Protective Effect of *Taraxacum officinale* against Oxidative Damage Induced by lead (Pb) in Rats Exposed to Contaminated Diet

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**ABSTRACT**

This work aims to evaluate the protective effect of dandelion *Taraxacum officinale* against lead toxicity. Female wistar rats were exposed to a diet containing 600 mg lead acetate/Kg food (Pb), or combined with 20 g fresh dandelion leaves/kg food (Pb-DD) and then they have been compared to a control group for 6 consecutive weeks. Some hematological and serum biochemical markers were evaluated. The obtained results indicated a significant decrease in RBC and hemoglobin levels accompanied with a significant increase in methemoglobin of Pb group compared to the control. Regarding the group treated with Pb-DD, RBC counts and hemoglobin level were unchanged, but methemoglobin percentage was significantly higher than the control. Results of Pb group indicated a significant increase in the activities of AST, ALT and alkaline phosphate as well as in the level of total bilirubin and uric acid. However, dandelion supplementation has kept the previous markers within their biochemical ranges, except uric acid. On the other hand, no pronounced variation was seen concerning albumin, cholesterol, creatinine and calcium concentration neither in Pb nor in Pb-DD treated animals. Histological examinations of liver, kidney and ovary have showed no real change in the group treated with Pb-DD compared to control. Contrary, Pb has provoked a cystic structures and vacuolization of liver accompanied with necrosis and micro calcifications of kidney. Degeneration of the ovary tissue with an absence of corona radiata and an apoptosis of granulosa cells was also seen in Pb group. In conclusion, dandelion supplementation to diet contaminated with Pb has remarkably reduced the metal toxicity in female rats.

**INTRODUCTION**

In recent decades, the potential impacts of the environment on our health have become a major concern of our societies worldwide. Lead is considered to be one of the oxidative stress inducer in different cells and organs [1]. Recently, it has been reported that chronic exposure to lead has provoked hematological, reproductive, renal and hepatic disturbances [2, 3, 4, 5, and 6]. Liver, kidneys have been considered as the target organs for the toxic effects of lead [7]. Many factors contribute to the absorption of lead as the deficiencies of iron, calcium, phosphorus, zinc, vitamin B1, vitamin D, magnesium and fibers [8, 9]. There are various natural and safe ways to remove body wastes and toxins that disturb the normal functioning of organs. In the current study the wild herbaceous species of dandelion, known among botanists under the name of *Taraxacum officinale* has been chosen. such herb is used medicinally since the days of Arab physicians of the 11th and 12th centuries. In Europe, tradition dictates that dandelion is taken in the spring to clean the body after a long winter [10, 11]. The products of dandelion are indicated for the treatment of bile duct obstruction and some disorders of the gallbladder because it improves liver function and stimulates the secretion of bile. However, it can be used in the supportive treatment of gallstones, under supervision of a doctor [12]. Supplementation of this plant protects the cells from oxidative damage and plays vital role in detoxification [13, 12]. Root extracts were widely used as a diuretic to promote urinary excretion. Other disorders that were treated with this herb in the past include fever, insomnia, rheumatism, eczema and other skin conditions, cancers and tumors [14]. The chemical composition of...
this herb is rich of many important components such as flavonoids, phenols, carotene, phosphorus, calcium iron, magnesium, manganese, and sulfur with high potassium content [15].

The present investigation was conducted to evaluate the possible protective efficiency of *Taraxacum officinale* for lead toxicity on some hematological and serum biochemical markers and also on the histological profiles of liver, kidney and ovary of female wistar rats.

**MATERIALS AND METHODS**

In this experiment, three groups of female adult rats 175±12g comprising seven individual each were chosen and given standard diet obtained from ONAB (Bejaia). The first group was used as a control, while the second one has received 600 mg lead acetate/kg food, whereas the third group has received 600 mg lead acetate + 20 g dandelion/kg food) for a period of 6 weeks. *Taraxacum officinale* leaves were freshly collected from unpolluted site, cleaned with distilled water, homogenized in the laboratory and then immediately added to rat diet. Animals were sacrificed at the end of the experiment and the blood was immediately collected in polyethylene tubes containing the anticoagulant EDTA for the immediate measurement of hematological markers (red blood cell counts, haemoglobin and methemoglobin), and in dry tubes which have been centrifuged at 4000 rpm/min for 15 minutes, to evaluate serum biochemical markers (total bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, uric acid, creatinine, cholesterol and calcium). Biochemical markers were measured by an automated apparatus METROLAB 2300 (Random Access Clinical Analyzer). Rats then were dissected where liver; kidneys and ovaries were removed carefully, weighed and then fixed in the 10% formalin solution and treated according to the classical method of [16].

Statistics analysis was performed by using Students’-t-test to compare each of the treated group with that of the control with the Minitab program (version15). Results were presented as means ± SD. The significant difference at p<0.05 was considered.

**Results:**

**Haematological and biochemical markers:**

Results of the haematological and biochemical markers are presented in table 1. A significant decrease of red blood cell counts and hemoglobin concentration in the group exposed to Pb alone compared to the control was observed. On the other side, this difference was not significant between Pb-DD group and the control.

Concerning methemoglobin, results have indicated a significant increase of its percentage in females exposed to Pb and Pb-DD alike and that compared to the control.

Total bilirubin concentration showed a significant elevation in females treated with Pb compared to the control, but the Pb-DD group has showed only a slight increase. Albumine, cholesterol, calcium and creatinine concentrations were unchanged in both treated groups and that compared to the control.

Uric acid concentration was highly elevated in Pb group and also in Pb-DD group.

The transaminases (AST and ALT) and alkaline phosphatase were remarkably raised in Pb treated group, although they were statistically not different in Pb-DD group when compared to the control.

**Table 1:** Mean (±SD) hematological and biochemical markers of female treated for a period of 6 weeks.

<table>
<thead>
<tr>
<th>Markers (Means±SD)</th>
<th>Control (n=7)</th>
<th>Pb (n=7)</th>
<th>Pb+DD (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells(10^6/µl)</td>
<td>8.0±0.81</td>
<td>4.4±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9±1.34</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>10.5±2.37</td>
<td>8.83±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5±2.20</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td>1±0</td>
<td>3±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.83±0.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total bilirubin (mg/l)</td>
<td>10.28±0.75</td>
<td>12.62±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.24±0.85</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>40.10±13.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.42±5.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.28±1.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST (UI/L)</td>
<td>128±28.32</td>
<td>246±77.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102±16.2</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>51±17.45</td>
<td>170±35.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76±15.67</td>
</tr>
<tr>
<td>PAL (UI/L)</td>
<td>144±8.91</td>
<td>265±56.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>160±17.66</td>
</tr>
<tr>
<td>Uric acid (mg/g)</td>
<td>16.83±1.37</td>
<td>34.20±1.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.57±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/g)</td>
<td>10.10±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.97±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.29±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol (g/l)</td>
<td>4.35±1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.80±1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>119.28±12.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.10±8.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.16±8.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Line having different superscript letter are significantly different (p<0.05) when compared to the control.

**Histological study:**

Microscopic study of female rat liver indicates that in the control group, the tissue parenchyma within the liver cells is well organized. In the Pb group, the photograph shows a vacuolization and a cystic structure. In the combined group, the photograph shows an improvement in the hepatic parenchyma with the presence of few vacuoles (Fig 1).
Kidney:
Microscopic study of kidney showed the following:
Control group: The renal tubules and glomeruli are normal and the kidney tissue is well organised. Pb group: The photograph indicates a slight tubular necrosis and tubular microcalcification (beginning of lithiasis). Pb-DD group: Kidney tissue is almost the same as that of the control (Fig 2).

Ovary:
Microscopic study of the ovary indicates the following:
Control group: The ovarian tissue, follicles and oocytes are normal. Pb group: The empty Graafian follicles without oocytes (degeneration of the oocyte). Pb-DD group: Ovarian tissue is almost the same as that of the control (Fig 3).

Fig. 1: Histological study of the female rat liver showing the control, the Pb and the Pb-DD groups after 6 week treatments, with a magnification of x100 (left) and x400(right).
HC: hepatocyte; CY: cyst; V: vacuole.

Fig. 2: Histological study of the female rat kidney showing the control, the Pb and the Pb-DD groups after 6 week treatments, with a magnification of x100 (left) and x400(right).
GL: glomerule; MC: Microcalcification.
Fig. 3: Histological study of the female rat ovary showing the control, the Pb and the Pb-DD groups after 6 week treatments, with a magnification of x100 (left) and x400(right).

Discussion:

Lead poisoning is an insidious disease which is often detected late after being confused with other digestive, hepatic, hematologic and behavioral disorders where it reduces the activity of certain enzymes by binding their sulfhydryl groups, or even to replace other metal ions [5].

In this study, the exposure of female rats to a Pb contaminated diet has caused some kind of anemia that is characterized by a significant drop of red blood cell counts and hemoglobin level. Such anemia is probably due to the inhibition of heme production by this metal [17]. Red blood cells are very sensitive to lead in reducing their life span by nearly 20% and disrupting heme biosynthesis [18]. Pb inhibits the three enzymes responsible for heme synthesis; the heme: D-aminolevulinic acid dehydratase (ALAD), the coproporphyrinogen decarboxylase and the ferrochelatase [19]. Some studies have suggested that the damage induced by lead could be originated from oxidative stress due to the ALA accumulation, which could generate the formation of peroxide ion [20]. However, Pb was known to disturb the active transport of red blood cell membrane by inhibiting the Na/K ATPase [20]. As a result, the gradients of ions, the water exchange, the osmotic pressure between cell and its surrounding are no longer controlled, leading to cell deformations, until a possible rupture of membrane, causing its cell death by lysis [18, 21].

However, the anemia caused by lead is corrected in the group treated by dandelion. It seems therefore that this herb acts as an antidote due to the presence of polyphenols, manganese, sulfur, calcium, potassium, including the existence of inulin which allows a better assimilation of calcium and magnesium in the body. The herb is rich in vitamin B allowing the body to prevent anemia on the one hand [22], and on the other hand, it allows fighting the free radicals effectively, which are the most responsible of various diseases, by reducing Pb toxic effects [15].

The actual results show a remarkable increase of methemoglobin in female rats contaminated with lead, which is probably due to the effects of oxidative stress induced by this metal [23]. The supplementation of dandelion has reduced the methemoglobin percentage slightly. Accordingly, Kemper et al., [48] have shown that the treatment of rats by dandelion had preserved methemoglobin rate. This effect is certainly due to the chemical composition of this herb, which can cause a reduction of methemoglobin by transforming the ferric iron (Fe³⁺) of hemoglobin to ferrous iron atom (Fe²⁺) [23]. However, acquired methemoglobinemia can result from the absorption of oxidising agents as Pb.

The high bilirubin concentration in female rats of the Pb group is in parallel with the significant drop of red blood cell counts and hemoglobin level, indicating some red blood cell lysis resulted from lead intoxication. However, this result agrees with that reported earlier concerning bilirubin level in female rats [24]. Lead can damage the hepatocytes’ baso-lateral membrane, which contain the bilirubin and glutathione transporter protein MRP2 (multidrug resistance-associated protein2) [25], affecting therefore the normal functioning of the latter, leading to the reduction of its level, causing hyperbilirubinemia [26].
On the other hand, rats treated by the combination of Pb-DD have normal level of serum bilirubin. It has long been shown to use dandelion for the stimulation of bile secretion [27], as it was reported to increase bile production in dogs and rats [28, 29]. Moreover, the latex of dandelion bars would have a specific action on the gallbladder and may also be a suitable solvent to treat the gallstones.

The Pb contaminated diet has no substantial effect on serum albumin level synthesized by liver, though that this metal can interact with many proteins by binding reversibly with thiol groups or by inhibiting the initiation of protein synthesis at the ribosomes [30].

The present study has showed a notable increase of serum transaminases and alkaline phosphatase activities of rats exposed to lead. These enzymes, however rises in the serum following a lesion of various organs mainly liver. The presence of dandelion has preserved the activity of these enzymes, probably by reducing hepatic injuries provoked by lead. Such herb is thought to stimulate the elimination of bile, which improves the liver's ability to clean up the blood and reabsorb nutrients [22]. An in vitro test using rat liver homogenate has demonstrated that dandelion leaf extract has an action against the free radicals higher than the extract of roots [31].

Cholesterol concentration in this study seems unaffected after the exposure of females to the Pb contaminated diet. Accordingly, plasma cholesterol (HDL) and triglyceride levels have not been affected in rats exposed to Pb for a period of two weeks [42]. Contrary, it was found that the exposure of rats to Pb has remarkably elevated serum cholesterol concentration [43]. Moreover, dandelion was beneficial even for diabetic rats, where noticeable decline of triglycerides and cholesterol were recorded [44].

It looks in this study that Pb intoxicated females have a non significant decrease in the concentration of serum calcium, which could be explained on the basis that Pb ions might bind to different tissues where they compete with calcium ions [45, 46, and 47]. Apparently, calcium level of females received the combined treatment of Pb and dandelion was close to that of the control group, which is an indication of the richness of this herb in many components capable of chelating Pb as that of sulfur and zinc.

A significant increase in uric acid level was recorded in rats exposed to lead. Even the group supplied with dandelion showed a significant increase despite the diuretic effect of this herb. Uric acid is a substance which results from the degradation of nucleic acids. It has been confirmed that Pb reduced the urinary excretion of uric acid and there were a positive correlation between blood lead levels and uric acid [33]. In cases of an acute poisoning, lead causes lesions to the kidneys and exactly at the proximal tubes, a hypophosphatemia and glucosuria or (Fanconi syndrome) have been recorded [34 and 35] whereas the intense chronic exposure to Pb caused a nephroslcerotic problem and a cortical atrophy [37, 38, and 39]. Moreover, [40] have reported from workers occupationally exposed to lead (PbB 160-320 mg/l) an elevation of uremia and a reduction in creatinine clearance. Renal failure can be occurred to workers occupationally exposed to high doses of Pb for long period [41].

The gross organ pictures of liver has recorded a number of 3 to 7 cysts/liver with a diameter ranges from 0.5 to 2.0 cm in rats intoxicated with Pb. Such cysts are completely absent in the control and even in the Pb-DD group. Moreover, liver histological study of the Pb group is marked with vacuolization and cystic structure, but it has a remarkable improvement in its parenchyma with the presence only of few vacuoles in the Pb-DD treated group; this is undoubtedly due to the beneficial effect of this herb. Such results are in line with the perturbation observed in the liver enzymes. Moreover, Pb had provoked cancer and hepatocytolysis in mice, hamsters and rabbits and even gliomas in rats [32].

The histological profile of this study indicates that rats exposed to Pb presents a minimal tubular necrosis and tubular microcalcification, perhaps it is the beginning of nephrolithiasis, which is a disease characterized by the presence of stones in the excretory channel of kidneys. Interestingly, rats supplied with a combination of lead and dandelion have a homogeneous and normal kidney tissue structure.

The histological study in this work indicates that rats exposed to Pb presents a minimal tubular necrosis and tubular microcalcification, it is the beginning of nephrolithiasis, which is a disease characterized by the presence of stones in the excretory channel of kidneys. It is assumed that the cause of the lithiasis is due to metabolic disorders of the body. Interestingly, rats supplied with a combination of lead and dandelion have a homogeneous and normal kidney tissue structure.

The female reproductive tract is a witness of Pb intoxication, where it was marked with follicular alteration (atresia) and oocyte degenerations as well. Such results are in accordance with the works of [37, 20 and 7]. Nevertheless, there has been an improvement of the ovarian tissue in the group given dandelion fresh leaves, thanks to the protective role of this herb.

Conclusions:

It can be concluded that Pb contaminated diet provoked haematological and certain biochemical perturbations of female rats. Pb has also altered the histological structure of liver, kidney and the ovary. On the other hand, dandelion supplementation has generally reduced such alterations. It can be concluded that
dendelion leaves are a cheap natural protective herb that may play a beneficial role in the prevention and in the phytotherapy of lead intoxication.

ACKNOWLEDGMENTS

The research present the national research project (PNR) funded by the Ministry. We thank the head of the laboratory animal eco physiology Pr. BOULAKOUD M. and KHALILI K. and for helpful assistance.

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