Biological Effect of Mallow and Jew's Mallow on Toxic Heavy Metal (Lead) In Vivo

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ABSTRACT

The main target of the present investigation was to study the effect of some calcium sources on the level of lead in albino rats. At the end of the experiment, feed intake (FI), body weight gain (BWG), feed efficiency ratio (FER) Also, serum liver enzymes (GOT & GPT) and kidney functions (Urea and creatinine) determined. Histopathological changes in the liver were examined. The results indicated that rats infected lead, which fed on a diet with mallow and Jew's mallow leaves significant increase in BWG, FI, and FER. The same groups improved the liver enzymes and kidney functions when compared with positive control group. Treating rats with mallow and Jew's mallow at the level of 10% decreased the toxic effect of lead on tested parameters and decreased the toxic changes in the cell structure of liver tissues when compared with positive control group. For histomorphometric measures of bone density, Corchorus olitorius and Malva sylvestris improved measures of bone density than that rats exposed for the lead.

Keywords: Mallow, Jew’s mallow, liver enzymes, kidney functions, Bone density.

INTRODUCTION

Human activity in the last few decades has led to global contamination by organic and inorganic compounds (Sahu et al., 2007 and Chaerun et al., 2011). The presence of the pollutants generated from industrial and agriculture activities in the waterways has been identified to produce potential harmful effects on the aquatic living organisms and the food webs (Oliveira et al., 2004 and Katnoria et al., 2011). Heavy metals contamination 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 a high affinity to the disulfide bridge between two cysteine residues in any protein compound. Heavy metals are very dangerous to live organisms especially for humans since they can cause DNA damage and carcinoenic effects.

The medicinal plants play an 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. Alkaloids, tannins, flavonoids, and phenolic compounds are important for bio-active ingredients of plants (Hill, 1952). The state of medicinal plant research has been emphasized in many developing countries (Edeoga et 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 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 includes the elimination 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 mental 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).

Jew’s mallow nutrition is basically due to its green leaves. Like all other green leafy vegetables, its leaves are a rich source of vitamins, and minerals. Its leaves contain a high amount of vitamin A (as β-carotene) and vitamin C. It contains vitamin C up to 53mg/100 g and source of pro-vitamin A up to 12 mg/100 g. It is very low in saturated fat and cholesterol and a source of thiamine, zinc, and dietary fiber. Jew’s mallow contains 500 mg calcium/100g (Dayal and Singh, 2015). The biological activity of this plant may be attributed to antioxidants, such as polyphenols, vitamin C, vitamin E, beta carotene, and other important phytochemicals. In a previous investigation gossypetin, 3-sulphate-8-O-b-D-glucoside and hypolaetin 3-sulphate were identified as the major flavonoid constituents in the leaf tissue of Jew's mallow. Other compounds with chemotaxonomic significance for this plant are the 8-hydroxyflavonoids so far the isolation of 8-hydroxyflavonoid sulphates has been reported from Jew’s mallow leaves. A comparative study of the composition in nutraceuticals (phenolics, flavonoids, carotenoids, ascorbic acid, tocopherols sugars, and fatty acids) and antioxidant properties of leaves was done. Jew’s mallow extracts are...
reported for their radical scavenging effect and had a negative correlation with the risks for chronic diseases such as cardiovascular diseases, arthritis, chronic inflammation and cancers (Tulio et al., 2002).

Mallow, the biological activity of this plant attributed to antioxidants, such as polyphenols, vitamin C, vitamin E, beta carotene, and other important phytocompounds (Barro et al., 2010). In a previous investigation gossypetin 3-sulphate-8-O-b-D-glucoside and hypolactin 3-sulphate were identified as the major flavonoid constituents in the leaf tissue of mallow (Nawwar and Buddrus, 1981). Other compounds with chemotaxonomic significance for the mallow, the 8-hydroxyflavonoids so far the isolation of three 8-hydroxyflavonoid sulphates has been reported from mallow leaves (Billeter et al., 1991). A comparative study of the composition in nutraceuticals (phenolics, flavonoids, carotenoids, ascorbic acid, tocopherols sugars and fatty acids) and antioxidant properties of different parts of mallow (leaves, flowers, immature fruits and leafy flowered stems) evaluated by (Barro et al., 2010). Mallow extracts are reported for their radical scavenging effect (Karakaya, 2004) as well as E. camaldulensis and C. sativa; the latter demonstrated also antineoplastic activity in B16 cells (Calliste et al., 2001). Studies reveal a negative correlation between the consumption of diets rich in fruit and vegetables and the risks for chronic diseases such as cardiovascular diseases, arthritis, chronic inflammation and cancers (Chen et al., and Zhang et al., 2017).

So, this study was designed to investigate the effect of Corchorus olitorius and Malva sylvestris to get rid of lead toxic in albino rats.

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 ± 2 °C and kept under normal healthy conditions. All rats (30 rats) were fed on the basal diet for one week before starting the experiment for adaptation. After this week, they were divided into six main groups:

**Group(1):** Rats were fed on basal diet as negative control group.

**Group(2):** Rats were fed on basal diet with lead (0.5% as lead chloride) (Frasco et al., 2005) as a positive control.

**Group(3):** Rats were fed on diet with lead (0.5%) and mallow at the level 5%.

**Group(4):** Rats were fed on diet with lead (0.5%) and mallow at the level 10%.

**Group(5):** Rats were fed on diet with lead (0.5%) and Jew's mallow at the level 5%.

**Group(6):** Rats were fed on diet with lead (0.5%) and Jew's mallow at the level 10%.

D. Biological evaluation

During the experiment period (28days), the quantities of the diet consumed and/or wasted were recorded every day. Besides, the 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, histopathological examination and histomorphometric measures of bone

At the end of the experiment period, the rats have fasted overnight before sacrifice and the blood samples were collected from each rat and centrifuged to obtain the serum. Serum carefully separated and transferred into dry clean Eppendorf tubes and kept frozen at-20°C for analysis as described by (Scherner, 1967). Liver's rats 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 liver section kept in formalin solution (10%), according to the method described by (Drury and Walling,1980). Bone mineral density (BMD) (mg/mm²) of the left femur of each rat measured by dual-energy X-ray absorptiometry (DXA; model DCS-600A; Aloca,Tokyo, Japan). The measure of bone density (MBD) calculated by the measured bone area (BA) according to the method described by Ohta's method (2002).

F. Hematological analysis

Different tested parameters in serum were determined using specific methods as follow: Glutamicoxalacetetranasimas (GOT), glutamic pyruvic transaminas (GPT), urea and creatinine according to Kakkar et al. (1984); Aebi (1974) ; Ellman (1959) and 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 software (Version 20; Untitled– SPSS Data Editor). The results were expressed as mean ± Standard deviation (mean ± S.D.). Data were analyzed using one way classification, analysis of variance.
The differences between means were tested for significance using the least significant difference (LSD) test at p<0.05 (Sendcor and Cochran, 1986).

RESULTS AND DISCUSSION

Data presented in Table (1) show the effect of mallow and Jew's mallow with high doses of lead on body weight gain (BWG). It could be noticed that differences between all mean values of these groups were significantly decreased when compared to the negative control group. With expecting, a 0.5% lead group was the lowest of body weight gain. There were no significant differences in BWG among groups 3 and 5. The best result was group 6 which fed on lead 0.5% and Jew's mallow at the level 10%.

Potential health problems associated with a high intake of products that contain a salt of Lead 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 vegetable fertilizer and meat additives provides a rising intake of lead which can contribute to weight loss and underweight. Also, the study done by Frasco et al. (2005) increased lead consumption would decrease total energy intake by decreased appetite and decreased fat intake. Dayal and Singh (2015) found that a high intake of Jew's mallow which used as food additives in soft foods has been linked to increasing body weight. This effect led to the high content of phenols as many vegetables and fruit to high doses of lead and mercury on serum urea and creatinine levels (mean ± SD). Regarding data present in the Table (1) show the effect of Mallow and Jew's mallow with high doses of lead on feed intake (FI) and feed efficiency ratio (FER). There are no significant differences in feed intake (FI) between group 6 and the negative control group. From the same table, it could be noted that the differences in values of feed intake between all treated groups were considered 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 following those reported by (Frasco et al. 2005) who found that the lead decreased appetite and decreased fat intake. Calliste et al. (2001) reported that Jew's mallow leaves are a source of antioxidants provitamin A and vitamin C that prevents damage caused by free radicals that may cause some forms of cancer.

According to data present in the same Table (1), it could be observed that there is no significant changes in feed efficiency ratio (FER) of group 3 and 4. These results denote that there were increases in feed efficiency ratio (FER) for group 5 when compared with the positive control group. From obtained results, it could be observed that treating rats with the tested calcium sources led to high doses of lead and kidney functions (serum urea and creatinine levels) It could be observed that the highest value of serum urea levels found in rats that received lead as a positive control group was significant when compared with all groups. Non-significant changes were found in serum urea level between groups 3, 4 and 5 also, there is no significant between groups 1 and 6.

The negative control group creatinine level was 0.46 ± 0.02 mg/dl which significantly decreased when compared with positive control and treated groups. Meanwhile, rats of groups 3, 4, and 5, creatinine level of these groups were non-significant between each other and showed significantly increased as compared to the negative control group. Groups 6 was the lowest creatinine value which showing a significant decreased as compared to the other groups and a significant increase when compared with control negative group.

Table 1. Effect of Mallow and Jew’s mallow to high doses of lead on body weight gain (BWG); feed intake (FI) and feed efficiency ratio (FER) (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>BWG g / 28 days</th>
<th>FER</th>
<th>FI (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (G1)</td>
<td>46.54± 4.21</td>
<td>0.124±0.001</td>
<td>11.71±0.22</td>
</tr>
<tr>
<td>Lead 0.5% as a positive control (G2)</td>
<td>46.1±0.13</td>
<td>0.058±0.001</td>
<td>2.83±0.11</td>
</tr>
<tr>
<td>Lead 0.5% and Mallow at the level 5% (G3)</td>
<td>10.74±0.21</td>
<td>0.081±0.002</td>
<td>4.76±0.12</td>
</tr>
<tr>
<td>Lead 0.5% and Mallow at the level 10% (G4)</td>
<td>19.5±1.11</td>
<td>0.081±0.002</td>
<td>8.68±0.27</td>
</tr>
<tr>
<td>Lead 0.5% and Jew’s mallow at the level 5% (G5)</td>
<td>16.68±1.2</td>
<td>0.104±0.001</td>
<td>5.7±0.24</td>
</tr>
<tr>
<td>Lead 0.5% and Jew’s mallow at the level 10% (G6)</td>
<td>25.15±2.19</td>
<td>0.091±0.002</td>
<td>9.86±0.37</td>
</tr>
<tr>
<td>LSD</td>
<td>3.48</td>
<td>0.016</td>
<td>2.32</td>
</tr>
</tbody>
</table>

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

Data in the Table (2) show the effect of mallow and Jew’s mallow to high doses of lead on kidney functions (serum urea and creatinine levels) It could be observed that the highest value of serum urea levels found in rats that received lead as a positive control group was significant when compared with all groups. Non-significant changes were found in serum urea level between groups 3, 4 and 5 also, there is no significant between groups 1 and 6.

The negative control group creatinine level was 0.46 ± 0.02 mg/dl which significantly decreased when compared with positive control and treated groups. Meanwhile, rats of groups 3, 4, and 5, creatinine level of these groups were non-significant between each other and showed significantly increased as compared to the negative control group. Groups 6 was the lowest creatinine value which showing a significant decreased as compared to the other groups and a significant increase when compared with control negative group.

Table 2. Effect of some vegetables and fruit to high doses of lead and mercury on serum urea and creatinine levels (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (G1)</td>
<td>36.3±4.13</td>
<td>0.46±0.32</td>
</tr>
<tr>
<td>Lead 0.5% as a positive control (G2)</td>
<td>47.33±1.21</td>
<td>1.45±0.42</td>
</tr>
<tr>
<td>Lead 0.5% and Mallow at the level 5% (G3)</td>
<td>43.33±2.12</td>
<td>1.33±0.25</td>
</tr>
<tr>
<td>Lead 0.5% and Mallow at the level 10% (G4)</td>
<td>41.6±1.4</td>
<td>1.31±0.64</td>
</tr>
<tr>
<td>Lead 0.5% and Jew’s mallow at the level 5% (G5)</td>
<td>41.23±1.72</td>
<td>1.27±0.05</td>
</tr>
<tr>
<td>Lead 0.5% and Jew’s mallow at the level 10% (G6)</td>
<td>39±2.7</td>
<td>1.15±0.04</td>
</tr>
<tr>
<td>LSD</td>
<td>3.03</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Means in the same column with different litters are significantly different (P <0.05).
These results agree with Zhang et al. (2005) who reported that high content of lead for a long time caused promote cellular apoptosis and necrosis in the liver and kidney. They found a negative correlation between the consumption of diets with mallow decreased the risks for chronic diseases such as cardiovascular diseases, arthritis, chronic inflammation, kidney disease, and cancers. Leaves of Jew’s mallow are a rich source of vitamins and minerals. Its leaves contain a high amount of vitamin A as (β-carotene) and vitamin C. It had a good source of thiamine, zinc and dietary fiber. Also, they found that the Jew’s mallow had protective effects in the kidney by restoring the normal kidney architecture (Dayal and Singh, 2015).

Finding presented in Table (3) show the effect of mallow and Jew’s mallow with a high dose of lead on levels of serum GOT and GPT.

It could be observed that the negative control group GOT was 38± 1.41 u/l which significantly increased in the positive control group which it was being 102 ± 4.14 u/l.

The level of GOT in groups 3, 4, 5, and 6 showed significant increases as compared to the negative control group and significant decreases in comparison the positive control group. Also, there were no significant changes between groups 4 and 5. The strongest effect in serum GOT levels recorded for group 6 which fed on a basal diet with 10% Jew’s mallow leaves.

The serum level of (GPT) in group 6 was the lowest level which being 57.66 ± 3.34 U/L. At the same time, rats that received a lead dose with 10%mallow and 5% of two plant leaves didn’t significantly different in serum level of GPT.

Edeoga et al. (2005) reported that lead had a potential role to cause injuries in several organs and tissues. The increased consumption of 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 oxidation. It could be hypothesized that increased lead sources exposure contributes to the development of non-alcoholic fatty liver disease (NAFLD) that can progress to cirrhosis over time in some individuals.

Calliste et al. (2001) showed that Jew’s mallow is one of the most important food which contain phenolic antioxidant compound and calcium (20.3% from Jew’s mallow leaves) which protected the liver from any free radicals.

Dayal and Singh (2015) found that Jew’s mallow extraction contained dietary fiber or essential oils, the flavonoids hesperidin and calcium which reduced the residual lead level and the degree of lipid oxidation.

Acute absorbed lead is distributed first to the blood where 98% of the lead becomes bound in the red blood cells; the remainder is available for redistribution to the soft tissues such as the liver, kidneys, lung, brain, muscles, and heart (Ide and Parker, 2005). Franket et al., (2005) investigated the influence of high dietary calcium source (Ca) (1.1%) on the biochemical and morphological manifestations of Pb and Zn toxicity, and to determine the effect of excess Zn on Pb toxicity. The results indicate that high dietary Ca source has a protective effect against the adverse effects of diet Pb and Zn, and that Zn aggravates Pb toxicity in growing pigs. It is known for many years that the amount of calcium in food influences tissue lead accumulation. Epidemiological studies on a general population have shown that calcium content in the food as mallow and Jew’s mallow is inversely correlated with lead in blood, bone or hair (Haser et al., 2016).

<table>
<thead>
<tr>
<th>Groups</th>
<th>GOT(U/L)</th>
<th>GPT(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (G1)</td>
<td>38±1.41</td>
<td>44±4.56</td>
</tr>
<tr>
<td>Lead 0.5% as a positive control (G2)</td>
<td>102±4.12</td>
<td>86±2.31</td>
</tr>
<tr>
<td>Lead 0.5% and Mallow at the level 5% (G3)</td>
<td>93±5.09</td>
<td>76±3.76</td>
</tr>
<tr>
<td>Lead 0.5% and Mallow at the level 10% (G4)</td>
<td>85±3.76</td>
<td>68.66±3.34</td>
</tr>
<tr>
<td>Lead 0.5% and Jew’s mallow at the level 5% (G5)</td>
<td>83±7.34</td>
<td>64±3.23</td>
</tr>
<tr>
<td>Lead 0.5% and Jew’s mallow at the level 10% (G6)</td>
<td>75±5.23</td>
<td>57.66±3.04</td>
</tr>
<tr>
<td>LSD</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

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

Histopathological examination

Liver:

Liver’s rats which fed on the basal diet show the normal histological (photo 1). In the photo (2), the liver’s rat which fed on a basal diet with lead 0.5% showed that congestion of central vein and hepatic sinusoids and kupffer cells activation and with local hepatic necrosis associated mononuclear cells infiltration. The liver’s rat which fed on a basal diet with lead 0.5% and 5% mallow showed that hydropic degeneration of hepatocytes. The liver’s rat which fed on a basal diet with 0.5% lead and mallow 10% showed that slight hydropic degeneration of hepatocytes (Photo 4). The liver’s rat which fed on a basal diet with lead 0.5% and Mallow 5% showed that slight activation of kupffer cells (Photo 5). The liver’s rat which fed on a basal diet with lead 0.5% and Mallow leaves 10% showed that no histopathological changes (Photo6).
Lead modulated the vasomotor action of soft muscle by its effect on Ca ATPase. It can affect the genetic reproduction of DNA by interaction with nucleic acid binding proteins with potential consequences for gene regulation. Acutely absorbed lead is distributed first to the blood where 98% of the lead becomes bound in the red blood cells; the remainder is available for redistribution to the soft tissues such as the liver, kidneys, lung, brain, muscles, and heart (Frasco et al., 2005). Jew's mallow is a rich source of vitamins and minerals. Its leaves contain a high amount of vitamin A (β-carotene) and vitamin C which protect the liver from toxic material as lead (Dayal and Singh, 2015).

Examination of bones

The positive control group recorded the highest incidence of lead poisoning (Subchondral sclerosis) and the emergence of common space (Narrow joint space) compared to a negative control group with no significant differences. Where the lowest incidence was recorded with 10 Molokia powder followed by Malva. The bone composition was significantly altered by this contaminant: the mineralization degree decreased as lead concentration levels increased. Histomorphometry of the growth plates of lead-exposed rats shows defective remodeling, altered growth plate thickness due to the loss of proliferating cells, and disorganization of the growth plate architecture. They found that histomorphometric measures of bone density (i.e., bone volume, trabecular number, and trabecular thickness) were lower in lead-exposed rats than controls. Lead-exposed rats (mean blood lead level 21 μg/dL) had lower DEXA-based BMD than controls. Other researchers have also shown that lead exposure decreases BMD or bone strength in rats, and inhibits fracture healing in mice (Gwiazda et al., 2005).
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Results of bones
From Photo (1) the BMD of the positive control group recorded the highest incidence of lead poisoning (Subchondral sclerosis), increased the medullary area, trabecular thickness, increased marrow area and the emergence of common space (Narrow joint space) while in photo (2) BMD of negative control group, there is no significant differences. In photos (3 and 4) which show the BMD of rats fed on 5% and 10% Mallow the lowest incidence and increase the mineral density and the strength of bone. In case of photos (5 and 6) of rats fed on 5% and 10% Jew's mallow respectively, show the highest bone density which near to the negative control group, decreased the medullary area, increased trabecular thickness and decreased marrow area. Gwiazda et al. (2005) showed that the bone composition was significantly altered by this contaminant. The mineralization degree decreased as lead concentration levels increased. Histomorphometry of the growth plates of lead-exposed rats shows defective remodeling, altered growth plate thickness due to the loss of proliferating cells, and disorganization of the growth plate architecture. They found that histomorphometric measures of bone density (i.e., bone volume, trabecular number, and trabecular thickness) were lower in lead-exposed rats than controls. Lead-exposed rats (mean blood lead level 21 (μg/dL.) had lower DEXA-based BMD than controls.

CONCLUSION
This study investigated the effect of Corchorus olitorius and Malva sylvestris on lead toxicity in albino rats. It could be concluded that rats infected with lead which fed on a diet with mallow and Jew's mallow leaves significant increase in body weight gain, feed intake and feed efficiency and improved the liver enzymes and kidney functions when compared with the positive control group. Treating rats with Corchorus olitorius and Malva sylvestris at 10% decreased the toxic effect of lead in rats' body on tested parameters, decreased the toxic changes in cells structure of liver and improved measures of bone density.

REFERENCES
التأثير البيولوجي لنباتات الملوخية ونباتات الخبيزة على السمية لمعن الصرص في الجسم الحي: "الجرذان"

باحث على تأثير بعض نباتات الملوخية ونباتات الخبيزة على السمية لمعن الصرص في الجسم الحي: "الجرذان"

 Millennium University, Mansoura, Egypt


