Beneficial Effects of Aristolochia Longa and Aquilaria Malaccensis on Lead-Induced Hematological Alterations and Heart Oxidative Stress in Rats

Derouiche Samir*, Zeghib Khaoula, Gharbi Safa and Khelef Yahia

Department of Cellular and Molecular Biology, Faculty of natural science and life, University of Echahid Hamma Lakhdar-Eloued, El-oued, Algeria

ABSTRACT

The current studies evaluated whether powder A. longa and A. malaccensis (widely used in Algerian traditional medicine) has the ability to protect against lead induced haematological changes and heart oxidative stress in rats. Twenty five female rats (Wistar albino) equally divided into five groups; control and four treated groups, received either Pb, Pb + Aristolochia (Ar), Pb+ Aquilaria (Aq) and Pb+Ar+Aq. lead acetate (100mg / kg bw) is exposed for 75 days and the duration of treatment with plants (1% of diet) is 15 days. some biochemical and hematological parameters are analysed. Phytochemical screening results revealed that A. longa and A. Malaccensis aqueous extract contained various bioactive compounds, including polyphenols, saponins, terpenoids, glycosides and flavonoids. Lead acetate exposure caused a significant decrease in the red blood cell count, hemoglobin concentration, granulocytes count and heart GSH level, GST and HGOT heart activities and a significant augmentation in the heart MDA level, heart CAT and serum GOT activities in rats . Our results revealed that treatment with Aquilaria and aristolochia causes a partial correction of all of this parameters. In Conclusion, Results confirmed the beneficial effects of A. longa and A. malaccensis treatment in Pb-induced oxidative stress in heart and suggest that Aristolochia longa could therefore be considered a promising source of novel treatments for heart alteration.

Keywords: A. Longa; A. Malaccensis; Heart; Lead; Oxidative stress

INTRODUCTION

Lead (Pb) is a non-essential toxic heavy metal widely distributed in the environment; various dysfunctions in physiological, biochemical and behavioral systems are induced by the exposure of the minute concentration of lead in a chronic way [1]. Lead is known to induce a broad range of physiological, biochemical, and behavioral dysfunctions in laboratory animals and humans, including central and peripheral nervous systems, haemopoietic system, cardiovascular system, hepatic, and kidney [2]. Previous studies have reported that exposure to low level Pb has been associated with several disease outcomes such as cardiovascular disease and hypertension [3]. A positive link between lead poisoning and cardiovascular disease, including peripheral arterial disease and fatal coronary heart disease and stroke. In addition tibia lead was significantly correlated with an increased odds ratio of intra-ventricular conduction defect [4]. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress [5]. The toxic heavy metals increase the libration of free radicals and decrease the activity antioxidants which cause oxidative stress and damage in the cells [6]. Several data show that metals are capable of associating with nuclear proteins and DNA, resulting in oxidation and alteration of biological macromolecules, eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes [7,8]. Thus, there has been an increased interest in the therapeutic potential of plant products or medicinal plants having antioxidant properties in reducing free radical-induced tissue injury, such as vitamins, phenolic compounds, nitrogen compounds, terpenoids, and some other secondary metabolites have potent antioxidant activity [9]. Aristolochia longa L. (Aristolochiaceae) locally called “Beroustoum” is a species commonly used in Algerian traditional medicine. It has multiple applications and virtues; it is recommended for ovarian failure, healing, diuretic, analgesic, anti-inflammatory, anti-mitotic [10]. Aquilaria malaccensis (Agarwood) is a species widely distributed south-east Asia and considered to have a broad spectrum of therapeutic effects. These include
antioxidant activities, analgesic, anti-inflammatory, antihyperglycemic, and antimicrobial [11]. The aim of the present study was to explore the benefit effect of Aristolochia longa and Aquilaria malaccensis on hematological alteration and heart toxicity induced by acetate lead and to evaluate phytochemical composition and some antioxidant parameters.

EXPERIMENTAL SECTION

Collection and Preparation of the Aqueous Extract
The roots of A. Longu and the heartwood of A. malaccensis were collected at herbalists' stores of a local El-oued market. The plants were washed with distilled water, then dried at room temperature for 48 to 92 hrs, then ground into powder and stored at room temperature until use. The aqueous extract was prepared by adding 500 ml of distilled water to 50 g dry powder of A. longa or A. malaccensis. After 24 h of maceration at room temperature, the mixture was filtered by filter paper and then dried in a rotary evaporator under vacuum [12].

Phytochemical Screening
In our study Mantta and Usman methods [13] were used to identify the different phytochemicals in extracts such as flavonoids, alkaloids, saponins, tannins, terpenoids and glycosides.

Animals and Treatment
Twenty five adult females rats, Wistar albino, with weight 224–230 g, were brought from the Animal Service of the Pasteur Institute in Algeria. The animals were installed in the faculty SNV University of El Oued, Algeria, in plastic cages in five groups of 5 rats each. Standard feeding of rats and water was available at all times. The aqueous extract was prepared in accordance with National Institute of Health guidelines for animal care and approved by the ethics committee of our institution.

Experimental Design
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 either lead (Pb), Pb + Aristolochia longa, Pb + Aquilaria malaccensis, or Pb + AL+AM. Lead (100 mg/kg b.w) as Pb(C2H3O2)2 added in their drinking water for 75 days. Rhizome powder of Aristolochia longa (Ar) (at a dose 1% of diet) and heartwood powder of Aquilaria malaccensis (Aq) (at a dose 1% of diet) were supplemented to the diet during the 15 days after lead exposure in the animals. Evaluate the food intake; drinking water and body weight were monitored during the whole experiment.

Blood Collection and Preparation of Tissue Samples
At the end of 2 weeks of Aristolochia longa and Aquilaria malaccensis the animals were fasted for 16 hours, anesthetized with chloroform by inhalation and then sacrificed by decapitation and blood was transferred into EDTA tubes for haematological studies and in non-heparinised tubes for serum GOT analysis. The blood obtained is centrifuged at 3000 rpm for 10 min and the serum is recovered and then rapidly frozen at -20 °C until use. After dissection, the heart was rapidly excised, washed, weighed and stored at -20 °C to analyze oxidative stress markers.

Measurement of Biochemical and Oxidative Stress Markers
Serum and heart GOT activities were determined by commercial kits from Spinreact (Girona, Spain) (GOT-1001161). Heart Malondialdehyde (MDA) was measured according to the method described by Sastre et al. [15]. The concentration of reduced Glutathione (GSH) in heart was performed with the method described by Ellman [16]. Glutathione-S-transferase (GST) and Catalase (CAT) activities in heart were measured by the method of Habig et al. [17] and Sinha [18] respectively.

Statistical Analysis
Statistical Analysis was performed using Student's t-test to compare the means of different groups. The differences were considered statistically significant at p<0.05.
RESULTS AND DISCUSSION

Phytochemical Screening
Results of Phytochemical analysis showed the existence of alkaloids, glycosides, phenolic compounds, tannins, flavonoids, Terpenoids and saponins in the extract plants(Table 1). These compounds are antioxidant activities which could play a major role in the capture of free radicals induced by heavy metal such as lead [19].

Table 1: Phytochemical composition of water extracts of rhizome Aristolochia longa and heartwood Aquilaria malaccensis

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>A. longa</th>
<th>A. malaccensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Body Weight and Relative Heart Weight
Pb(C2H3O2)2 exposure at a dose (100 mg/kg b.w) leads a significant diminution (p<0.001) in body weight and a rise in Relative heart weight compared to the control rats. Whereas A. malaccensis or A. longa treatment corrected these changes (Table 2). Similar types of findings were observed in a study conducted by Reckziegel et al. [20]. However, overall loss of body weight with continuous exposure to lead might be explained on the basis of anorexia which is induced by heavy metal ingestion [21]. Another possible explanation for the loss of body weight may be the decreased muscle mass and cachexia due to the oxidative stress induced by lead [22]. After the treatment time, the animals that received A. longa and A. malaccensis showed a partial amelioration of this less body weight, may be due to the improvement of behavior and metabolic parameters. Our study detected elevation in the relative heart weight of Pb group was thought to be due to necrosis which was attributed to accumulation of lipid [23]. This is consistent with our pathological result as lead acetate intake caused myocardial necrosis of Pb group. However, the A. longa and A. malaccensis treatments reverted partially this change. This improvement is probably due to the inhibitory effect of these plants against the accumulation of lead in heart which reduces their harmful effect on heart and therefore decreases their relative weight. Also, the beneficial effect of these plants could be due to the presence of anti-inflammatory compounds such as flavonoids and phenolic acids [24].

Table 2: Mean initial Body weight (g), final Body weight (g) and relative heart weight of control and experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=5)</th>
<th>Pb (n=5)</th>
<th>Pb+ Aq (n=5)</th>
<th>Pb+ Ar (n=5)</th>
<th>Pb+Aq+Ar (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Body weight</td>
<td>225±5.06</td>
<td>225.2±5.69</td>
<td>226.60±23.71</td>
<td>221±8.76</td>
<td>225.2±3.51</td>
</tr>
<tr>
<td>Final Body weight</td>
<td>291±3.07</td>
<td>198±2.32***</td>
<td>196±5.00***</td>
<td>215±1.15* a</td>
<td>231±1.61* a</td>
</tr>
<tr>
<td>Relative heart weight</td>
<td>0.36±0.0007</td>
<td>0.44±0.024***</td>
<td>0.31±0.007*** c</td>
<td>0.32±0.024c</td>
<td>0.33±0.015* c</td>
</tr>
</tbody>
</table>

Caption: * p<0.05, ** p<0.01, ***p<0.001: significantly different from control group; a p<0.05, c p<0.001: significantly different from Pb group
Hematological Parameters and GOT Activity
As shown in Table 3, Pb induced a significant decrease (p<0.001) in RBC, HGB, GRN and HGOT activity and a significant rise (p<0.05) in SGOT activity compared to control. Treatment with powder of heartwood A. malaccensis or of rhizome A. longa partially restored the levels of RBC, HGB, GRN and HGOT and SGOT activities. Similar results have been reported by Amal et al. [25] who reported the effect of exposure by lead and anemia. Lead causes anemia by disrupting the synthesis of heme and destroying red blood cells [26]. On the other hand, lead-induced iron deficiency through competitive inhibition is a proven cause of anemia [27]. Indeed, in our study a protective effect of A. malaccensis and A. longa was reported against hematotoxicity and indicates that these plants can be protected against lead anemia. The results of heart and serum GOT activities indicate that A. malaccensis and A. longa are capable of stabilizing the plasma membrane as well as repair of tissue damage caused by lead acetate.

Table 3: Hematological parameters and GOT activity in heart and serum of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=5)</th>
<th>Pb (n=5)</th>
<th>Pb+ Aq (n=5)</th>
<th>Pb+ Ar (n=5)</th>
<th>Pb+Aq+Ar (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB (g/dL)</td>
<td>13.0±0.141</td>
<td>11.35±0.184***</td>
<td>11.82±0.16***b</td>
<td>11.8±0.17***b</td>
<td>11.37±0.22***b</td>
</tr>
<tr>
<td>RBC (10^6/µL)</td>
<td>7.36±0.042</td>
<td>6.87±0.02***c</td>
<td>7.36±0.047c</td>
<td>7.458±0.12 c</td>
<td>7.173±0.14a</td>
</tr>
<tr>
<td>GRN (10^3/µl)</td>
<td>0.825±0.094</td>
<td>0.80±0***</td>
<td>1.375±0.126**b</td>
<td>0.975±0.0726a</td>
<td>0.725±0.031*a</td>
</tr>
<tr>
<td>SGOT (U/l)</td>
<td>205.8±10.3</td>
<td>218.7±20.7*</td>
<td>211.67±4.16*</td>
<td>208.0±4.62a</td>
<td>220.0±8.08*</td>
</tr>
<tr>
<td>HGOT (U/l)</td>
<td>37.8±4.96</td>
<td>8.80±1.06***</td>
<td>17.8±0.929***c</td>
<td>21.6±2.05 ***c</td>
<td>20.40±1.71***c</td>
</tr>
</tbody>
</table>

Caption: RBC: Red blood cell count; HGB:Haemoglobin concentration; GRN: Granulocyte; SGOT: serum glutamic-oxaloacetic transaminase; HGOT: heart glutamic-oxaloacetic transaminase; *p<0.05, ** p<0.01, ***p<0.001: significantly different from control group; a p<0.05, b p<0.01, c p<0.001: significantly different from Pb group

MDA Concentrations
As showed in Figure 1, results indicate a significant (p<0.05) augmentation in MDA concentrations in heart tissues after Pb treatment for 75 consecutive days compared to the control group. The treatment of Pb exposed rats with A. malaccensis or A. longa decrease significantly the MDA heart level (p<0.001 and p<0.05) respectively as compared to the Pb exposed group. Interestingly, our results showed that co-treatment with A. malaccensis and A. longa partially reversed this change (p=0.001). From the results of our study it is seen that exposure of lead to rats caused a significant rise in lipid peroxidation as indicated by increase in MDA. Our results also corroborate well with that of Markiewicz et al. [28] who demonstrated that lead increases the rate of lipid peroxidation in heart. Oxidative stress is caused by a relative overload of oxidants, reactive oxygen species [29]. Lead causes oxidative stress through the generation of ROS, including hydroperoxides, singlet oxygen and hydrogen peroxide. and by inhibition of antioxidant system [30]. Pb stimulated lipid peroxidation resulted in the formation of aldehydic by-products, which in turn caused a decrease in reduced glutathione content. In addition, the higher level of heart MDA of Pb group indicates increased lipoperoxidation and potential neuronal membrane damage. In fact, the increased lipid peroxidation detected in the heart of lead treated rats in the present study confirms previous results [31]. The antioxidant activity of A. longa and A. malaccensis extracts is mainly related to their higher level of phenolic compounds. Several studies have shown that lipid peroxidation is inhibited by flavonoids and other compounds of plant origin [32]. These latter are well known for their ability of scavenging free radicals such as superoxide radical (O2), hydroxyl radical (OH) and others ROS. These elements are the prilam source of antioxidant ability of A. longa and A. malaccensis reducing the free radicals as hydroxyl radical which is the major cause of lipid peroxidation [33].
Figure 1: Level of MDA in heart tissues of control (C) and experimental groups. Means ± SE from 5 animals in each group. Significance from C: *p=0.05, ***p=0.001. Significance from Pb: a p<0.05, b p<0.01, c p=0.001

GSH Concentrations
The GSH level was significantly decreased (p=0.001) in Pb-exposed rats as compared to control rats. The treatment of Pb-exposed rats with Aq or Aq+Ar, we noticed a partial prevention from the Pb-induced decrease in GSH heart level. Whereas the treatment With A. longa alone a complete prevention from the Pb-induced decrease in GSH heart level (Figure 2). Le glutathion reduit (GSH) impliqué dans le mécanisme contre le stress oxydatif en récupérant les ROS et l'oxygène réactif intermédiaires [34]. It is a sulfhydryl peptide widely found in all biological systems. It is the first line of defense against free radical attacks as a non-enzymatic antioxidant. Its sulfhydryl (SH) group can interact directly with the radicals or as a cofactor or coenzyme of enzymatic antioxidant [35]. This decrease in GSH levels may be due to its consumption in the scavenging free radicals generated by lead, also Pb binds exclusively to the thiol groups which decrease the GSH levels thereby interfering with the antioxidant activity [36]. Secondary metabolites of plants possess several biological activities, and are a source of pharmacologically active [37]. Since biological activities of medicinal plants are closely related to their chemical compounds [38].

Figure 2: Level of GSH in heart tissues of control (C) and experimental groups. Means±SE from 5 animals in each group. Significance from C: **p<0.01, ***p<0.01. Significance from Pb: b p<0.01, c p=0.001

CAT Activity
As shown in Figure 3, exposure to Pb induced a significant (p=0.001) increase in CAT activity compared with control rats. When rats were concomitantly exposed to Aq, Ar or Aq+Ar, activity of CAT was significantly lower than in Pb-exposed rats (p=0.05, p=0.001 and p=0.001) respectively. Aristolochia treatment alone entirely reversed the Pb-induced increase in CAT activity. similar results have been reported [39]. It is not known the
direct oxidative role of lead, but indirectly by the inhibition of the enzyme delta amino levulinic acid dehydratase which cause a delta-levulinic acid delta acid accumulation leading to the formation of superoxide radicals and hydrogen peroxide[40].

Figure 3: Activity of CAT in heart tissues of control (C) and experimental groups. Means ± SE from 5 animals in each group. Significance from C: *p<0.05, **p<0.01 ***p<0.001. Significance from Pb: a p<0.01, c p<0.01

GST Activity
The data presented in Figure 4 showed that the exposure to Pb led to a significant decrease (p=0.001) in GST activity compared to the control. Aq treatment alone or in combination with Ar increase significantly the GST activity in Pb group, whereas Ar treatment alone entirely reversed the Pb-induced decrease in GST. The involvement of antioxidative enzymes such as GST play a considerable mission in protecting cells from oxidative stress [41]. So, assessment of activities of this enzyme may supply important informations about oxidative stress that cells exposed. We determined that lead used in this study were decreased enzyme activity. Similar results have been also reported by Sarkar et al. [42]. Flavonoids possess strong cytotoxic and apoptogenic activities against several cancer lead induced. This fact is confirmed by the significant increasing GSH and GST antioxidant levels in hear.

Figure 4: Activity of GST in heart tissues of control (C) and experimental groups. Means±SE from 5 animals in each group. Significance from C: *p<0.05, **p<0.01. Significance from Pb: a p<0.05

CONCLUSION
Data from this study suggest that rhizome powder *A. longa* or heartwood powder of *A. malaccensis* supplementation attenuates lead induced cardio toxicity by mechanisms related, at least in part, to its ability to decrease oxidative stress
and cardiac tissue damage and preserve the activity of antioxidant enzymes. \textit{A. longa} and \textit{A. malaccensis} could serve as a true functional food and may positively affect health promotion via reducing cardiovascular risk.

**ACKNOWLEDGEMENT**

The first author would like to thank the Faculty of Sciences of Nature and Life, University of El Oued, Algeria for the permission to utilize the facilities to make this work.

**REFERENCES**