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Ameliorative effect of *Aristolochia longa* and *Aquilariamalaccensis* on lead acetate induced nephrotoxicity in female rats

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Abstract

Background: *Aquilariamalaccensis* And *Aristolochia longa* are plants used extensively in traditional medicine for the treatment of cancer in several regions in Algeria. We investigate the ameliorative effect of *Aristolochia longa* and *Aquilariamalaccensis* on kidney toxicity induced by lead in female albino rats. **Methods:** Twenty five (25) apparently healthy female Wistar rats were randomly divided into five groups of five rats in each: control, Pb, Pb + *A. longa* (Ar), Pb+ *A. malaccensis* (Aq), and Pb+Ar+Aq. lead (100 mg/kg b.w) as Pb(C₂H₃O₂)₂ added in their drinking water for 75 days. *A. longa* (rhizome powder at a dose 1% ^{w/w} of diet) and *A. malaccensis* (heartwood powder at a dose 1% ^{w/w} of diet) were added to the feed during the last 15 days of lead exposed in the animals. **Results:** Obtained results revealed that lead treatment caused a significant increase in blood glucose level, serum urea, creatinine and uric acid level and in kidney of MDA level and CAT activity. In contrast, it led to a decrease in the kidney GST activity and GSH level in rats. Also, the results clearly showed that lead causes alterations of glomerular and tubular structure in comparison with controls. Our results showed that treatment with *A. malaccensis* and *A. longa* a partial correction of the previous parameters. The histological observations confirmed the nephroprotection results by the biochemical parameters. **Conclusion:** Results demonstrated beneficial effects of *A. longa* and *A. malaccensis* treatment against acetate lead-induced toxicity and kidney damage in rats.

Key Words: *A. longa*, *A. malaccensis*, nephrotoxicity, acetate lead, stress oxidative.

Introduction

Lead is a chemical element that exists in nature. It is considered a major pollutant in the environment[1]. It is recognized as one of the most toxic and harmful heavy metals, even in small amounts [2].As one major heavy metal pollutants, lead is normally generated from industry and continuously present in soil, drinking water and aquatic environments[3].The non-biodegradable nature of lead is the prime reason for its prolonged persistence in the environment [4].Lead can transfer into the trophic chain by causing deleterious effects for humans and other living organisms. It is a toxic metals in the environment and target most organs of the human body[5]. The kidneys play an essential role in the elimination of lead from the body and the bioaccumulation of lead is mainly in the kidneys and then in the liver and brain in animals exposed by lead [6]. This metal induces a wide range of physiological, biochemical and behavioral dysfunctions in laboratory animals and humans, including respiratory, neurological, hepatic and male and female reproductive systems [7]. Renal toxicity of lead is characterized by alteration in proximal tubular, glomerular sclerosis and in interstitial fibrosis. Renal dysfunction in humans exposed to the excessive amount of lead includes enzymeuria, low- and high-molecular weight proteinuria, transport of organic anions and glycemic filtration rate. In addition, at the histopathological state, renal damage has been shown in humans, including intranuclear inclusion bodies and cellular necrosis in the proximal and interstitial tubule fibrosis [8]. Lead can cause oxidative stress in the blood and other tissues which is considered as one of the possible mechanisms of the harmful and toxic effects induced by this metal in the organism [9]. It has been postulated that lead causes oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides [10], leading to the development of cancer, diabetes, neurodegenerative diseases and cardiovascular diseases [11].*Aquilaria malaccensis* and *Aristolochia longa* Are plants used extensively in traditional medicine in the treatment of cancer in several regions in Algeria. *Aquilaria malaccensis* has numerous biological activities, including activity, anti-microbial, anti-tumor, anti-allergic, anti-oxidant [12].*Aristolochia longa* also has several therapeutic uses as against ovarian insufficiencies, healing, diuretic [13] , analgesic, anti-inflammatory, anti-hyperglycemic [14]. The aim of present study was designed to investigate the effect of *Aquilaria malaccensis* and *Aristolochia longa* on nephrotoxicity, stress oxidative and kidney tissue alteration induced by lead in rats.

Materials and methods

Chemicals

Lead acetate and all other chemicals used in the study were obtained from Sigma-Aldarich Corporation (St. Louis, Missouri, USA).

Plant materials

Aristolochia longa roots and *Aquilariamalaccensis* heartwood were collected in herbalists shops from a local market of El-Oued. (voucher specimen no: FSNV/DB/consult/2016/78-15-02 for *Aristolochia longa* and voucher specimen no: FSNV/DB/consult/2016/81-12-06 for *Aquilariamalaccensis*) The vegetal materials were washed with water, and then dried at room temperature for 48 to 92 h. grounded into powder and stored at room temperature until use.

Animals and Experiments

Female albino rats aged 8 weeks old weighting approximately 220g, were obtained from the animal house of Pasteur institute, Algeria. Animals had free access to standard diet and were provided distilled water. After the adaptation period, rats were divided into five 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 diet and water were available ad libitum for the duration of the experiments unless otherwise noted. Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 65.3 % and room temperature of 23 ± 2 C°. 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. The experiment was conducted over a period of 70 days. After a period of adaptation, the animals were divided into five experimental groups of 5 animals each:

Groupe I (control group): animals served as normal control.

Groupe II (lead-treated group): animals received acetate lead (100 mg/kg b.w) as $Pb(C_2H_3O_2)_2$ added in drinking water for 70 days in animals.

Groupe III (lead+ Aq-treated group): animals received a powder diet containing powder of heartwood of *AquilariaMalaccensis* (1% w/w in feed) for 15 days.

Groupe IV (lead+Ar-treated group): animals received a powder diet containing powder of rhizome of *Aristolochialongua* (1% w/w in feed) for 15 days.

Groupe V (lead+Aq+Ar-treated group): animals received a powder diet containing powder of both heartwood of *Aquilariamalaccensis* and rhizome of *Aristolochialonga* for 15 days.

Blood was collected, some of which was centrifuged at 3,000 rpm for 10 min to obtain the plasma which was kept frozen at -20°C until used for biochemical analysis of urea, creatinine, uric acid and total protein level.

2.4. Tissue preparation

1g of kidney tissue of each rat of different groups was used. After milling and homogenizing the tissues in TBS (50 mM Tris, 150 mM NaCl, pH 7.4), and centrifuged at $10\,000 \times g$ for 15 min at 4°C, then the supernatant obtained was stored at -20 °C while waiting for the determination of oxidative stress parameters.

Plasma Biochemical parameters

Biochemical markers were assayed according to standard methods. The levels of serum urea, creatinine, uric acid and total protein were estimated using commercial kits from Spinreact laboratories, Spain (urea, ref. 1001332; creatinine, ref. 1001113; uric acid, ref. 1001013 and total protein, ref. 1001291).

Tissue oxidative stress parameters

Estimation of lipid peroxidation levels

The principle of this assay is based on the condensation of MDA in acidic and hot medium with thiobarbituric acid according to Yagi et al.[15]. The reaction results in the formation of a pink complex between two molecules of thiobarbituric acid which can therefore be measured by Absorption spectrophotometry at 532 nm and the level of MDA in liver was expressed as nmol/mg protein.

Determination of reduced glutathione (GSH) level

GSH concentration was performed with the method described by Ellman[16]. The complex formed between GSH and 5,5'-dithiodis-2-nitrobenzoic acid (DTNB) releases thionitrobenzoic acid (TNB) which has an absorbance at 412 nm. Total GSH content was expressed as nmol GSH/mg prot.

Determination of Glutathione-S-transferase (GST) activity

The activity of GSTs was measured spectrophotometrically according to the method of Habig et al. [17], based on the formation kinetics of a complex between a GST substrate: 1-chloro-2-4-dinitrobenzene (CDNB), And glutathione (GSH). The complex formed can be visualized by increasing the optical density at a 340 nm. The enzyme activity was expressed as nmol CDNB conjugate/min/mg protein.

Determination of Catalase activity

The catalase activity consists in measuring the disappearance of catalase-induced H₂O₂ contained in the sample by measuring the absorbance of H₂O₂ at 560 nm using a spectrophotometer according to the method of Aebi, 1984 [18].

2.7.Histopathological Analysis

The kidney from each rat was removed immediately and conserved in a sample flask containing 10% formalin solution. Liver was processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin for histological examination.

Statistics Analysis

Mean data values are presented, with their standard deviations (mean ± SD). All statistical comparisons were made by Student's t-test using Minitab 13 software, and statistical significance was defined as $P < 0.05$.

Results

body weight, body weight gain and relative kidney weight

The results obtained (Table 1) show that Pb(C₂H₃O₂)₂ treatment at a dose (100 mg/kg b.w) caused a significant decrease ($p < 0.001$) in body weight and a significant increase ($p < 0.01$) in Relative kidney weight compared to the control rats. But After the treatment time, the animals that received the powder of rhizome *A. longa* and heartwood *A. malaccensis* alone or combined showed reversion partial of this change.

Table 1

Mean initial Body weight (g), final Body weight (g) and relative kidney weight of control and experimental groups

parameters	Control (n=5)	Lead (n=5)	Lead + Aq (n=5)	Lead + Ar (n=5)	Lead +Aq+Ar (n=5)
Initial Body weight	225±5.06	225.2±5.69	226.60±23.71	221±8.76	225.2±3.51
Final Body weight	291±3.07	198±2.32***	196±5.00***	215±1.15** ^a	231±1.61* ^a
Relative kidney weight	0.56±0.01	0.73±0.04**	0.65±0.02* ^a	0.67±0.01*** ^b	0.59±0.01 ^c

* 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.

Values are mean ± SEM, n=number of observations.

3.2. Biochemical parameters

The results obtained in Table 2 show that a significant increase ($p < 0.05$) in blood glucose, serum urea, creatinine and uric acid level and no significant difference in serum total protein concentration in the group exposed to acetate lead compared to the control group. Treatment with powder of heartwood Aq or of rhizome Ar totally restored the level of blood glucose, serum urea, creatinine and uric acid Compared to lead group.

Table 2

Changes in biochemical parameters of control and experimental groups

parameters	Control (n=5)	Lead (n=5)	Lead + Aq (n=5)	Lead + Ar (n=5)	Lead +Aq+Ar (n=5)
Blood glucose (g/l)	0.752±0.051	0.905±0.107*	0.80±0.053 ^b	0.81±0.067 ^a	0.775±0.027 ^c
Urea (g/l)	0.64±0.067	0.81±0.085*	0.60±0.037 ^c	0.67±0.030 ^c	0.67±0.033 ^b
Creatinine (mg/l)	4.40±0.40	4.55±0.320*	4.40±0.245 ^a	3.80±0.200* ^a	4.00±0.00 ^a
Uric acid (mg/l)	17.60±1.94	18±2.00*	18.8±1.32*	17.6±1.60 ^a	18.6±1.36*
Totale protein (g/l)	50.6±1.40	48.33±1.76	47.60±2.60	48.2±1.12	47.4±1.53

* p<0.05, ** p<0.01 : significantly different from control group.

^a p<0.05, ^b p<0.01: significantly different from Pb group.

Values are mean ± SEM, n=number of observations.

Stress oxidative parameters

Our results illustrated in Table 3 show a significant increase ($p < 0.01$ and $p < 0.001$) in lipid peroxidation level and catalase activity respectively and a significant decrease ($p < 0.001$) in GSH concentration and GST activity in kidney of rats of the lead group compared to the control. In contrast our results also show a significant decrease in kidney MDA level ($p < 0.001$) and catalase ($p < 0.01$) activity in lead+Aq, lead+Ar and lead+Ar + Aq treated groups and a significant increase in kidney GSH concentration ($p < 0.01$) and GST activity ($p < 0.05$) in only lead +Ar group Compared to lead group.

Table 3.

MDA, GSH levels and antioxidant enzyme activities in kidney tissue of control and experimental groups

parameters	Control (n=5)	Lead (n=5)	Lead + Aq (n=5)	Lead + Ar (n=5)	Lead +Aq+Ar (n=5)
MDA ($\mu\text{mol/mg pro}$)	3.16 $\pm$ 0.41	4.37 $\pm$ 0.46**	2.12 $\pm$ 0.32** ^c	2.11 $\pm$ 0.13*** ^c	1.717 $\pm$ 0.09*** ^c
GSH (nmol/mg pro)	0.48 $\pm$ 0.09	0.248 $\pm$ 0.01***	0.212 $\pm$ 0.03***	0.38 $\pm$ 0.040* ^b	0.227 $\pm$ 0.01***
GST (nmol/min/mg pro)	3.75 $\pm$ 0.78	2.48 $\pm$ 0.02***	2.44 $\pm$ 0.17**	3.01 $\pm$ 0.14** ^a	2.62 $\pm$ 0.53**
CAT (UI/mg pro)	8.61 $\pm$ 0.82	18.75 $\pm$ 0.22***	14.21 $\pm$ 0.88*** ^b	11.78 $\pm$ 0.77** ^c	11.51 $\pm$ 0.18 ^b

* p<0.05, ** p<0.01, *** p<0.001 : significantly different from control group.

a p<0.05, b p<0.01: significantly different from Pb group.

Values are mean $\pm$ SEM, n=number of observations.

Histopathological studies

Microscopic observation of histological sections of kidney from the control rat showed normal renal parenchyma with glomeruli, tubules and normal cortical and medullar areas (Fig. 1A). However, histological sections of the lead-exposed rat kidney revealed Degeneration represented by necrosis and glomerular atrophy apparent to their architecture with tubular necrosis, dilatation and tabular vacuolation. In addition, haemorrhage, foci of inflammation and large space of Bowman, was observed in the kidney sections of this group (Figure 1B). Treatment with *Aquilariamalacensis* and / or *Aristolochia longa* in diets in lead-contaminated rats for 15 days provided protection against tubular and glomerular histological damage induced by this metal. These plants specially *Aristolochia longa* induce a remarkable regeneration of kidney tissue. However, *Aquilariamalacensis* partially decreased severe damage to kidney tissue (Figure 1C, 1D, 1E).

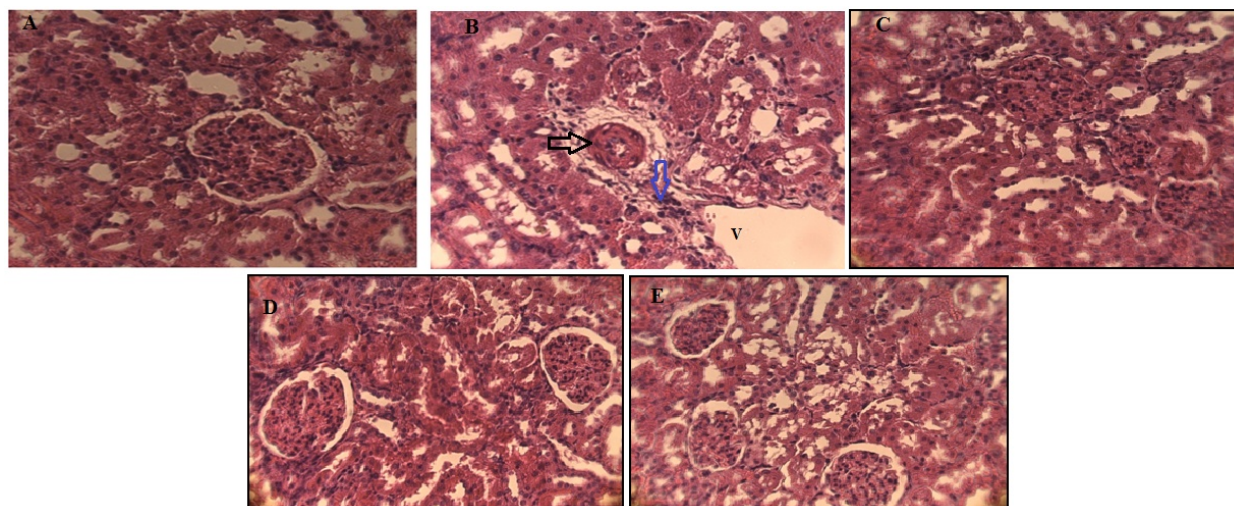


Figure 1. The histological examination of Kidney from a control rat showing normal architecture (Figure 1A, $\times 400$). Acetate lead treated rats kidney showing Focus of inflammation (blue arrow), Glomerular necrosis (black arrow) and vacuolation (V) (Figure 1B, $\times 400$). Powder rhizome of *A. longa* -treated rat kidney showing normal appearance of tubule and glomerulus (Figure 1D, $\times 400$). kidney sections of the animals administered powder of heartwood *A. Malaccensis* alone (Figure 1C, $\times 400$) or combined with rhizome of *A. longa* (Figure 1E, $\times 400$) of showed moderate degree of kidney damage, necrosis and inflammatory cell, protection from tubule and glomerulus degradation.

Discussion

The present study investigated the effects of treatment with powder of rhizome *A. longa* and heartworm *A. malaccensis* on biochemical markers of kidney function, oxidative stress and kidney histology outcome of acetate lead-induced kidney damage in rats. In the present study, the reduction in body weight is used as an indicator of the deterioration of the overall health of the rat. Exposure of lead acetate to doses (100 mg / kg) in rats resulted in a decrease in body weight and increase in relative liver weight. This result is consistent with several studies [19, 20] who suggested that reduction in body weight is due to reduced food consumption by lead effects on nerve centers responsible for the regulation of satiety and hunger. Also the loss of body weight may be due the reduced in mass of muscle and cachexia due to the oxidative stress induced by lead [21]. Increase in relative kidney weight may be due to lead-induced necrosis and apoptosis on this organ [22]. After the treatment time, the animals that received *A. longa* and *A. malaccensis* showed reversion partial of this less body weight, due probably the improvement of behavior and biochemical parameters. Improvement of relative kidney weight was probably due to the inhibitory effect of these plants against the accumulation of lead in kidney which reduces their injurious effect on kidney and therefore decreases their relative weight. Also, the protective effect of these plants could be due to the presence of anti-inflammatory compounds such as

flavonoids and phenolic acids [23]. In the present study, lead exposure results in a significant increase in blood glucose level. Our results are in agreement with study of Ibrahim et al. (2012) [24]. Lead enters into competition with calcium in metabolic processes exactly on the insulin excretion by several actions: inhibitory action on voltage-dependent calcium channels, decreased adenylate cyclase activity [25], Reduces the release of certain neurotransmitters such as serotonin [26]. Moreover, lead causes adverse effects on the pancreas exactly on the excretion of insulin by islets of Langerhans which increases blood glucose level [27]. Our results also demonstrated a significant decrease in the blood glucose level in rats treated with *A. malaccensis* and *A. Longa* alone or in combination compared with the lead group, the polyphenolic and flavonoid compounds of plants can influence glucose metabolism by several mechanisms such as inhibition of carbohydrate digestion and absorption of glucose in the intestine, Stimulation of insulin producing cells, modulation of glucose release from the liver, activation of glucose uptake in insulin-sensitive tissues, and modulation of hepatic glucose production [28,29]. In our study, exposure of lead acetate to rats resulted in nephrotoxicity characterized by a significant increase in the serum urea, creatinine and uric acid level. These results are similar to those published in previous studies [30, 31, 32]. The results clearly show that this metal affects the excretory function of nephrons, the structural and functional unit of the kidneys. Treatment of rats with *A. Malaccensis* and / or *A. Longa* restored renal function. The protective effect of *A. Malaccensis* and *A. Longa* was reflected by decreased serum urea and creatinine level in rats treated with these plants compared to rats exposed to lead. In our study, the results show a significant decrease in GSH level and GST activity and a significant increase in MDA level and CAT activity in the kidney of rats exposed with lead acetate. These results are in agreement with several studies [33,34, 35]. There is a complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress [36]. These changes are explained by the increase in the of free radicals, which can be due to the accumulation of 5-aminolevulinic acid (ALA) in kidney under the effect of lead [37]. These also shows that lead is able to promote the generation of ROS, which results in lipid peroxidation in renal tissues, suggesting their deleterious effects in this tissue. Thus, another mechanism, lead can have a direct peroxidation activity that has a high binding affinity on the cell membrane [38]. Since oxidative stress is the first response to the environmental pollutants, liver cells may stimulate antioxidant and detoxification responses to counter heavy metal damages. The involvement of anti-oxidative enzymes such as GST play a considerable mission in protecting cells from oxidative stress [39]. Increased CAT activity can be explained as a tissue defense

mechanism against increased H₂O₂ fluxes during Pb-induced oxidative stress [40]. Our results show that treatment with *A. malaccensis* and / or *A. longa* could prevent lead-induced alteration, increasing the antioxidant activity of GSH and GST and decreasing lipid peroxidation. Suggest that both plants possess antioxidant activity based on the elimination of free radicals and the restoration of the oxidizing / antioxidant balance during toxicity induced by this metal. The presence of polyphenols and flavonoids in *A. Malaccensis* and *A. Longa* may be responsible for antioxidant activity and are considered good chelating agents for metal ions. In addition, the activity of phenolic acids depends on the number and position of the group (-OH) and (-OCH₃) groups [41], and the flavonoids inactivate and stabilize the free radicals by their group Hydroxyl (C₃-OH). They are also capable of chelating metal ions (released from their binding or transport proteins) [42]. According to the results of the present study, lead exposure produces histological damage in the kidney including glomerular necrosis, tubular dilation, and inflammation. These alterations may be due to the excessive production of free radicals and as a result of lipid peroxidation induced by lead acetate [43]. Treatment with *A. malaccensis* and / or *A. longa* in intoxicated rats has been able to regenerate the structure of the kidney. Our results suggest that these plants, especially *A. longa*, could reduce kidney lesions induced by (Pb (C₂H₃O₂)₂). The biochemical and oxidative stress parameters are also correlated with the histological study. This can be attributed to the anti-radical effect of *A. Malaccensis* and *A. longa*. These plants reduced the oxidative stress caused by lead, allowing the reduction of histological alterations and restoration of the normal physiological state of the body.

Conclusion

Lead is a strong nephrotoxic agent by inducing renal dysfunction and oxidative stress in kidney. *Aristolochia longa* and *Aquilariamalaccensis* were able to moderate this toxicity by decreasing oxidative stress and decreasing the alteration of the kidney tissue.

Author's contribution

All research done by the authors

Conflict of interest statement

We declare that we have no conflict of interest.

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