Evaluation of the Chelating Efficacy of *Aquilaria malaccensis* and *Aristolochia longa* Against Biochemical Alterations Induced by Lead Bioaccumulation in Rats

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**Abstract**

The current study was evaluated the chelating effect of *Aquilaria malaccensis* and *Aristolochia longa* against lead induced biochemical alterations and bioaccumulation of lead in tissues of rats. 25 adult female Wistar albino rats, equally divided into control and four treated groups, received either lead, lead + *A. malaccensis*, lead + *A. longa* and lead + *A. malaccensis* + *A. longa* lead (100 mg/kg b.w) as Pb(C₂H₃O₂)₂ added in their drinking water for 75 days. *A. malaccensis* and *A. longa* (at a dose 1% of diet) were added to the feed during the last 15 days of lead exposure in the animals. Result showed that in lead–intoxicated rats, an increase of lead accumulation in serum, bone and liver of rats. Results also revealed that lead affected metabolic system by increasing blood glucose and serum urea concentrations and decreasing serum calcium concentration. Treatment with *A. malaccensis* and *A. longa* alone or combined significantly reduced the adverse effects related to most of serum and tissue lead accumulation and restored biochemical parameters alterations in animals treated with lead. The present study shows that *A. malaccensis* and *A. longa* are an effective chelating agents for the removal of lead and it has proved efficient in restoring both the biochemical alteration and toxicity after a sub lethal exposure of lead acetate in rats.

**Keywords:** *Aquilaria malaccensis*, *Aristolochia longa*, Chelating Agents, Lead Toxicity, Rats

1 Introduction

Lead is an environmental pollutant that has a toxic effect on humans. Lead has been used for a long time in the metal products industry, pipelines, cables, as well as in paints and pesticides¹. The lead-contaminated bio systems have undergone a massive modification at the cellular, molecular and functional levels. The mammal is a target of lead toxicity causing damage in different behavioral, metabolic and physiological systems². The study of Burroughs and Rollins (2017), showed that lead is responsible for several cardiovascular diseases like hypertension⁵. The liver and kidney are preferred targets for many xenobiotics including lead which is bioaccumulated in the liver in a very important way (33%), oxidative stress is an important agent responsible for different liver tissue damage by lipid peroxidation (LPO) induced by reactive oxygen species (ROS)⁶. The medicinal plants have pharmacological property against several diseases by their richness in antioxidant and bioactive substances like polyphenol, vitamins, ternenoids, and other secondary metabolites⁷. *Aquilaria malaccensis* and *Aristolochia longa* are very well known plants by their use in the popular medicine against cancer in several regions in Algeria. *A. malaccensis* has numerous biological activities, including activity, anti-microbial, anti-tumor, anti-allergic, anti-oxidant⁸. *Aristolochia longa* L. (Aristolochiaceae) known in our region “Beroustoum” is a medicinal plant very used by women against ovarian insufficiency, also for cicatrization and diuretic and also used for the analgesic and anti-inflammatory effect⁹. The present study evaluated microstructural changes following simultaneous administration of powder of rhizome of *Aristolochia longa* on lead–induced metabolic disorders and lead bioaccumulation in tissues of rats.

2 Materials and Methods

2.1 Animals and Handling

Twenty five adult females albino rats, weighing 224–230 g, were brought from the animal house of Pasteur institute, Algeria.
They were placed in three groups of 5 rats in each and kept in animal’s house of Molecular and cellular biology Department, University of El Oued, Algeria. Standard rat food and tap water were available ad libitum for the duration of the experiments. Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12 h light/12 h dark) with a relative humidity 62.3 % and room temperature of 25 ± 2 C⁰.

The experimental procedures were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our Institution.

2.2 Experimental design

The experiment was conducted over a period of 10 weeks. After a period of adaptation, the animals, at the age of 08 weeks, were divided into five experimental groups of 5 animals each: the control group was not treated with Pb and the remaining four experimental groups received Lead + Aquilaria malaccensis (Lead +Aq), Lead + Aristolochia longa (Lead +Ar). and (Lead+Aq+Ar). lead (100 mg/kg b.w) as Pb(C2H3O2)2 added in their drinking water for 75 days. Aquilaria malaccensis (heartwood powder) and Aristolochia longa (roots powder) were added to the feed (at a dose 1% of diet ) during the last 15 days of lead exposure in the animals. Evaluate body weight were monitored during the whole experiment.

2.3 Preparation of serum and tissue samples

At the end of 2 weeks of Aquilaria malaccensis and Aristolochia longa treatment, rats were fasted for 16 hrs, anaesthetized with chloroform by inhalation, rats were decapitated and blood was transferred into non-heparinized tubes for serum biochemical analysis. Serum was obtained by centrifugation of the blood at 3000 rpm for 10 min and then quickly frozen at −20C⁰ until used. Liver and bone samples were rapidly excised, weighed and rinsed in ice cold saline (0.9% NaCl (w/v) and treated for measurement of lead level.

2.4 Measurement of biochemical parameters

Blood glucose, serum urea, serum protein and serum Calcium levels were determined by use commercial kit from Spinreact, Spain (ref: glucose-20121, urea-20141, calcium: 20051 and total proteins-1001291).

2.5 Determination of lead concentration

2.5.1 In the serum samples

In the serum samples, lead was determined after 10-fold dilution. In this case, the lead standards were prepared from a 1 mg/mL lead acetate standard solution. All tubes were soaked in HNO₃ (10% v/v)³.

2.5.2 In the tissues samples

Dry calcination of liver and bone is carried out in a muffle furnace at a temperature of 600 °C for 6 hours. The ash obtained is dissolved by an attack of 3ml of pure nitric acid (HNO₃). The liquid obtained is filtered on filter paper in a 20 ml flask and completed to its final volume with the demineralized water. For the lead assay, lead standards are prepared from a 1000 ppm stock solution, using a nitric acid (1%) solution for dilution³.

2.6 Statistical Analysis

Carried out by using 1-way analysis of variance (ANOVA) followed by Dunet’s test to compare means among the groups. Differences were considered statically significant at p<0.05.

3 Results

3.1 Initial body weight, body weight gain and relative liver weight

Pb(C2H3O2)2 treatment at a dose (100 mg/kg b.w) caused a decrease (p<0.05) in body weight and an increase in Relative liver weight in the rats compared to the control rats. Whereas the animals that received the powder of rhizome A. longa and heartwood A. malaccensis alone or combined showed partial reversion of this change (Fig 1).

3.2 Lead concentration

Table 2 shows a significant increase (p <0.05) in the concentration of lead in serum, bone and liver in lead-contaminated rats compared to control rats. Treatment with A. malaccensis powder decreased significantly (p <0.05) the concentration of lead in serum, bone and liver. On the other hand the administration of A. longa powder in lead exposed rats significantly reduced (p <0.05) the concentration of lead in serum and bone but not in the liver. Co-treatment with powder of both plants decreased (p <0.05) the lead concentration in serum, bone and liver compared with lead group.

3.3 Biochemical parameters

Our results (Table 3) show a significant increase (p <0.05) in blood glucose and serum urea concentration and a very highly significant (p <0.001) decrease in serum calcium in the lead group compared with the control. But no change in the serum protein concentration has been reported. On the other hand, in the A. malaccensis and A. longa treatment groups, we observed a significant decrease in blood glucose level (p <0.01 and p <0.05) and serum urea concentration (p<0.001), a significant increase (p <0.05 and p <0.01) in serum calcium level respectively and no significant effect on serum protein concentration in comparison with the lead group. For rats treated with both plants, the results indicate an effect similar to the effect of two separate plants in comparison with the lead group.

4 Discussions

Through this study, we investigated the potential benefit of treatment with rhizome powder of A. longa in reversing Pb-induced liver oxidative stress in rats orally exposed to Pb.
In our study, we observed that lead administration resulted in a striking reduction in body weight of the albino rats. Similar types of findings were observed in a study conducted by Reckziegel et al. (2016)\(^1\).

Fig 1: Mean initial body weight (g), final body weight (g) and relative liver weight of control and experimental rats

Values are mean ± SEM, n=5: number of observations. *p<0.05 significantly different from control group. a p<0.05 significantly different from Lead group

### Table 2: Mean lead concentration in serum, bone and liver of control and experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Lead</th>
<th>Lead + Aq</th>
<th>Lead + Ar</th>
<th>Lead + Aq+Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Pb (µg/l)</td>
<td>27.42±2.25</td>
<td>54.28±5.27</td>
<td>35.25±6.51</td>
<td>30.36±6.02</td>
<td>32.22±8.3</td>
</tr>
<tr>
<td>Bone Pb (µg/g)</td>
<td>2.73±1.25</td>
<td>204.31±1.57</td>
<td>144.6±21.5</td>
<td>155.68±6.02</td>
<td>176.3±10.8</td>
</tr>
<tr>
<td>Liver Pb (µg/g)</td>
<td>20.00±2.89</td>
<td>55.49±6.06</td>
<td>42.07±4.92</td>
<td>54.12±8.22</td>
<td>27.89±6.06</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5: number of observations. *p<0.05: significantly different from control group. a p<0.05: significantly different from Lead group

### Table 3: Mean blood glucose, urea, protein and calcium concentration in serum of control and experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Lead</th>
<th>Lead + Aq</th>
<th>Lead + Ar</th>
<th>Lead + Aq+Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (g/l)</td>
<td>0.752±0.051</td>
<td>0.905±0.107</td>
<td>0.805±0.053</td>
<td>0.805±0.067</td>
<td>0.775±0.027</td>
</tr>
<tr>
<td>Serum urea (g/l)</td>
<td>0.64±0.067</td>
<td>0.81±0.085</td>
<td>0.60±0.037</td>
<td>0.67±0.030</td>
<td>0.67±0.033</td>
</tr>
<tr>
<td>Serum protein (g/l)</td>
<td>50.6±1.40</td>
<td>48.33±1.76</td>
<td>47.60±2.60</td>
<td>48.2±1.12</td>
<td>47.4±1.53</td>
</tr>
<tr>
<td>Serum Calcium (mg/l)</td>
<td>122.5±0.95</td>
<td>105.67±0.44</td>
<td>115±0.97</td>
<td>125.6±0.62</td>
<td>116.2±0.61</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=5: number of observations. *p<0.05: significantly different from control group. a p<0.05: significantly different from Lead group

However, it is possible to explain the drop in body weight due to an eating disorder (anorexia) induced by heavy metal contamination such as lead in our study\(^1\) or explain by lead induced oxidative stress causing a decrease in muscle mass and adipose (cachexia) that reflect directly on body weight\(^1\). After the treatment time, the animals that received \(A.\) malaccensis and \(A.\) longa showed reversion partial of this less body weight, due probably the improvement of behavior and biochemical parameters. In our result, the elevation of the lead-induced hepatic hypertrophy has been shown to cause necrosis due to lipid accumulation\(^1\). However, the \(A.\) malaccensis and \(A.\) longa treatment reverted partially this change. The richness of the plants by phenolic compounds, steroids and flavonoids have concerning the lead analysis at the tissue level, anti-inflammatory character it is probably the origin of this improvement\(^1\). The results obtained show a significant increase in the level of Pb in serum, bone and liver tissue, which reflects on the accumulation (or fixation) of this metal on these targets tissues. We found that lead concentrations in bone are higher than in serum and liver. The bone behaves like a cumulative dosimeter of lead in intoxication, which has become a good marker for assessing the level of Pb pollution and especially the duration of exposure\(^1\). Lead contamination of bone can be explained by several methods, lead has a high affinity for calcium binding sites and can therefore replace calcium in bone. Indeed, delayed calcification and osteoporosis attributable to the inhibitory effect of lead on intestinal calcium absorption on one side and competition between lead and calcium in bone formation on the other hand, by reducing calcium deposits\(^1\). On the other hand, the administration of \(A.\) malaccensis and / or \(A.\) longa of lead resulted in a significant improvement in serum calcium concentration in treated rats, which is probably due to the anti-inflammatory effect of lead on intestinal calcium absorption.

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longa has reduced the level of lead in serum, bone and liver tissues in favor of a detoxification process. Indeed, this reduction of lead means that these plants have beneficial effects against the accumulation of lead in the body and therefore can be considered as chelating agents of Pb. These effects of plants are explained by the reduction of absorption of lead in the gastrointestinal tract or increased excretion. As a result, reduced lead absorption promotes bone growth. For blood glucose, the results obtained show a significant increase in the concentration of serum glucose in rats contaminated with lead acetate compared to control rats. Lead competes with calcium-dependent metabolic processes and more precisely insulin excretion by inhibitory action on voltage-gated calcium channels and by decreased activity of adrenylate cyclase.

Our results also demonstrated a significant decrease in blood glucose concentration in rats treated with A. malaccensis and A. longa in combination more (than other treatments) compared to the Pb group. Hypoglycemia may be due either to increased insulinemia or elevated blood glucose transport to peripheral tissues. Many investigations have reported that polyphenol compounds, phenol and flavonoids are responsible for hypoglycemic activity and other pharmacological activities of plants. Compared to the control, the results show a remarkable decrease in serum calcium level of lead exposed rats. These results are in agreement with those published by Missoun et al. (2010), who demonstrated a significant decrease in blood calcium levels in rats exposed to 1000 ppm of lead acetate for 90 days.

Our results may be due to the competition between lead and calcium for the same sites in the body by several mechanisms; it inhibits the gastrointestinal absorption of Ca\(^{2+}\), increases the excretion of Ca\(^{2+}\) by the kidneys, also Pb\(^{2+}\) and Ca\(^{2+}\) interact for storage in the bone, which leads to the alteration of calcium homeostasis. The improvement effect of the A. malaccensis and A. longa against the reduction of calcium shows the effect of chelating of these plants against the accumulation of lead in organs which decreases their level which improves the level of body calcium.

5 Conclusion

Data from this study suggest that rhizome powder A. longa treatment attenuates lead induced liver and bone toxicity and lead accumulation. A. malaccensis and A. longa could serve as a true functional food and may positively affect health promotion via reducing lead toxicity.

6 Conflict of interest

We declare that we have no conflict of interest.

7 Author’s contributions

SD and KZ performed whole experimental procedures. All authors read and approved the final manuscript.

8 Acknowledgements

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9 References


