The efficiency of *Syzygium aromaticum* essential oil against renal intoxication by lead in rats during development.

Djallal Eddine Houari Adli, Kadda Hachem, Mokhtar Benreguieg, Mustapha Brahmi, KAHLOULA Khaled and Slimani Miloud

Laboratory of Biotoxicology, Pharmacognosy and Biological recovery of plants, Department of Biology, Faculty of Sciences, University of DrMoulayTahar, Saida, Algeria;

*Correspondence: Djillou2006@yahoo.fr Accepted: 24 May 2018 Published online: 20 Sep. 2018*

The present study was aimed to investigate the protective effect of essential oil of *Syzygium aromaticum*, on Lead acetate (0.2%) induced nephrotoxicity, in wistar rats pups during the gestation and lactation period. The animals wistar rat pups were exposed to Lead acetate via their dams' drinking water from postnatal day (PND) 1 to (PND) 21. After weaning, the lead exposed rats received injections of essential oil of *Syzygium aromaticum* (0.1 ml/kg) for 20 days. Lead concentration in the digested tissues of kidney was significantly increased compared with control rats. However, the levels of kidney markers such as urea, uric acid, creatinine were significantly increased in blood following lead acetate administration. Lead-induced oxidative stress in kidney tissue was indicated by a significant decreased in the level of superoxide dismutase, catalase and glutathione peroxidase respectively. Histologically, the kidney showed several histological alterations such as necrosis of glomeruli and altered tubules. Administration of essential oil clove markedly attenuated the previous lead-induced biochemical alterations in serum and renal tissues as well as the histological and cellular changes. From this study, it can be concluded that the essential oil of *Syzygium aromaticum* showed effective a nephro-protective effect.

**Keywords:** Antioxidant, Essential oil, Lead acetate, Nephrotoxicity, *Syzygium aromaticum*.

**INTRODUCTION**

Lead (Pb) poisoning occurring in humans, either due to occupational or environmental exposure has become a great public problem. This metal causes a broad range of biochemical, physiological and behavioral dysfunctions. However, the lead is in the bloodstream, it is distributed into soft and hard tissues (Gerhardsson et al., 1995). Milk is the most important food source for newborn, however, also be a pathway of maternal excretion of toxic elements such as lead, and these toxins impact most severely on the newborn at a time of rapid development of the central nervous system (Astrup-Jensen et al., 1991). In rodents Pb mobilized from the skeleton is transferred to the suckling offspring during lactation (Keller and Doherty, 1980), and that lactational transfer after current or recent exposure to lead in dams was considerably higher than placental transfer (Hallen et al., 1995). Exposure to lead can result in significant alterations in multiple organs (Rendón et al., 2007). Nephropathy and renal failure in workers exposed to lead has been reported (Hong et al., 1980) and alterations in the nucleus, mitochondria and membranes of proximal tubule
Adli et al., Nephro-protective effect of *Syzygium aromaticum* against lead intoxication

cells have been observed in lead-exposed individuals (Bernard and Becker, 1998). Rats exposure to lead in drinking water or by intraperitoneal injection induces kidney histopathological lesions, principally in proximal tubule cells (cytomegaly, karyomegaly, necrosis, vacuolization, mitochondrial swelling, lysosomalpleomorphism and nuclear inclusion bodies) (Fowler et al., 1980). The healthy use of natural products rich in bioactive substances has promoted the growing interest of the pharmaceutical, food, and cosmetic industries. The clove plant *Syzygium aromaticum*, native to tropical Asia, is a source of essential oils widely used in both medicine and cosmetics. Previous research has shown that clove has several therapeutic properties, such as anti-inflammatory, neuroprotective action, antihyperglycemic, nervous stimulant ,tonic antimutagenic, aphrodisiac , bactericidal , nematicidal , fungicide, anti-toothache , anti-oxidant , analgesic, anesthetic and hypotensive properties (Mani et al.,2012; Dashti-R and Morshedli,2009). The aims of the present study were to further investigate in vivo properties of *Syzygium aromaticum* (clove) essential oil, against a nephro-toxicity induced by lead acetate during the gestation and lactation period.

**MATERIALS AND METHODS**

**Plant Material and Preparation of Aqueous Suspension**

Dried clove buds *Syzygium aromaticum* were purchased from local herb market in Saida (Algeria) and were identified and authenticated by an expert taxonomist. We used 50 g of dried clove buds *Syzygium aromaticum* that were processed by steam distillation, over a period of four hours, in an all-glass apparatus, to obtain essential oil with 5.5% yield.

**Animals and Treatment**

This study was carried out on Wistar rats weighing between 200 and 250g. All through the experiment duration, the animals were housed in separate cages, fed with standard laboratory food and allowed free access to water, maintained at 22–25 °C under a well regulated light and dark (12 h:12 h) in an animal room. They were mated two weeks after their arrival (three females and one male per cage). On pregnancy day 0 the dams were divided into four groups: One group (n = 7) received 0.2% lead acetate in drinking distilled water during gestation and lactation (Pb) , The second group (n = 7) received intraperitoneal injection of (0.1ml/kg) clove essential oil (Kahloula et al.,2013; Halder et al., 2011). The third group (n = 7) received lead acetate and clove essential oil (CEO). whereas, rats in the control group (n = 7) received distilled water without lead acetate (C), as described previously. At birth, the Pb pups continued to receive lead acetate during lactation until postnatal day (PND) 21 and the rats were weaned on this day. The number of suffering animals were minimised in accordance with the guidelines of the European Council Directive (86/609/EEC).

**Kidney weight**

The body weight of each animal was daily recorded throughout the duration of the experiment. The left kidney weights of different groups of animals were registered.

**Biochemical analysis**

In the end of the treatment, the animals of different groups were sacrificed under light anesthesia (chloral). The blood sample from each rat was collected from the orbital vein in heparinized tubes. Then, they were centrifuged at 5000 rpm for 10 min for plasma separation.

**Uric acid, urea and creatinine levels**

The concentrations of kidney enzymes activities uric acid, blood urea and creatinine were measured using kits (Chronolab, Spain) plasma activities of uric acid and blood urea were determined according to Kaplan,(1984) and creatinine was according to Murray,(1984).

**Determination of the anti-oxidant enzymes**

Superoxide dismutase (EC 1.15.1.1) (SOD) was analyzed in the supernatant using the technique of Kakkar et al. (1984). This method is based on the inhibition of the formation of nicotinamide adenine dinucleotide, and phenazinemethosulfate from amino blue tetrazoliumformazan. The activities and levels of renal antioxidants as catalase (CAT), glutathione peroxidase (GPx) were determined by the methods of Sindha, (1972), Rotruck et al., (1973), respectively.

**Lead analysis in tissues of kidney**

Depositing 1g fresh weight of each sample with 1 ml of nitric acid (HNO₃) at 65% purity, we bring the temperature to 95 ° C for one hour, after cooling; we supplement the content to 4ml of
double distilled water. The lead concentration was determined in the organs by atomic absorption spectrophotometry (Perkin-Elmer model 800) and the values were expressed in µg/g.

**Histopathological examination**

After scarification, small pieces of kidney were taken from all rats groups fixed in buffered formalin solution, dehydrated in ascending grades of ethanol, cleared in xylol and casting, blocking, cutting at 8–10m thickness. To observe the histological changes in control and treated stages two stains, that is, hemotoxylin/eosin and thionin were used (Bancroft ,1975).

**Statistical analysis**

Results were expressed as mean ± standard error of the mean (SEM). Data were analysed by the two-way analyses of variance (ANOVAs). When a significant difference was found, the Student-Newman-Keuls post-hoc test was conducted. For all analyses, a difference was considered significant at P ≤ 0.05.

**RESULTS**

Table 1: Effect of clove oil on the body and the kidney weights in rats intoxicated.

<table>
<thead>
<tr>
<th>Groups (g)</th>
<th>Pb</th>
<th>CEO</th>
<th>Pb+CEO</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weights</td>
<td>76.85±0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>96.68±0.015</td>
<td>80.250±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>93.347±0.248</td>
</tr>
<tr>
<td>Kidney weights</td>
<td>0.441±0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.380±0.001</td>
<td>0.424±0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.371±0.0022</td>
</tr>
</tbody>
</table>

Data are mean ±S.E.M. ab P<0.001, (Pb vs. Control).

Table 2: Effect of clove oil on the level of blood urea, uric acid and creatinine in serum of rats intoxicated.

<table>
<thead>
<tr>
<th>Groups (mg/dl)</th>
<th>Pb</th>
<th>CEO</th>
<th>Pb+CEO</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea</td>
<td>4.40±0.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.89±0.20</td>
<td>3.99±0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.72±0.18</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>6.11±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.017±0.26</td>
<td>4.14±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50±0.56</td>
</tr>
<tr>
<td>Créatinine</td>
<td>11.99±0.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.33±0.005</td>
<td>9.84±0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.40±0.38</td>
</tr>
</tbody>
</table>

Data are mean±S.E.M. ab P<0.001, bP<0.01, (Pb vs. Control)

Table 3: Effect of clove oil in the activity of anti-oxidant enzymes and lead acetate concentration (Pb) in kidney of rats intoxicated.

<table>
<thead>
<tr>
<th>Groups (mg/dl)</th>
<th>Pb</th>
<th>CEO</th>
<th>Pb+CEO</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/g)</td>
<td>0.61±0.0359&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93±0.0191</td>
<td>0.82±0.0191&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.020</td>
</tr>
<tr>
<td>CAT (U/g)</td>
<td>0.46±0.024&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.63±0.081</td>
<td>0.60±0.018&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.65±0.049</td>
</tr>
<tr>
<td>GPx (U/g)</td>
<td>0.850±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.211±0.004</td>
<td>0.951±0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.393±0.32</td>
</tr>
<tr>
<td>Pb (µg/g)</td>
<td>360±6,5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.188±0.008</td>
<td>163.37±4,52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.241±0.005</td>
</tr>
</tbody>
</table>

Data are mean±S.E.M. ab P<0.001, bP<0.01, aP<0.05, (Pb vs. Control)
The kidney lead concentration was significantly increased ($^{ab}P < 0.001$) in exposed-animals compared to controls. We also noticed a significant decrease ($^{ab}P < 0.001$) of lead levels in rats treated with clove essential oil reports to the intoxicated rats (Table 3).

Lead poisoning entraîn a significant disruption of renal function parameters (creatinine, urea) in the blood. Indeed, histological study of kidney tissue intoxicated by lead with a dose of 0.2% rats showed necrosis of glomeruli exposed to their architecture, with tubular dilation and altered tubules (Figure 1(C)). Furthermore, the optical microscope observation of kidney tissue from control and treated rats with CEO showed the normal structure of the renal tubules and glomeruli(Figure 1 (A, A2) and Figure 1 (B)). In the same context, the animals exposed to lead and treated with CEO show more or less normal structure of glomeruli and tubules (Figure 1 (A)).

Figure 1: Photomicrograph of kidney tissue stained with hematoxylin and eosin. (A) and (A1) in kidney sections from control rats appeared with normal architecture. (B) and (B1) Rats exposed to lead with a dose 0.2% showed tubular necrosis (black arrow), nucleus pyknosis (yellow arrow) and tubular dilation and glomerular atrophy. (C) and (C1) intoxicated rats treated with CEO show tubular regeneration (black arrow) and glomerular and the tubular Réobtenions gauge (D)and (D1) kidney sections from treated rats with CEO.
DISCUSSION
This study was carried out to provide, on the one hand, a comprehensive assessment of the Pb exposure effects during pregnancy and lactation on the kidney function, and on the other hand, to attenuate the Pb nephro-toxicity, which is translated into structural lesions in the nephron by using Syzygium aromaticum essential oil treatment on rats. Since body weight is considered as a putative indicator of health, the final body weight of lead poisoning rats with lead was significantly lower than the normal group (C). These results clearly indicated that lead during the pregnancy and lactation period caused a significant decrease in the gain of body weight. Similar observations have been reported by different authors reporting that weight reduction is intimately linked to the reduction of food intake following a Pb intoxication and induces an anorectic effect by directly acting on the nervous centers responsible for the regulation of satiety and hunger (Herak-Kramberger and Sabolic, 2001; Djebli et al., 2005). However, this heavy metal is known to act on the body weight of the animals even when intoxication levels remain low (Auclair et al., 2004). In the same way, a significant increase in the weight of the kidney is a result of lead toxicity. Abdel Moneim et al., (2011) observed increases in both kidney weights and the relative kidneys weights in rats treated with lead acetate while O’Flaherty et al., (1986) observed increases in both kidney wet weight and dry weight in rats given varying doses of lead. Further, lead modifies cellular function by disrupting many different metabolic pathways and physiological processes (Haito et al., 2008). However, the results of the present study clearly demonstrate an increase of urea, uric acid and creatinine in the blood. These results show that lead affects the glomerular filtration causing disturbances in concentration urea and creatinine in the blood (Fowler and Du Val, 1991). Furthermore, Bonsignore et al., (1965) made two assumptions regarding the increase in serum uric acid. In the first, the increase is due to the direct effect of lead on the metabolism of uric acid. In the second, it is the result of morphological disorders of the nephron. These changes may be related to one or more factors, including increased serum levels of Pb or decreased Pb reabsorption by alteration in tubular transport mechanisms, as well as structural lesions in the nephron (Murata et al, 2009). The current work revealed that the levels of Pb in tissues of kidney were significantly higher in Pb treated group than controls. These results are also in agreement with the work of Thylamal and Saroja, (2004) which showed that the kidneys are among the most sensitive to lead organs. Some works suggested that this element is strongly bound to macromolecules in the intracellular compartment because Pb binding proteins have been isolated from the kidney, liver, blood and brain explained the high concentration of Pb in a kidney tissue (Thylamal et al., 2004). In fact, histological examinations revealed that chronic Pb exposure causes progressive necrosis glomerular and tubular alterations and interstitial changes (El-Nekeety et al., 2009). These tubular alterations caused by Pb toxicity might be a result of a hydrolic changes in the renal tissue and suggest that Pb intoxication yields to a partial failure in the ions pump transport of tubules cells which in turn produces tubular swelling and causes necrosis of the tubules. The loss of cell integrity, cell death and renal tissue damage is the result of free radical attack in response to oxidative stress (Ekor et al., 2006; Sener et al., 2002). The oxidative stress has also been implicated to contribute to lead-associated tissue injury in the liver, kidney, brain and other organs (Sener et al., 2002).

However, the antioxidant enzyme such as SOD, CAT and GPx play an important role in regulation and maintenance of metabolism in the body against oxidative stress (Wu et al., 2004). Reactive oxygen species (ROS) generated are scavenged by the antioxidant defense system. In issues, the cellular antioxidant defense system includes enzymes such as SOD, CAT and GPx. The productions of free radicals or ROS in the tissues exceed the ability of the antioxidant system to eliminate them, result in oxidative stress (Husain and Somani, 1997). These enzymes play a vital role during the process of scavenging (ROS) and preventing their formation (Veerappan et al., 2004). Therefore, Pb may affect the antioxidant barrier via inhibiting the functional thiol groups of enzymes such as SOD (Jurczuk et al., 2006; Patrick, 2006). Our results showed a decreases activity of SOD, CAT and GPx in chronic Pb exposure rats. It has been reported that this metal impairs the antioxidant system of the tissues in proportion to the amount of lead ingestion (O’Flaherty et al., 1986).

In the other hand, the traditional use indicates that clove has several therapeutic properties (Dashti-R and Morshed, 2009). So the major
The efficiency of clove oil on oxidative damage. Antioxidant property of clove oil has protective effect against lead intoxication. Clove essential oil has been reported to stimulate glucose transport into cells (Judentlich and Pertunen, 2004). The current results clearly indicate that clove oil treatment with induce significant decreased in kidney markers (urea, uric acid and creatinine) compared with Pb poisoning group. Moreover, it succeeded to induce an improvement in kidney function. Our results are in agreement with the works of Abdel Wahhab and Aly, (2005). Histological investigations revealed that clove oil could improve to some extent the altered kidney histopathology. Said, (2011), reported that eugenol reduced tubular necrosis in Gentamicin intoxicated kidneys. The efficiency of clove oil to reduced accumulation of Pb in kidney was perhaps due to the presence of chelating agent such as eugenol (Nassar et al., 2007). These biologically active compounds might have chelated lead and enhanced its excretion from the body, resulting in reduced Pb accumulation in renal tissue. The activities of antioxidants enzymic such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in renal tissue were significantly elevated on clove oil supplementation as compared to the Pb intoxicated rats. Clove essential oil has been reported in previous studies as one of the strongest antioxidants, even higher than some synthetic antioxidants like BHT or butylatedhydroxyanisole (Misharina et al., 2008; Wei et al., 2010). The strong activity of clove essential oil can be due to the presence of eugenol, the main constituent of this essential oil, which is known to have antioxidant activity and has protective effect against oxidative damage induced by Pb (Wei et al., 2010). Additionally, some studies suggest that clove essential oil has a kidney protective effect (Abozid and EL-Sayed, 2013; Said, 2011).

CONCLUSION
The obtained results showed that Pb intoxication resulted in a significant decrease in body weight, the levels of SOD, CAT and GPx activities in tissue homogenates of kidney. Moreover, Pb significantly increased the lead concentration in the kidney. Pb markedly increased urea, uric acid and creatinine. Regarding to the histopathological examination, Pb exposure induced necrosis glomeruli of the kidney. The clove essential oil administration caused a significant improvement in histopathological structure of the kidney as well as the kidney function and antioxidant status. Therefore, this oil may play a protective role against lead-mediated kidney injury.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT
The authors are grateful to member of the Pathology Laboratory, University Hospital Complex of Sidi-Bel-Abbes, Sidi-Bel-Abbes, Algeria for all contribution to the realization of this work.

AUTHOR CONTRIBUTIONS
DEHA,KH and BM designed and performed the experiments and also wrote the manuscript. MB, KK and MS performed the biochemical and statistical analysis and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Abdel-Wahhab MA, 2005. Antioxidant property of Nigella sativa (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. Aly SE. J ApplToxicol
Adli et al., Nephro-protective effect of *Syzygium aromaticum* against lead intoxication


Herak-Kramberger and CM Sabolic I, 2001. The integrity ofrenal cortical brush-border and basolateral membrane vesicles is damaged in vitro by nephrotoxic heavy metals. Toxicology 156: 139-147.


