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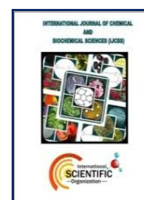

Effectiveness of a Novel SeNPs synthetized by *Aquilaria malaccensis* extract compared to selenium acetate on lead induced metabolic disorder and oxidative stress in pregnant rats

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Abstract

The aim of this study was to evaluate the effect of selenium and selenium nanoparticles on some biological parameters in pregnant rats exposed to lead acetate. Twenty-five (25) female rats were randomly divided into four groups (n=5): control, pregnant rats, pregnant rats+lead (pregnant Pb), pregnant Pb+Se and pregnant rats+SeNPs. Lead (200 mg/kg b.w.) as Pb $(C_2H_3O_2)_2$ added in their drinking water for 20 days. Se and SeNPs, at a dose 10 g/kg of diet and 3mg/kg of diet respectively, were added to the feed during the last 10 days of lead exposed in the animals. The results obtained show that pregnancy does not significantly affect the most of parameters studied. Our results showed also that a significant increase ($p<0.05$) in cholesterol and liver glycogen concentration and in GOT and GPT activities in the pregnancy Pb group compared to the pregnancy rats. The results obtained also reveal a significant decrease ($p<0.05$) in the level of GR, GB and Hb, tissue GSH and SOD activity and a significant increase ($p<0.05$) in heart and liver MDA levels in comparison with pregnancy group. The treatment with Se and SeNPs partially improves the biochemical and hematological parameters with protection of the tissues against radical attacks (oxidative stress) caused by lead in pregnant rats. In conclusion, this work shows that selenium and SeNPs has beneficial effects against lead toxicity in rats during gestation.

Keywords: Lead, Pregnancy, Se, SeNPs, oxidative stress, Wistar rats

Full length article **Corresponding Author*, e-mail: dersamebio@gmail.com

1. Introduction

Pregnancy is an important and complex stage in a woman's life. The body has metabolic and physiological changes simultaneously with the development and growth of the fetus [1]. In the case of pregnancy, the body's resistance to diseases is very weak, especially those related to poisonings such as lead poisoning [2], which is a non-essential toxic heavy metal widely distributed in the environment. Lead as xenobiotic is known to induce a wide range of dysfunctions of the central and peripheral nervous systems and the hemopoietin system [3]. Pregnancy exposes too many complications that can be related to an alteration of oxidative stress that is also associated with the appearance of several pathologies during pregnancy [4]. Radical phenomena play an important role in reproduction, implantation of the fertilized egg and the development of the embryo. But an imbalance between their production, intense during gestation, and their elimination can generate oxidative stress [5]. Toxic metals increase the production of free radicals and reduce the availability of antioxidant stores in order to respond to stress resulting from damage [6]. A growing body of data proves that metals are able to interact with nuclear proteins and DNA, causing oxidative deterioration of biological macromolecules, eventually leading to many chronic diseases, such as atherosclerosis, cancer, and diabetes [7]. Selenium is essential for the life of living beings [8]. Recent data confirm the major role of selenium in many pathophysiological situations. Moderate selenium deficiency, especially when associated with low vitamin E status, appears to increase susceptibility to various diseases in which oxidative stress is involved: cardiovascular disease, inflammatory disease, viral infections, neurodegenerative diseases and cancers [9]. But it is reported that the degree of selenium absorption is much lower in ruminants than in non-ruminants when selenium supplementation is taken orally [10]. In order to avoid this negative phenomenon of reduced absorption of selenium, nanoparticles polymer systems with in vivo potential have been proposed, these nano-forms of selenium characterized

by their small size, which facilitates cell access and their antioxidant and anti-oxidant power-inflammatory [11]. Nanotechnology has been a known field of research since the last century [12]. The green synthesis of nanoparticles is a novel way to synthesize nanoparticles by using biological sources. It is gaining attention and attracted the scientists from different fields due to its simplicity and environment-friendly nature [13]. The biosynthesis for obtaining nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology [14]. To the extent of our knowledge, after reviewing the literature, there were no in-vivo studies that have evaluated the efficacy of biologically synthesized nano-selenium in lead toxic of pregnant rats. Thus, this study aim was to investigation of the toxic effects of lead in pregnant rats and to study the efficacy of selenium acetate and SeNPs supplementation in Wistar rats.

2. Materials and methods

2.1. Chemicals

Sodium chloride (NaCl), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), methanol, coomassie blue, butylate dihydroxy toluene (BHT), trichloroacetic acid (TCA), phosphate-buffered (KH_2PO_4, K_2HPO_4), ethylene diamin tetra-acetic acid (EDTA) were of analytical grade. A. malaccensis heartwood was collected in herbalists' shops from a local market.

2.2. Synthesis of nano-selenium with A. malaccensis extract

2.2.1. Preparation of A. malaccensis extract

"*A. malaccensis* extract was prepared by exactly weighting 10 g of *A. malaccensis* heartwood which was transferred into a 250 ml conical flask, already containing 100 mL of deionized water. The mixture was then heated at 50°C for 20 min, and the extract was macerated at room temperature for 24 hours and then filtered by using Whatman® filter paper grade 1. The filtered extract was used at the same session to synthesize selenium nanoparticles [15].

2.2.2. Green synthesis of selenium nanoparticles (SeNPs)

50 ml of selenium acetate dihydrate solution (0.5 M) were prepared with distilled water. 1 ml of aqueous extract of the trunk bark of *A. malaccensis* was added to the above solution after 10 minutes of stirring. To maintain the pH at 12, 2.0 mol-dm^{-3} sodium hydroxide was used to give a pale white aqueous solution (appendices). This was then placed in a magnetic stirrer for 2 hours [16]. The pale white precipitate was then removed and washed again with distilled water followed by ethanol to get rid of impurities. Then a pale white powder of selenium oxide nanoparticles was obtained after drying at 60°C. in the oven overnight (appendices) [17].

2.3. Characterization of SeNPs

The SeNPs nanoparticles prepared by the above method were characterized using Scanning Electron Microscopy (SEM) (Thermo Scientific Apreo S apparatus) for morphology analysis of the nanoparticles.

2.4. Animals and experimental design

Females rats with weight (187.32±2.54) were bought from animal's service of Pasteur institute in Algeria, they are installed in faculty SNV, University of El Oued, Algeria in plastic cages divided in five groups of 5 rats of each. They kept in the animals breeding house for adaptation. The animals were adapted to laboratory condition photoperiod (12 h of night/12h of darkness), an ambient temperature of 24±4°C and humidity of (62.2±11%) for two weeks. The standard diet and water are free for the animals during period of adaptation. The realization of the experimental part is respect to the ethical approval Committee referenced (105 EC/DCMB/FNSL/EU2019) of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El Oued, Algeria. Coupling method was assisted by placing the individual females overnight in the home cage of a singly-housed male of the same stock. Some normal non-pregnant rats and positively pregnant females were chosen and randomly divided into the following five groups (5 rats):

Group 1 (Control group): Non-pregnant rats served as normal control

Group 2 (Pregnant rats group): Pregnant rats received normal diet

Group 3 (Pregnant Pb group): Pregnancy rats received acetate lead (200 mg/kg b.w) as Pb(C2H3O2)2 added in drinking water for 20 days

Group 4 (Pregnant Pb + Se group): Pregnancy rats received acetate lead and treated with selenium (10 mg/kg diet) for 10 days

Group 5 (Pregnant Pb + SeNPs group): Pregnancy rats received acetate lead and treated with selenium nanoparticles (3 mg/kg diet) for 10 days

2.5. Blood collection and tissue preparation

At the end of 3rd week of experiment the animals were fasted for 16 h, anesthetized by chloroform inhalation then sacrificed by decapitation. The blood was collected in EDTA tubes for hematological analysis. The serum was obtained by blood centrifuging at 3000 rpm for 10 min and frozen at 20 °C until the use for urea, creatinine, calcium and electrolytes levels assay. Then, the liver and kidney of rats of different groups was rapidly excised, weighed and

stored at-20°C until use for lead and oxidative stress evaluation.

2.6. Determination of biochemical and hematological markers

Serum triglycerides, triglycerides, GOT and GPT parameters levels were determined by Autoanalysis (BIOLIS24j) use commercial kit from Spin react, Spain (ref: triglycerides -20141, triglycerides -20151, GOT-20051 and GPT-1001170). Hematological analysis (FNS) is performed by the hematology autoanalyzer (Sysmex).

2.7. Determination of liver lead concentration

Dry calcination of liver 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 (HNO_3). 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 dilution [18].

2.8. Preparation of tissue homogenate

One gram of liver or heart from each rats of different experiment groups was used. The tissues were milled and homogenized in 9 mL of buffer solution of TBS (50 mM Tris, 150 mM NaCl, pH 7.4). The tissue suspension was centrifuged at 9000 rpm for 15 min at 4C°, the supernatant obtained was stored at -20C° until use for glycogen or the oxidative stress marker assay.

2.9. Determination of liver glycogen concentration

The determination of glycogen in the liver is carried out according to the anthrone-sulfuric acid colorimetric method. 1 ml of anthrone reagent is added to 25 µl of the sample (liver homogenate) then the mixture is placed in a water bath at 80ºC for 10 min. A green color develops, the intensity of which is proportional to the amount of carbohydrate present in the sample. Reading the optical density at 620 nm against a reagent blank containing distilled water. The absorbance value is calculated and then converted into equivalent carbohydrate concentration using a calibration curve established with glucose (0.01-0.1 mg/ml), under the same experimental conditions [19].

2.10. Oxidative stress markers measurement

The method of malondialdehyde (MDA) assay is based on the reaction between the carbonyl compounds of malondialdehyde with thiobarbituric acid according the method of Yagi, (1976) [20]. The level of reduced Glutathion (GSH) was determined according the method of Weak and Cory, (1988) [21] by measuring the optical density results from the formation of 2-nitro-5-mercocapturic acid from the reduction of dithio-bis-2-nitrobenzoic acid, which is called Ellman reagent with SH groups exist in GSH. The assay method of superoxide dismutase (SOD) activity using the NBT by the superoxide anion (O_2), is used as a basis for detecting of presence of SOD by measuring the absorbance at 560 nm [22].

2.11. Statistical Analysis

The statistical evaluation is carried out by the student's T test using Minitab 17.1 statistical package. The values are given as mean and standard deviations (SD) (mean±SD) for four groups. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Scanning electron microscopy (SEM) analysis

The size of the SeNPs was analyzed through scanning electron microscopy (SEM) images (Figure1). The size of some selected biosynthesized nanoparticles was 49.98–87.26 nm according to SEM images.

3.2. Biochemical markers

Concerning the biochemical markers (table 1), results obtained show that no significant variation ($p \geq 0.05$) in serum triglycerides, VLDL, GOT and GPT levels in the serum and glycogen and lead in liver of pregnant rats group compared to the controls. Our results also show a significant increase ($p<0.05$) in serum triglycerides, total cholesterol, VLDL concentration, GPT and GOT activities and liver lead and liver glycogen in the pregnant Pb group compared to the pregnant rats group. Treatment with selenium acetate and SeNPs significantly ($p<0.05$) ameliorated the most of biochemical marker studied compared to the pregnant Pb and pregnant rats groups.

3.3. Hematological markers

Results obtained in table 2 shows that a ($p<0.05$) significant decrease in Platelet level and no significant variation ($p \geq 0.05$) in the red blood cells (RBC), white blood cells (WBC), MCV and hemoglobin (HB) levels in pregnant rats group compared to the controls. In the other hand, results show that exposure of pregnant rats to lead acetate caused a significant decrease ($p<0.05$) in red blood cells, white blood cells and hemoglobin levels but a significant increase in platelet level when compared to pregnant rats group. Treatment of pregnant rats exposed to lead with selenium acetate or SeNPs significantly ($p<0.05$) improved the number of red blood cells, white blood cell and platelet count and HB concentration, as compared to the pregnant Pb and pregnant rats groups.

3.4. Oxidative stress markers

The analysis of the MDA GSH and SOD levels in liver and heart was conducted. Our results showed that no significant variation ($p \geq 0.05$) in MDA and GSH levels and in SOD activity, in PR group compared to the controls. It

was found also that the pregnant rats treated with the lead acetate significantly ($p<0.05$) increased MDA levels, while SOD and GSH were revealed to have decreased in liver and heart tissues when a comparison is drawn with the pregnant rat's group. However, the selenium acetate and SeNPs treated group showed the exact reverse when compare with the pregnant Pb and pregnant groups as shown in table 3.

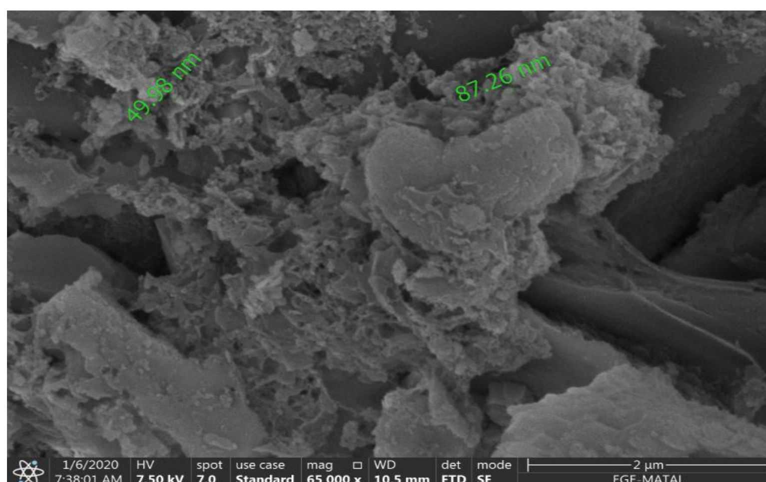

Figure 1. Scanning electron microscope images of Selenium-*A. malaccensis* extract nanoparticles

Table 1. Liver glycogen and lead and serum biochemical levels in control and experimental groups

Parameters	Control (n=5)	Pregnant rats (n=5)	Pregnant Pb (n=5)	Pregnant Pb+Se (n=5)	Pregnant Pb+SeNPs (n=5)
Blood glucose (mg/dl)	89.15±4.71[a]	156±2.54[b]	148±5.36[b]	152±1.78[b]	153±2.32[b]
Serum Triglycerides (mg/dl)	49.50±5.30[a]	41±2.97[a]	65±3.68[b]	35±4.61[c]	46±1.85a
Serum Cholesterol (mg/dl)	154.17±1.16[a]	65±8.02[b]	263±5.89[c]	75±3.12[b]	65±4.23[b]
Serum VLDL (mg/dl)	9.9±0.86[a]	8.2±0.13[a]	13.02±0. 38[b]	7.3±0. 21[a]	9.2±0.63[a]
Serum GOT (U/l)	205.80±10.30[a]	190±12.2[a]	450±25.4[b]	226±23.12[a]	184.3±31.2[a]
Serum GPT (U/l)	29.50±4.33[a]	33±1.32[a]	59±3.23[b]	61.6±4.16[b]	45.5±1.32[c]
Liver glycogen (µg/g tissue)	14.82±0.3[a]	15.31±0.26[a]	46.62±0.21[b]	29.72±0.75[c]	51.48±1.05[b]
Liver lead (µg/g tissue)	0.020±0.009[a]	0.031±0.002[a]	0.152±0.[007b]	0.184+0.003[b]	0.134±0.001[c]

The results are presented by mean±SEM. n=number of observations. [a,b,c] Different superscript within each line indicate significant difference between the means ($p<0.05$)

Table 2. Hematological markers levels in control and experimental groups

Parameters	Control (n=5)	Pregnant rats (n=5)	Pregnant Pb (n=5)	Pregnant Pb+Se (n=5)	Pregnant Pb+SeNPs (n=5)
RBC (10^6/µl)	7.17±0.3[a]	6.98±0.11[a]	6.41±0.09[b]	7.37±0.08[a]	**6.76±0.14[a]**
Hemoglobin (g/dl)	14.15 ± 0.12[a]	14.12±0.52[a]	11.23±0.91[b]	13.86±0.35[a]	**13.07±0.45[a]**
MCV (fL)	55±1.85[a]	56±2.36[a]	57±2.95[a]	54±3.61[a]	**55±5.42[a]**
WBC (10^3/µl)	6.60 ± 0.20[a]	7.22±0.63[a]	3.44±0.42[b]	5.01±0.15[c]	**5.20±0.32[c]**
Platelet (10^5/µl)	1.20 ± 0.020[a]	0.71±0.05[b]	0.85±0.08[c]	0.71±0.082[b]	**0.48±0.044[d]**

The results are presented by mean± SEM. n=number of observations. [a,b,c,d] Different superscript within each line indicate significant difference between the means ($p< 0.05$)

Table 3. Oxidative stress markers in liver and heart of control and experimental groups

	Parameters	Control (n=5)	Pregnant rats (n=5)	Pregnant Pb (n=5)	Pregnant Pb+Se (n=5)	Pregnant Pb+SeNPs (n=5)
Liver	**MDA (µmol/mg prot)**	0.48±0.037[a]	0.50±0.039[a]	0.56±0.022[b]	0.46±0.031[a]	0.43±0.082[a]
	GSH (nmol/mg prot)	120±1.7[a]	100.22±1.02[a]	55.81±0.92[b]	95.2±1.63[a]	140.1±8.38[a]
	SOD (nmol/min/mg prot)	0.25±0.002[a]	0.32±0.006[a]	0.16±0.003[b]	0.23±0.0081[a]	0.18±0.0009[b]
Heart	**MDA (µmol/mg prot)**	0.51±0.24[a]	0.53±0.24[a]	0.74±0.014[b]	0.47±0.069[a]	0.46±0.017[a]
	GSH (nmol/mg prot)	71.8±2.36[a]	75.07±7.08[a]	69.8±2.36[b]	79.35±6.82[a]	64.72±3.67[b]
	SOD (nmol/min/mg prot)	0.29±0.003[a]	0.28±0.003[a]	0.31±0.005[b]	0.33±0.002[b]	0.34±0.001[b]

The results are presented by mean± SEM. n=number of observations. [a,b,c] Different superscript within each line indicate significant difference between the means ($p < 0.05$)

4. Discussion

Recent studies show that nanocompounds may be more effective than parent compounds [23]. In addition, nanoscale compounds have various therapeutic advantages including low side effects. For this, selenium and its nano form (SeNPs) were chosen as the basis of biotherapy against lead toxicity. The results obtained show a very significant increase in the level of Pb in the liver during gestation, which reflects on the binding of this metal to these target tissues. The liver is an important target for lead. Lead has a high affinity for the protein thiol groups of hepatic cell membranes, which leads to hepatic lyses and necrosis [24]. But on the other hand, our results do not indicate any effect of selenium or nano selenium on the level of Pb which concludes that from the above results that these elements act against tissue damage induced by Pb but not on the lead level itself. On the other hand, the results obtained from our study show a metabolic disturbance characterized by a significant increase in serum cholesterol and transaminases and of hepatic glycogen levels in pregnant rats intoxicated by lead. The high cholesterol level could be due either to an increase in the synthesis or to a decrease in the elimination of lipoproteins. Decreased elimination may result from alteration of cell surface lipoprotein receptors or from inhibition of hepatic lipase lipoprotein activity [25]. An increase in serum transaminases activities due to hepatic dysfunction after exposure to several toxic metals [26]. Leakage of transaminases into the bloodstream may be the result of liver dysfunction [27]. On the other hand, the results of metal therapy show that used Se and SeNPs supplementation in feed restored glycogen level, transaminases activities and lipid profile. Selenium salt and SeNPs influenced significantly on cholesterol. These results could be attributed to lipolysis, which was elevated under the influence of Se [28]. Supplementation with SeNPs increases the levels of 15-deoxy-Δ-12, 14-prostaglandin J2 [29], a receptor activated by proliferator-γ ligand. Activation of this receptor will decrease the level of sterol regulatory element binding protein 2, resulting in cholesterol depression [30]. For Selenium, which is an essential trace element, has been shown to protect against cardiovascular disease [31]. Improvement in glycogen level by selenium supplementation could be due to acceleration of glucose metabolism and inhibition of glucose synthesis in the liver, suggesting a decrease in a source of precursor supply for gluconeogenesis, a decrease in the passage from the liver to blood and muscles [32]. Our results showed a significant change in hematological parameters in pregnant rats exposed to lead acetate. These results are in accordance with the study of Alain et al., (2015) [33] and the study of Orji et al., (2016) [34]. These changes could be attributed to the toxic effect of lead on blood cells where it interacts with certain reactions where calcium is a secondary mediator, causing the inhibition of a number of enzymes like aminolevulinic acid dehydratase, plays a key role in the biosynthesis of the heme necessary for the formation of hemoglobin [35]. The decrease in WBC may be the result of inactivation of the immune system related to the toxic action of lead which can induce leukopenia and thrombocytopenia [36]. In addition, the treatment of pregnant rats exposed to lead by Se and SeNPs significantly improved hematological parameters. SeNPs is a small-scale, mobile-effect chemoprevention agent [37]. Selenium increases immunity and has a role in inflammation by increasing the production of antibodies thus enhancing the effect of their synthesis in response to antigenic stimulation [38]. The results of the effect of lead on oxidative stress markers show that lead affects these markers by increasing the level of tissue MDA, and the activity of hepatic and renal GST and by decreasing GSH and tissue SOD in pregnant rats. Radical phenomena play an important role in the reproduction, the nesting of the fertilized egg and the development of the embryo. But an imbalance between their production, intense during gestation, and their elimination can generate oxidative stress [39]. Oxidative stress has been suggested as one of the main mechanisms of Pb toxicity. Lead may induce oxidative damage possibly due to inhibition of 5-aminolevulinic acid (ALA) dehydratase leading to accumulation of ALA, a

potential endogenous source of free radicals or due to the direct interaction of Pb with biological membranes, inducing lipid peroxidation [40]. Furthermore, treatment of pregnant rats exposed to lead by Se or SeNPs improved oxidative stress levels in liver and heart. Our results suggest that the Se possesses the protective effect in Pb poisoning by reducing oxidative stress through antioxidant properties [41].The SeNPs could prevent lead-induced damage, and thus play an important role in the prevention of diseases resulting from lipid peroxidation. This effective defense can delay the oxidation of lipids or other macromolecules, neutralizing free radicals, thereby reducing reactive oxygen species or chelating metal ions [42]. Selenium induces the expression of selenium peroxidase-dependent glutathione through the formation of seleno-phosphate which is an integral part of seleno-cysteiyl tRNA. Glutathione-dependent glutathione peroxidase plays a central role in treatment by lowering the level of ROS inside the cell [43]. Additionally, selenium increases the activity of selenium dependent antioxidant enzymes such as GPx and TrxR. This can therefore reduce the peroxidation of lipids by free radicals, and regenerate GSH [44].

5. Conclusion

In conclusion, lead is a strong toxic agent in metabolic system during pregnancy by inducing oxidative stress in rats which causes several health problems in pregnant rats. Selenium salts and nanoparticles form supplementation in food were able to moderate this toxicity by decreasing oxidative stress and restoration of the biochemical and hematological parameters which protects rats and their fetus during pregnancy.

Conflict of interest statement

All authors have approved the manuscript with no conflict of interest.

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