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Protective effects of *Aristolochia longa* and *Aquilaria malaccensis* against lead induced acute liver injury in rats

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ABSTRACT

Objective: To investigate the protective effects of *Aristolochia longa* (*A. longa*) and *Aquilaria malaccensis* (*A. malaccensis*) on acute hepatotoxicity 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+ *A. longa* (Ar) + *A. malaccensis* (Aq) lead (100 mg/kg b.w.) as Pb $(C_2H_3O_2)_2$ added in their drinking water for 75 days. *A. longa* (rhizome powder at a dose of 10 g/kg of diet) and *A. malaccensis* (heartwood powder at a dose 10 g/kg 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 serum GOT, GPT and ALP activities and in liver of MDA level and CAT activity. In contrast, it led to an decrease in the liver GOT, GPT and GST activities and in GSH level in rats. Also, the results clearly showed that lead causes alterations of hepatic tissue 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 hepatoprotection results by the biochemical parameters. **Conclusions**: Results demonstrated beneficial effects of *A. longa* and *A. malaccensis* treatment in Pb-induced oxidative stress and tissue damage in liver.

1. Introduction

Lead is among the most known and abundant heavy metals. it is present in the different biological systems and in the environment. It is recognized by its toxicity and its harmful effect, even in small amounts [1]. Lead is present in soil and aquatic environments including drinking water also present in several products manufactured in industry [2]. This metal causes a disturbance in biochemical and physiological aspects in many living species, including humans. such as disturbances in respiratory, neurological, hepatic and reproductive systems [3]. Lead causes an imbalance between the release of free radicals and the antioxidant system in the cell which generates a state of oxidative stress which is the origin of lead toxicity in the body. Macromolecules are the main targets of ROS. The latter cause lipid peroxidation, protein oxidation and DNA alterations, leading to the development of cancer, diabetes, neurodegenerative diseases and cardiovascular diseases [4]. *Aquilaria malaccensis* (*A. malaccensis*) and *Aristolochia longa* (*A. 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-oxidant [5]. *A. longa* also has several therapeutic uses as against ovarian insufficiencies, healing, diuretic [6], analgesic, anti-inflammatory, anti-hyperglycemic [7]. The purpose of

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this study is to evaluate the effect of *A. malaccensis* and *A. longa* on hepatotoxicity, oxidative stress and liver tissue alteration induced by lead in rats.

2. Materials and methods

2.1. Chemicals

Lead nitrate $Pb(C_2H_3O_2)_2$ and all other chemicals used in the study were obtained from Sigma-Aldarich Corporation (St. Louis, Missouri, USA).

2.2. Plant material

A. longa roots and *A. malaccensis* heartwood were collected in herbalists shops from a local market of El-Oued. The plant material was washed using distilled water and then drying in room temperature for 48 to 92 h and then grounded into powder and stored at room temperature until use.

2.3. Animals and Experiments

Female albino rats aged 8 weeks old weighting approximately 220 g, were obtained at the Animal Service of the Pasteur Institute, Algeria. Animals have free access to water and food. After the adaptation period, rats were divided into five groups (n=5) and kept in animal's house of biology Department, El Oued University, Algeria. Standard diet and water were available *ad libitum* for the duration of the experiments unless otherwise noted. The animals were carried under the same conditions, photoperiod (12h of light/12h of black) with a relative humidity of 65.3% and an ambient temperature of (23 ± 2) ℃ for two weeks. The experiments on rats were performed according to the National Institute of Health's standards for animal care and authorized 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:

Group I (control group): animals served as normal control; Group II (Pb-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; Group III (Pb+ Aq-treated group): animals received powder of heartwood of *A. malaccensis* alone at a dose of 10 g/kg diet for 15 days; Group IV (Pb+Ar-treated group): animals received powder of rhizome of *A. longua* alone at a dose of 10 g/kg diet for 15 days. Group V (Pb+Aq+Ar-treated group): animals received both powder of heartwood of *A. malaccensis* and rhizome of *A. longua* 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 ℃ until used for biochemical analysis of GOT, GPT and ALP activities.

2.4. Tissue preparation

For each lot, 1 g of liver was taken from the rats. the tissues are bryoed and homogenized in a TBS buffer solution (50 Mm Tris, 150 mM NaCl, pH 7.4), then centrifuged for 15 min at 10 000 g and 4 ℃, the supernatant obtained is stored at – 20 ℃ while waiting for the determination of liver GOT and GPT activities and the oxidative stress parameters.

2.5. Plasma Biochemical parameters

Enzymes marker were measured according to classic methods. Alkaline phosphatase (ALP) and transaminases (GOT and GPT) activities were estimated using commercial kits from Spinreact laboratories, Spain (ALP, ref. 1001130; GOT, ref. 1001160 and GPT, ref. 1001170).

2.6. Tissue stress oxidative parameters

2.6.1. Estimation of lipid peroxidation levels

The malondialdehyde dosage is based on the condensation of MDA in acidic and hot medium with thiobarbituric acid according to Yagi *et al.* [8]. 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.

2.6.2. Determination of reduced glutathione (GSH) level

The GSH level was measured by Ellman method [9]. The complex formed between GSH and 5,5'dithiodis-2-nitrobenzoic acid (DTNB) releases thionitrobenzoic acid (TNB) quantify by reading the absorbance at 412 nm. The GSH rate results were expressed as nmol GSH/mg prot.

2.6.3. Determination of Glutathione-S-transferase (GST) activity

The activity of Glutathione S Transferase was measured by the method of Habig *et al.* [10] using the spectrophotometric technique, which is based on the kinetics of formation of a complex between CDNB (1-chloro-2-4-dinitrobenzene) and GSH (glutathione). The formed complex can be detected by increasing the optical density at 340 nm. The enzymatic activity of GST was expressed as nmol CDNB conjugate/min/mg protein.

2.6.4. Determination of the enzymatic activity of catalase

The assay of the catalase enzyme activity consists in measuring the catalase-induced loss of H_2O_2 contained in the sample by the measurement of H_2O_2 absorbance at 560 nm using a spectrophotometer according to the Aebi, 1984 method [11].

2.7. Histopathology analysis

Part of the liver of each group was removed and stored immediately in a 10% formalin solution, treated by the technique of the paraffin and then cut (5 µm thickness), and stained with hematoxylin and eosin for histological examination.

2.8. Statistics analysis

The values are presented by the means with their standard deviations (mean ± SD). Statistical comparison between means was done by Student's t-test, and statistical significance was defined as P <0.05.

3. Results

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

The results obtained (Table 1) show that treatment with 100 mg/kg bw of lead acetate results in a significant reduction (P<0.001) in body weight and a significant increase (P<0.05) in the liver relative weight compared to controls. But after the treatment time, the animals that received the powder of rhizome *A. longa* and heartwood *A. malaccensis* alone or combined showed partial reversion of this this change.

3.2. Biochemical parameters

The results shown in Table 1 showed that the GOT and ALP activities are significantly increased (P<0.05 and P<0.01) in the serum and significantly decreased (P<0.05 and P<0.01) in the liver respectively. But no significant difference for serum GPT activity in the group exposed to lead acetate compared to the control group. Treatment with powder of heartwood *A. malaccensis* or of rhizome *A. longua* partially restored the activity of serum GOT GPT and ALP activities. However, our results clearly show a significant increase (P<0.01) in the activity of liver transaminases (GOT and GPT) in *A. malaccensis*, *A. longua* and *A. longua* + *A. malaccensis* treated groups compared to Pb group.

3.3. Stress oxidative parameters

Our results illustrated in Table 2 showed a significantly elevated (P<0.05 and P<0.01) in lipid peroxidation level and catalase activity respectively and a significant decrease (P<0.001) in GSH concentration and GST activity in the Pb group compared to the control. In contrast our results also show that the level of MDA and the catalase activity are significantly decreased (P<0.01) in the liver and that the hepatic GSH concentration and GST activity are significantly increased in *A. malaccensis*, *A. longua* and *A. longua* + *A. malaccensis* groups compared to Pb group.

3.4. Histopathological studies

Observation of histological sections of the liver of the control rat by the light microscope showed a normal structure with clear liver cells (Figure 1A). However, histological sections of the Pb-exposed rat liver revealed hepatic damage and lysis as haemorrhage, necrosis, inflammatory dilation, and sinusoidal dilatation (Figure 1B). Treatment with *A. malacenssis* and / or *A. longa* in diets in lead-contaminated rats for 15 days provided protection against liver histological damage induced by this metal. These plants induce a remarkable regeneration of liver tissue. However, *A. malacenssis* partially decreased severe damage to liver tissue (Figure 1C, 1D, 1E).

Table 1

Changes in body weight and biochemical parameters of control and experimental groups (n=5).

Parameters	Initial body weight	Final body weight	Relative liver weight	Serum GOT (U/L)	Serum GPT (U/L)	Serum ALP (U/L)	Liver GPT (U/L)	Liver GOT (U/L)
Control	225.00±5.06	291.00±3.07	2.519±0.057	205.80±10.30	89.50±4.33	40.16±6.07	1140.20±30.74	55.80±2.90
Pb	225.20±5.69	198.00±2.32**	3.206±0.246*	218.70±20.70*	90.50±7.79	74.10±4.25**	890.52±40.79**	40.75±6.37*
Pb+ Aq	226.60±23.71	196.00±5.00**	2.578±0.056$^{\#\#}$	211.67±4.16*	80.50±7.08$^{*\#\#}$	58.48±9.35$^{*\#}$	1120.90±190.00$^{\#}$	42.40±5.88*
Pb+ Ar	221.00±8.76	215.00±1.15$^{**\#}$	2.529±0.046$^{\#\#}$	208.00±4.62$^{\#}$	86.25±5.73	76.60±14.90**	1390.00±100.90$^{\#\#}$	83.40±9.22$^{*\#\#}$
Pb+Aq+Ar	225.20±3.51	231.00±1.61$^{*\#}$	2.491±0.023$^{\#\#}$	220.00±8.08*	86.67±7.80	76.93±4.23**	2280.20±350.80$^{*\#\#}$	75.60 ±10.50$^{\#\#}$

$^{*}P$<0.05, $^{**}P$<0.01, significantly different from control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01; significantly different from Pb group; Values are mean ± SEM, n=number of observations.

Table 2

Liver tissue MDA, GSH levels and antioxidant enzyme activities of control and experimental groups (n=5).

Parameters	MDA (μmoL/mg pro)	GSH (nmoL/mg pro)	GST (nmoL/min/mg pro)	CAT (UI/mg pro)
Control	1.837±0.372	0.220±0.017	14.30±1.28	14.030±0.369
Pb	2.263±0.398*	0.098±0.006***	6.27±0.57***	17.651±0.493**
Pb+ Aq	1.306±0.416$^{\#}$	0.190±0.021$^{\#\#}$	9.21±1.67*	12.330±0.407
Pb+ Ar	1.662±0.258$^{\#}$	0.305±0.029*$^{\#\#}$	13.03±1.96$^{\#\#}$	6.824±0.437$^{***\#\#}$
Pb+Aq+Ar	1.115±0.232$^{\#\#}$	0.238±0.037$^{\#\#}$	10.84±0.87$^{**\#\#}$	11.310±0.227$^{\#}$

GOT: glutamic-oxaloacetic transaminase, GPT: serum glutamic-pyruvate transaminase, ALP: serum alkaline phosphatase. $^{*}P$<0.05, $^{**}P$<0.01, significantly different from control group; $^{\#}P$<0.05, $^{\#\#}P$<0.01; significantly different from Pb group; Values are mean ± SEM, n=number of observations.

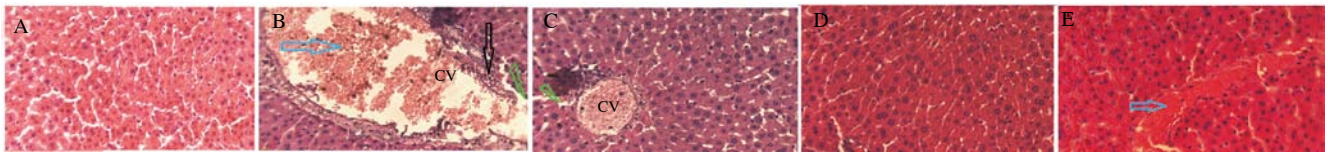

Figure 1. The histological examination of livers.

Histological examination from a control rat showing normal architecture (Figure 1A, ×400); Acetate lead treated rats liver showing necrosis and hemorrhage (blue arrow), inflammatory (black arrow) and sinusoidal dilatation (green bolt) (Figure 1B, ×400). Powder rhizome of *A. longa* -treated rat liver showing normal appearance of hepatocytes (Figure 1D, ×400). Liver sections of the animals administered powder of heartwood *A. malaccensis* alone (Figure 1C, × 400) or combined with rhizome of *A. longa* (Figure 1E, ×400) of showed moderate degree of liver damage, hemorrhage and inflammatory cell, protection from hepatocyte degradation.

4. Discussion

This study evaluated the effects of treatment with powder of rhizome *A. longa* and heartworm *A. malaccensis* on enzymes markers, oxidative stress and liver histology outcome of acetate lead induced hepatotoxicity in rats. In the present study, The reduction in body weight is a sign of loss of the overall physiology of the rat. Exposure of lead acetate to doses (100 mg/kg) in rats resulted in a decrease in body weight and an increase in the relative weight of the liver, which is in agreement with several studies [12, 13] which suggest that lead induces a reduction in body weight and food consumption by its effect on nerve centers Responsible for the regulation of satiety and hunger. The reduction in body weight can also be explained by a reduction in muscle mass and cachexia due to lead-induced oxidative stress [14]. On the other hand the origin of the increase in the liver relative weight probably due to lead-induced necrosis and apoptosis on this organ [15]. 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 liver weight is probably due to the inhibitory effect of these plants against the accumulation of lead in liver which reduces their injurious effect on liver and therefore decreases their relative weight. Also, the protective effect of these plants may be because of the existence of anti-inflammatory compounds such as phenolic acids and flavonoids [16]. In the present study, lead exposure results in a significant decrease of transaminases (GOT and GPT) activities in liver and a significant increase in GPT, GOT and ALP activities in serum. Our results in agreement with several studies [17-19]. Disruption of the activity of these enzymes may be the result of many factors, including hepatic lysis by cell necrosis, cell leakage, and loss of functional integrity of the tissue membrane of liver cells[20]. Also, these effects are due to lipid peroxidation induced by the generation of ROS under the effect of lead toxicity and alterations of the cell membrane causing the leakage of enzymes in the blood [21]. Then, our results indicates that the use of rats by *A. longa* or *A. malaccensis* or both significantly restored the enzymatic activity in serum or tissues compared to exposed lead acetate rats, which means that these plants inhibit hepatic damage caused by Pb. This is due to the reduction of accumulation of free radicals and protection against oxidative stress by Aristolochia. The anti-inflammatory property of Aristolochia species is probably the result of a direct interaction between aristolochic acid and phospholipase A2 derivatives. Aristolochic acid is the main constituent of *A. longa*. In addition, phospholipase A2 is an enzyme that catalyses the hydrolysis of the membrane arachidonic acid, which leads to the formation of local inflammatory mediators such as prostaglandins (PG), leukotrienes, and thromboxanes. Arachidonic acid is a key biological intermediate that is converted into a large number of eicosanoids with potent biological activity [22]. In our study, the results show in our results lead induces an increase in MDA levels and CAT activity and a decrease in GSH level and GST activity in the liver tissue. These results are consistent with several studies [23-25]. There is a complex interaction between antioxidants and pro-oxidant like ROS , which modulates oxidative stress generation [26]. These changes are explained by the increase in the of free radicals, which can be due to the increase of 5-aminolevulinic acid (ALA) under the effect of lead[27]. These also shows that lead is able to promote the generation of ROS, which results in lipid peroxidation in hepatic 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 [28]. Since oxidative stress is the first response to the environmental pollutants, Hepatic cells stimulate the antioxidant and detoxification system to limit the damage caused by heavy metals. The involvement of enzymatic antioxidants such as GST play an important role in protecting cells against the release of free radicals and therefore limit oxidative stress[29]. Increased CAT activity can be explained as a tissue defense mechanism against increased H_2O_2 fluxes during Pb-induced oxidative stress [30]. 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 existence of (-OH) and (-OCH_3) groups (number and position) in the structure of phenolic acids explains their powerful antioxidant power of these molecules [31], and the flavonoids inactivate and stabilize the free radicals by their group Hydroxyl (C_3-OH). They are also capable of chelating metal ions (released from their binding or transport proteins) [32]. According to the results of the present study, exposure to lead causes tissue toxicity of the liver by causing histological lesions, including necrosis, inflammation and hemorrhage. These results are in agreement with the study of Shalan *et al.* [33]. These alterations may be due to lipid peroxidation induced by the excessive production of free radicals under the influence of exposure to lead[34]. Treatment with *A. malaccensis* and/or *A. longa* in intoxicated rats has been able to regenerate the structure of the liver. Our results suggest that these plants, especially *A. longa*, could reduce hepatic lesions induced by (Pb $(C_2H_3O_2)_2$). 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 limit of histological lesions and the normalization of the physiological state of the body.

Lead is a strong hepatotoxic agent by inducing oxidative stress. *A. longa* and *A. malaccensis* were able to moderate this toxicity by decreasing oxidative stress and decreasing the alteration of the liver tissue.

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Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Mishra S, Ghosh D, Dutta M, Chattopadhyay A, Bandyopadhyay D. Melatonin protects against lead-induced oxidative stress in stomach, duodenum and spleen of male Wistar rats. *J Pharm Res* 2013; **1**(11): 997-1004.

[2] Derouiche S, Djouadi A. Control of lead and cadmium in cosmetic product (Kohl) of Pits dates by cyclic voltammetry. *J Chem Pharm Res* 2017; **9**(3):319-323.

[3] Ponce-Canchihuamán JC, Pérez-Méndez O, Hernández-Muñoz R, Patricia V, Juárez-Oropeza MA. Protective effects of *Spirulina maxima* on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. *Lipids Health Dis* 2010; **9**:35.

[4] Wang J, Yang Z, Lin L, Zhao Z, Liu Z, Liu X. Protective effect of Naringenin against lead-induced oxidative stress in rats. *Biol Trace Elem Res* 2012; **146**:354–359.

[5] Chena HQ, Wei JH, Yanga JS, Zhanga Z, Yangb Y, Gaoa ZH, et al. Chemical constituents of agarwood originating from the endemic genus aquilaria plants. *Chem Biodivers* 2012; **9**(2): 236-250.

[6] Cherif HS, Saidi F, Guedioura A. Toxicological evaluation of *Aristolochia longa* L. extract in mice. *Indian J Appl Res* 2014; **4**(5): 26-30.

[7] Benarba B, Meddah B. Ethnobotanical study, antifungal activity, phytochemical screening and total phenolic content of Algerian *Aristolochia longa*. *J Intercult Ethnopharmacol* 2014; **3**:150-154.

[8] Yagi K. Simple fluorimetric essay for lipoperoxide in blood plasma. *Biochem Med* 1976; **15**(2): 212-216.

[9] Weckbercker G, Cory JG. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells *in vitro*. *Cancer Lett* 1988; **40**(3): 257-264.

[10] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; **249**: 7130-7139.

[11] Aebi H. Catalase *in vitro*. *Meth. Enzymol* 1984; **105**: 121-126.

[12] Berrahal A, Nehdi A, Hajjaji N, Gharbi N, El-Fazâa S. Antioxidant enzymes activities and bilirubin level in adult rat treated with lead. *Pharmacol Toxicol* 2007; **330**: 581–588.

[13] Reckziegel P, Dias VT, Benvegnú DM, Boufleur N, Barcelos RC, Segat HJ, et al. Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. *Toxicol Rep* 2016; **3**: 351-356.

[14] Yeh YC, Liu TJ, Wang LC, Lee HW, Ting CT. A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. *Br J Pharmacol* 2009; **156**: 48-61.

[15] Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. The effect of lead acetate toxicity on experimental male albino rat. *Biol Trace Elem Res* 2011; **144**: 1120-1132.

[16] Keerthi M, Prasanna J, Aruna M, Rao N. Review on polyphenols as nature's gift world. *J Pharm Pharm Sci* 2014; **3**: 445-455.

[17] Kilikdar D, Mukherjee D, Mitra E, Ghosh AK, Basu A, Chandra MA, et al. Protective effect of aqueous galic extract against lead-induced hepatic injury in rats. *Indian J Exp Biol* 2011; **49**: 498-510.

[18] Attia A, Ibrahim F, Nabil G, Aziz S. Antioxidant effects of ginger (*Zingiber officinale* Roscoe) against lead acetate-induced hepatotoxicity in rats. *Afr J Pharm Pharmacol* 2013;**7**(20):1213-1219.

[19] El-Ashmawy I, El-Nahas AF, Salama O. Protective effect of volatile oil, alcoholic and aqueous extracts of *Origanum majorana* on lead acetate toxicity in mice. *Basic Clin Pharmacol Toxicol* 2005;**97**: 238–243.

[20] Sharma V, Singh M. Attenuation of N-nitrosodimethylamine induced hepatotoxicity by *Operculina turpethum* in Swiss Albino mice. *Iran J Basic Med Sci* 2014; **17**(1): 73–80.

[21] Okeke N, Emeka A, Johntel C. Biochemical taurine alleviated modification in male Wistar rats co-exposed to chlorpyrifos and lead. *Int J Environ Sci & Toxicol* 2014; **2**(9): 104-115.

[22] Houghton P. Anti-inflammatory plants ethnopharmacology of medicinal plants: Asia and the pacific. *Br J Clin Pharmacol* 2007; **64**(2): 248.

[23] Kansal A, Sharma V, Sharma A, Lodi S, Sharma H. protective role of coriandrum sativum (coriander) extracts against lead nitrate induced oxidative stress and tissue damage in the liver and kidney in male mice. *Int J Appl Biol Pharm* 2011; **2**(3): 65-83.

[24] Alya A, Bini Ines D, Montassar L, Najoua G, Saloua E. Oxidative stress, biochemical alterations, and hyperlipidemia in female rats induced by lead chronic toxicity during puberty and post puberty periods. *Iran J Basic Med Sci* 2015; **18**(10): 1034–1043.

[25] Jackie T, Haleagrahara N, Chakravarthi. Antioxidant effects of Etlingera elatior flower extract against lead acetate – induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. *BMC Res Notes* 2011; **4**(67): 8.

[26] Samir D, manel A, Abir H. Effect of extracts aqueous of phragmites australis on carbohydrate metabolism, some enzyme activities and pancreatic islet tissue in alloxane induced diabetic rats. *Int J Pharm Pharm Sci* 2017; **9**(6): 54-58.

[27] Ahamed M, Siddiqui MKJ. Low-level lead exposure and oxidative stress: current opinions. *Clinica Chimica Acta* 2007; **383**: 57–64.

[28] Hnde G, Nuran E. Can antioxidants be beneficial in the treatment of lead poisoning. *Free Radic Biol Med* 2000; **29**(10): 927–945.

[29] Djouadi A, Derouiche S. Study of fluoride-induced haematological alterations and liver oxidative stress in rats. *World J Pharm Pharm Sci* 2017; **6**(5): 211-221.

[30] Gurer H, Zgunes HO, Saygin E, Ercal N. Antioxidant effect of taurine against lead-induced oxidative stress. *Arch Environ Contam Toxicol* 2001; **41**: 397–402.

[31] Chusri S, Singthong P, Kaewmanee T. Antioxidant, anticancer, and cytotoxic effects of thai traditional herbal preparations consumed as rejuvenators. *J Food Sci* 2015; **13**(1): 40-48.

[32] Ghedira K. *Les flavonoïdes*: structure, propriétés biologiques, rôle prophylactique et emplois en thérapeutique. *Phytotherapy* 2005;**3**: 162.

[33] Shalan MG, Mostafa MS, Hassouna MM, Hassab SE, El-Nabi AE. Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. *Toxicology* 2005; **206**: 1–15.

[34] Aziz FM. Protective effects of latex of *Ficus carica* L. against lead acetate-induced hepatotoxicity in rats. *Jordan J Biol Sci* 2012; **5**(3): 175 –182.