation of samples:

Moringa leaves were cleaned for removing dust and impurities and dried at 50° C using a fan oven. Then, they were milled by a precession mill to give powder. A grinder mill and sieves were used to obtain a powder particle size of less than 0.2mm.

B. Biological Experiments

Basal diet was prepared from fine ingredients per 100g. The diet had the following composition :Corn starch 67%, Casein 13% (AIN, 1993), corn oil 10%, Fiber 5%, Salts mixture 4% (Hegsted*et al.*, 1941), vitamin mixture 1% (Campbell, 1963). Moringa leaveswere added to the tested diet at the level 2.5, 5 and 10%.

C. Experimental Design

Biological experimental was done at the central laboratory of Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. Rats (n = 30 rats) were housed individually in wire cages in a room maintained at $25 \pm 2^{\circ}$ C and kept under normal healthy conditions. All rats (30rats) were fed on basal diet for one – week before starting the experiment for to learn non-lethal dose. After this week, they were divided into nine main groups:

<u>Group(1)</u>:Rats were fed on basal diet as negative control group.

- <u>Group(2)</u>:Rats were fed on basal diet with toxin mixture at the level 0.2% as a positive control.
- <u>Group(3)</u>:Rats were fed on diet with toxin mixture and moringa at the level 2.5%.
- <u>Group(4)</u>:Rats were fed on diet with toxin mixture and moringa at the level 5%.
- <u>Group(5)</u>:Rats were fed on diet with toxin mixture and moringa at the level 5%

D. Biological evaluation

During the experiment period (28days), the quantities of diet which were consumed and / or wasted were recorded every day. In addition, rat's weight was recorded weekly. The body weight gain (BWG), feed intake(FI), feed efficiency ratio(FER) were determined according to (Chapman *et al.*, 1959).

E. Biochemical evaluation and Histopathological examination

At the end of the experiment period, the rats were fasted overnight before sacrifice and the blood samples were collected from each rat and centrifuged to obtain the serum. Serum was carefully separated and transferred into dry clean ebendorf tubes and kept frozen at-20°c for analysis as described by (Schermer, 1967). Liver, and Brain were removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution(0.9%), dried by filter paper, weighed and kept in formalin solution (10%), according to the method described by (Drury and Walling, 1980)

F. Hematological analysis

Different tested parameters in serum were determination using specific methods as follow: Glotamicoxaloacetictransaminas (GOT), glotamic pyruvic transaminas (GPT), urea and createnine according to Kakkaret al., (1984); Aebi (1974) ; Ellman (1959) & Reitman and Frankel (1957) respectively.

G. Statistical analyses

Statistical analysis was carried out using the programmer of Statistical Package for the Social Sciences (SPSS), PC statistical soft ware (Version 20; Untitled–SPSS Data Editor). The results were expressed as mean \pm Standard deviation (mean \pm S.D.). Data were analyzed using one way classification, analysis of variance (ANOVA).

The differences between means were tested for significance using least significant difference (LSD) test at p<0.05 (Sendcor and Cochran, 1979).

Results and Discussion

In the current study the effect of moringa leaves to get rid of toxic heavy metals in rat's body.

1-Effect of moringa leavesat different levels on body weight gain (BWG) of rats toxic with cadmium and lead mixture.

Data presented in table (1) showed the effect of different levels of tested leaveson body weight gain (BWG) of rats .

It could be noticed in table (1) that differences between all mean values of these groups were significant when compared to control negative group. With expecting, 0.02% toxins mixturegroup was the lowest value of body weight gain. There is no significant differences in BWG among group 3 and 4. The best result was group 5 which fed on mixture 0.02% and moringa at the level 10%.

Potential health problems associated with a high intake of vegetables and meat products which contain salt of Lead and cadmium have been linked to decreased energy intakes, weight gain and the weight loss epidemic as indicated by **Katnoria***et al.* (2011).

Meanwhile, **Oliveira** *et al.* (2004) found that the rising consumption of vegetables fertilizer and meat additives provides a rising intake of Lead which can contribute to weight loss and underweight.

Also, study done by **Frasco** *et al.* (2005) increased cadmium consumption would decrease total energy intake by decreased appetite and decreased fat intake.

Karakaya (2004) who found that moringa leaves increased weight gain to contained many biological active compounds including chymopapain and papain which is the ingredient that aids digestive system and a good supply of vitamin A and C that are highly essential for maintaining a good health.

 Table (1): Effect of moringa leaves at different levels on body weight gain (BWG) of rats toxic with cadmium and lead mixture .

Groups	BWG g / 28 days
Negative control(G1)	$43.64^{a} \pm 4.21$
Positive control (G2)	$4.61^{d} \pm 0.13$
Toxins mixture and moringaleaves at the level 2.5%(G3)	$9.12^{c} \pm 1.19$
Toxins mixture and moringa leaves at the level 5% (G4)	$12.74^{c} \pm 0.21$
Toxins mixture and moringa leaves at the level 10%(G5)	$17.54^{b} \pm 1.11$

Means in the same column with different litters are significantly different (P ≤ 0.05).

2- Effect of moringa leaves at different levels on Feed Intake (FI) and feed efficiency ratio (FER) of rats toxic with cadmium and lead mixture.

Data present in table (2) showed the effect of tested leaves to high doses of lead and cadmiumon feed intake (FI) and feed efficiency ratio (FER) (mean± SD).

It is clear that no significant differences in feed intake (FI) between positive controls and group 3. From the table, it could be noted that the differences in values of feed intake between all treated groups were considerable as compared to negative and positive control groups. The obtained data revealed a high variation in feed intake between treatments and the controls group, this may be due to the acceptability of the added material. These results are in accordance with those reported by **Frasco** *et al.*(2005) who found that leaddecreased appetite and decreased fat intake.**Calliste** *al.*(2001) reported that moringa is a source of antioxidants vitamin as A that prevents damage caused by free radicals that may cause some forms of cancer.

According to data present in the same table (2), these results denote that there were significant increases in feed efficiency ratio (FER)for all groups when compared with control positive group. The highest value of feed efficiency ratio (FER) was found in 10% group. It is noticed that a significant decreases in BWG% for control group compared to all groups, was indicated and confirmed that the real effect for BWG% due to moringa leaves administration.

From the obtained results, it could be observed that treating rats with the tested vegetables and fruit led to increase in BWG%, FI and FER when compared with both positive controls while lower than negative control. These results were in agreement with those reported by **Calliste***et al.*(2001) who said that dietary fiber (DF) derived from fruits and vegetables have a relatively high proportion of SDF.

Table (2): Effect of moringa leaves at different levels on Feed Intake		
(FI) and feed efficiency ratio (FER) of rats toxic with		
cadmium and lead mixture (mean± SD).		

Groups	FI (g/day)	FER
Negative control(G1)	$11.71^{a} \pm 0.22$	$0.133^{a} \pm 0.001$
Positive control (G2)	$2.63^{d} \pm 0.11$	$0.063^{d} \pm 0.001$
Toxins mixture and moringa leaves at the level 2.5% (G3)	$2.89^{d} \pm 0.02$	$0.112^{b} \pm 0.001$
Toxins mixture and moringa leaves at the level 5% (G4)	$4.76^{\circ} \pm 0.12$	$0.096^{b} \pm 0.002$
Toxins mixture and moringa leaves at the level 10%(G5)	$7.68^{b} \pm 0.27$	$0.082^{\rm c} \pm 0.002$

Means in the same column with different litters are significantly different ($P \le 0.05$).

3-Effect of moringa leaves at different levels on kidney functions of rats toxic with cadmium and lead mixture (mean± SD).

Data given in table (3)showed the effect of moringa leaves to high doses of lead and cadmium mixture on serum urea levels(mean \pm SD). It could be observed that the highest value of serum urea levels was found in rats which receivetoxins mixture as positive control group. No significant changes were found in serum urea levels between groups 2 and 3 also, there is no significant between group 4 and 5.

It is clear that in control negative group creatinine levels was $0.46 \pm 0.02 \text{ mg/dl}$ which significantly decreased when compared with rats which received the toxins mixture as positive control and group fed on moringa leaves at the level 2.5%. Meanwhile, rats of groups 4 and 5, creatinine levels of these groups were non significant between each other and showed significantly increasing as compared to control negative group. Groups 5 was the lowest creatinine value which showing a significant decreased as compared to the other groups and a significant increased when compared with control negative group.

Table (3): Effect of morin	nga leaves	at differen	t leve	els on	n kidney
functions of rats	toxic with	cadmium	and	lead	mixture
(mean± SD).					

Groups	Urea (mg/dl)	Creatinine (mg\dl)
Negative control(G1)	$27^{\circ} \pm 4.23$	$0.46^{\circ} \pm 0.12$
Positive control (G2)	$50.33^{a} \pm 3.21$	$2.45^{a} \pm 0.22$
Toxins mixture and moringa leaves at the level 2.5% (G3)	48.33 ^a ± 3.31	2.05 ^a ± 0.15
Toxins mixture and moringa leaves at the level 5% (G4)	43.33 ^b ± 4.12	$1.53^{b} \pm 0.35$
Toxins mixture and moringa leaves at the level $10\%(G5)$	40.3 ^b ± 1.1	$1.35^{b} \pm 0.50$

Means in the same column with different litters are significantly different ($P \le 0.05$).

4-Effect of moringa leaves at different levels on liver functions of rats toxic with cadmium and lead mixture (mean± SD).

Data presented in table (4) showed the effect of moringa leaves to high doses of lead and cadmium mixtureon levels of serum $AST(mean\pm SD)$.

It could be observed that in control negative group AST was $39\pm$ 3.00 u/l which significantly decreased when compared with positive control group. But, the levels of AST in groups 3,4 and 5 showed significant increasing as compared to control negative group and significant decreased as compared to control positive groups. The strongest effect in serum AST levels recorded for group 5 which fed on basal diet with 10% of moringa leaves.

Groups	AST(U\L)	ALT(U\L)
Negative control(G1)	39 ^e ± 3.00	$45^{c} \pm 0.6$
Positive control (G2)	$109^{a} \pm 3.21$	$95^{a} \pm 2.3$
Toxins mixture and moringa leaves at the level 2.5% (G3)	$102^{a} \pm 5.01$	$90^{a} \pm 5.4$
Toxins mixture and moringa leaves at the level 5% (G4)	$94^{b} \pm 5.34$	82.66 ^b \pm 0.34
Toxins mixture and moringa leaves at the level 10%(G5)	88 ^c ± 7.76	$77^{\ b}\pm 3.8$

It is clear that the serum level of (ALT) in group 5 which fed on toxins mixture with 10% moring a leaves was the lowest level which being 77 \pm 3.8 U/L and showing no significant change with group which fed on toxins mixture with 5% tested leaves which was 82 \pm 0.34 U/L. At the

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same time, rats which received toxins mixture with 2.5% leaves and positive control groupsignificantly didn't differ in serum level of ALT.

Edeogaet *al.*(2005) revealed that mercury had a potential role to cause injuries in several organs and tissues. The increased consumption of cadmium and Lead sources in foods and drinks is linked with the hepatic metabolism and caused lipogenesis and ATP depletion, which leads to fat accumulates in the liver by the primary effect of NO oxidation. It could be hypothesized that increased mercury and Lead sources consumption contributes to the development of non-alcoholic fatty liver disease (NAFLD) which can progress to cirrhosis over time in some individuals.

Anwar *et al.*(2007) showed that moring plant is one of the most important plants which containing phenolic antioxidant compound which protected liver from any free radical.

Khalafallaet al.(2010) found that the moringa extraction contained dietary fiber or essential oils, the flavonoids hesperidin and narirutin which reduced the residual mercury levels and the degree of lipid oxidation.

Anwar *et al.*(2007) found that moring leaves contained significant antioxidant activity and hadhepatoprotection effect by restoring the normal hepatic architecture.

Table (4): Effect of moringa leaves	at different levels on liver
functions of rats toxic with	cadmium and lead mixture
(mean± SD).	

Groups	AST(U\L)	ALT(U\L)	
Negative control(G1)	$39^{e} \pm 3.00$	$45^{\rm c} \pm 0.6$	
Positive control (G2)	$109^{a} \pm 3.21$	$95^{a} \pm 2.3$	
Toxins mixture and moringa leaves at	$102^{a} + 5.01$	$90^{a} \pm 5.4$	
the level 2.5% (G3)	102 ± 5.01		
Toxins mixture and moringa leaves at	$94^{b} \pm 5.34$	$82.66^{b} \pm 0.34$	
the level 5% (G4)	94 ± 3.34	82.00 ± 0.34	
Toxins mixture and moringa leaves at	$88^{c} + 7.76$	77 ^b ± 3.8	
the level 10%(G5)	00 ± 7.70	11 ± 3.0	

Means in the same column with different litters are significantly different. (P \leq 0.05).

Histopathological results:

Liver: Liver's rat which fed on basal diet, the Liver structure showing the normal histological (photo 1). In photo(2), Liver's rat which fed on basal diet with 0.2% toxins mixture as positive control group showed that congestion of central vien and hepatic simusoids and kupjjer cells activation and with local hepatic mecrosis associated mononuclear cells infiltration..In Photo (3), Liver's rat which fed on basal diet with 0.2% mixture with 2.5% tested leaves showed that slight vacuoligation of hepatocytes. In Photo (4), Liver's rat which fed on basal diet with 0.2% mixture and 5% from leaves showed that hydropic degeneration of hepatocytes . In Photo (5), liver's rat which fed on basal diet with mixture 0.02% and 10% leaves showed that no histopathological changes .

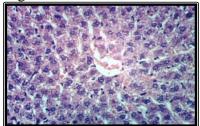
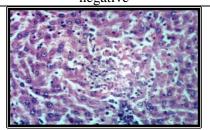


Photo (1): liver of rat fed on diet as control negative



Photo(3): liver of rat fed on diet with 0.02% toxin and 2.5% moringa leaves

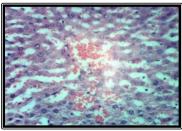
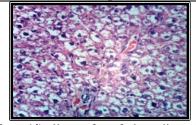
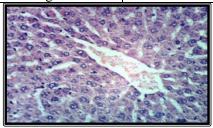


Photo (2) : liver of rat fed on diet with lead 0.2% as positive control



Photo(4): liver of rat fed on diet with 0.02% toxin and 5% moringa leaves



Photo(5): liver of rat fed on diet with 0.02% toxin and 10% moringa leaves

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تأثير المورينجا على خليط الرصاص والكادميوم في جسم الفئران

حمديه أحمد هلال ، نهاد رشاد الطحان ، فاطمة السيد عبد الرشيد قسم التغذية و علوم الأغذية، كلية الاقتصاد المنزلي، جامعة المنوفية

الملخص العربى:

الهدف الرئيسي لهذه الدراسة هو معرفة تأثير أوراق مورينجا اوليفيرا للتخلص من سموم المعادن الثقيلة (خليط الكادميوم والرصاص) في الفئران البيضاء استخدمفى هذه الدراسة عدد 30 من ذكور الفئران البيضاء من فصيلة (اسبراجودولى) وزنها 150 ± 5جم، وتم تقسيمهم إلى 5 مجموعات متساوية، المجموعة الرئيسية الأولى تم تغذيتها على الغذاء الاساسى واستخدمت كمجموعة ضابطه (سالبة)، في حين تم تغذية المجموعة الثانية (6فئران) على الغذاء الاساسى واستخدمت كمجموعة ضابطه (سالبة)، في حين تم تغذيتها على الغذاء الاساسى واستخدمت كمجموعة ضابطه (سالبة)، في حين تم تغذية المجموعة الثانية (6فئران) على الغذاء من الغذاء الماسي واستخدمت كمجموعة ضابطه (سالبة)، في حين تم تغذية المجموعة الثانية (6مئران) على الغذاء من الغذاء الإساسى واستخدمت كمجموعة ضابطة من الكادميوم والرصاص بنسبة 20% كمجموعة ضابطه (معابطه (موجبة).

و تغذية المجموعات (3و لمو5) على الغذاء الاساسى مع خليط السموم وأوراق المورينجا بنسب مختلفة 2.5، 5 و 10%. وفي نهاية فترة التجربة، تم قياس معدل اكتساب الوزن-معدل كفاءة الغذاء الغذاء المأكول – الوزن النسبى للأعضاء- مستوى الجلوكوز – أنزيمات الكبد (GOT,GPT,ALP) – وظائف الكلى(اليوريا – حامض اليوريك-الكرياتينين) - الكوليسترول الكلى(TC) الجليسريدات الثلاثية(TG)الليبوبروتينات (مرتفعه ، منخفضة).

تم فحص الهستوباثولوجى على الكبد وأشارت النتائج الى أن الفئران المصابة بخليط السموم والتى تتغذى على الغذاء الاساسى مع 10% من أوراق المورينجا أظهرت زياده كبيرة فى وزن الجسم ووزن الاعضاء وجلوكوز الدم والكوليسترول والدهون الثلاثية وظائف الكلى وانزيمات الكبد وانخفاض فىالليبوبروتينات المرتفعة حدث تحسن لكل التقديرات (أكد التشريح الهستولوجى هذه التحسنات).

الكلمات المفناحية: فئران اسبراجودوللي ، خليط السموم (الرصاص والكادميوم) ، اوراق المورينجا ، انزيمات الكبد ،وظائف الكلي .