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Original Research Article

Potentials of *Pisum sativum* Extract and *Cocos nucifera* Water in Mitigating Lead Toxicity in Body Weight and Serum Lipid Profile of Rat Model

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ABSTRACT

Many studies have recommended using plants and their extracts for abating heavy metals from cells and tissues. Most of these recommendations are, however, based on the rich antioxidant properties in them. Thus, this study investigated *Pisum sativum* extract (PS) and *Cocos nucifera* water's (CN) ameliorative potentials on the alterations in body weight and serum lipid profile of rats induced by lead nitrate ($Pb(NO_3)_2$). Lipid profile analyses were done on the serum collected from blood samples of forty-eight healthy albino rats (distributed into eight groups of 6 rats each) of average weight 154 g. This experiment lasted for 18 weeks and at the end of 3 weeks, one rat from each group was sacrificed. Results showed that the average values of total cholesterol, triglyceride, LDL-ch, coronary risk index, and LDL/HDL-ch significantly increased in rats administered with only ($Pb(NO_3)_2$) compared with rats in the control groups. Also, a significant reduction in the mean value of HDL-ch and body weight was observed in the group of rats administered with only ($Pb(NO_3)_2$) compared to the group of rats in the control groups. Furthermore, a decrease in the total cholesterol, triglyceride, LDL-ch, coronary risk index, and LDL/HDL-ch was observed from this study. In contrast, the mean value of HDL-ch and body weight of the rats increased when co-treated with PS and CN, especially in the combined form. Thus, this study showed that PS and CN have the potentials to ameliorate the alterations in body weight and lipid profile of the rat model induced by ($Pb(NO_3)_2$).

***Keywords*:** Atherosclerosis, Cardiovascular disorder, Lead nitrate, Medicinal plant.

Introduction

Lead exposure is related to adverse cardiovascular consequences and results in increased chances of adverse inflammatory clinical markers.[1]Several epidemiologic investigations have revealed a relationship between chronic low-level lead exposure with the risk of hypertension and the elevation of blood pressure.[2,3] Lead is known to affect organ/body weight ratio when given at toxic doses, which may depress weight gain.[4] Josthna, Geetharathan,[5] reported that accumulation of lead in rats' tissues, markedly decreased body weight gain in comparison to the control group of rats. Alteration in serum lipid profile due to lead exposure contributes significantly to cardiovascular disease progression as it leads to a rise in the amount of low-density lipoprotein cholesterol (LDL-ch), triacylglycerol (TG), total cholesterol (TC), and decrease in high-density lipoprotein cholesterol (HDL-ch).[6]The TC ratio to HDL-ch is referred to as the coronary risk index (CRI).[7]Coronary risk index can be a robust marker for envisaging the peril of coronary heart disease and atherosclerosis and reveal the occurrence of low-density lipoprotein cholesterol or triacylglycerol in the serum.[6]Lead toxicity employs a pathological free radical mechanism that results in lipid peroxidation and the breakdown of phospholipids, leading to loss of membrane integrity and damage of tissues and organs.[8]

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DMSA and $CaNa_2EDTA$ which stands for meso-2,3-dimercaptosuccinic acid and calcium disodium ethylenediaminetetraacetic acid respectively are widely used in mitigating lead toxicity,[9] however, Zhai, Narbad [10] reported from his review that there is a need to use medicinal plants in getting rid of lead from tissues as some chemical substances ($CaNa_2EDTA$ and DMSA) reported to expel lead from tissues, could cause nephrotoxicity, hepatotoxicity and neurotoxicity in experimental animal studies. Various types of medicinal plants like *Pleurotus tuberregium*,[11] *Allium sativum*,[12] and *Indigofera oblongifolia*[13]have been reported to neutralize or detoxify toxins and protect cells and tissues from the toxic effects of lead. *Pisum sativum* and *Cocos nucifera* water have been established to have medicinal application by scientific evidence.[14,15]*Cocos nucifera* water and its active components shikimic acid has been reported to reverse hydrogen peroxide-induced oxidative damage in the main functional cells of the liver, maybe via the obstruction of nuclear factor-κB, with the stimulation of the phosphorylated-phosphatidylinositol 3-kinase-Akt-glycogen synthase kinase 3 beta (p-PI3K/Akt/GSK3β) pathways and decrease in apoptosis by interfering the SAPK/JNK/Bax pathway.[16]*Pisum sativum* is an essential dietary source of vitamins and critical metals that can promote the vitamins and essential metals in the human body, which can decrease the risks of lead toxicity.[10] Sandström, Hansen[17] reported that triglyceride reduced when they examined the influence of fibre preparations prepared from fibre obtained from *Pisum sativum* cell wall on cardiovascular health. Hence, this research aimed to investigate the ameliorative potentials of the combined use of *Pisum sativum* extract and *Cocos nucifera* wateron lead-induced serum lipid and coronary risk indices alterations in rats. Based on the rich medicinal properties of *Pisum sativum* extract and *Cocos nucifera* water,[18-21] this research posited that the combined use of *Pisum sativum* extract and *Cocos nucifera* water will ameliorate the effect of lead on rats' serum lipid and coronary risk indices.

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Materials and Methods

Experimental animals, lead nitrate and dietary protocol

Albino rats of both sexes with a body weight of 154 ± 5 g were acquired from the Department of Animal and Environmental Biology breeding unit, University of Benin. They were kept in wooden cages (45 by 30 by 20 cm) with wire mesh covers on the cages and were fed regularly with rat pellet obtained from Ewu, Edo State, Nigeria, and water *ad libitum* for a period of 2 weeks for proper acclimatization. Upon clearance from the ethical committee (approval number: LS20261), animal care was performed according to the National Research Council's guidelines and the American Association of Accreditation for Lab Animal Care.[22]Lead nitrate ($Pb(NO_3)_2$) of laboratory reagent grade was procured from Paten Chemicals in Benin, Nigeria. In March, 2020, *Cocos nucifera* and *Pisum sativum* were obtained from a traditional non-industrialized area in Evbuotubu village and vegetable market, respectively in Benin City. The edible aerial part of *Pisum sativum* L. was shade dried and ground into fine particulate. Five hundred grams (500 g) of the particulate was soaked in 5000 ml of 98% ethanol, and after removal of debris, evaporation of the solvent was done using a rotary evaporator to obtain the crude extract (yield 2.8% *w*/*w*). *Cocos nucifera L* was cut open at the 'eye' of the coconut to extract the water, which was stored at 4°C until the experiment commenced.

Experimental procedure

A total of forty-eight (48) healthy albino rats of average weight 154 g were distributed indiscriminately into eight sets of 6 rats respectively as follows:

Group A – received 2 mL of 4 g/kg of ($Pb(NO_3)_2$)
Group B – received 2 mL of 4 g/kg of ($Pb(NO_3)_2$) + 2 mL of 15 mg/kg of *Cocos nucifera* water (CN) alone
Group C – received 2 mL of 4 g/kg of ($Pb(NO_3)_2$) + 2 mL of 15 mg/kg of *Pisum sativum* extract (PS)
Group D – received 2 mL of 4 g/kg of ($Pb(NO_3)_2$) + 2 mL of 15 mg/kg of *Cocos nucifera* water (CN) + 2 mL of 15 mg/kg of *Pisum sativum* extract (PS)
Group E – 2 mL of 15 mg/kg of *Cocos nucifera* water (CN)
Group F – 2 mL of 15 mg/kg of *Pisum sativum* extract (PS)
Group G – 2 mL of 15 mg/kg of *Cocos nucifera* water (CN) + 2 mL of 15 mg/kg of *Pisum sativum* extract (PS)
Group H – received 2 mL distilled water (DW)

Each group was administered orally as a medium to the rat twice weekly using a gavage needle, which lasted for 18 weeks. At the end of each 3 weeks, one rat from each group was sacrificed using chloroform as anaesthetic through inhalation.

Collection of Samples for lipid profile parameters

Feeding was stopped a night before the collection of blood samples. Blood was collected from the heart of dissected rats using 5 mL disposable sterile syringes and transferred into plain tubes for lipid profile analysis. Samples were allowed to clot for 15-30 minutes after collection by leaving them undisturbed at room temperature. Serum was separated by centrifuging blood samples for 5 min at 2000 rpm. Estimations of the biochemical parameters like TC, TG, HDL, LDL, were done using serums by means of commercially available autoanalyzer kits in an auto analyzer (Erba Mannheim, EM- 200) at the chemical pathology laboratory unit of the University of Benin Teaching Hospital. The LDL ratio to HDL-ch and the coronary risk index were calculated as TC/HDL-ch.

Statistical analysis

Data were analyzed using general descriptive statistics one-way analysis of variance (ANOVA) at a 95% probability level of significance. The experiment was done in triplicates (n = 3). If significant differences were found, Duncan's multiple range tests was employed to relate the various experimental categories.

Results and Discussion

Body weight

The lowest mean body weight value of 154.33 ± 9.05 g was measured in the category of rats administered with lead salt while the highest mean value of 198.33 ± 12.7 g was recorded in the group of rats administered with both *Pisum sativum* extracts and *Cocos nucifera L* water only. The mean body weight values for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* extracts and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* extracts were 166.83 ± 8.18 g, 173.17 ± 8.19 g and 179.50 ± 10.00 g respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* extracts and the control group were 187.33 ± 9.44 g, 196.50 ± 14.96 g and 213.33 ± 17.59 g respectively (Figure 1).

Total cholesterol

The lowest mean total cholesterol value of 73.87 ± 5.27 mg/dL was measured in the category of rats administered with both *Pisum sativum* extracts and *Cocos nucifera L* water only while the highest mean value of 98.67 ± 3.74 mg/dL was recorded in the group of rats administered with lead salt. The mean total cholesterol value for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* were 88.90 ± 4.01 mg/dL, 91.28± 4.41 mg/dL and 85.60 ± 6.38 mg/dL respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* and the control group were 77.23 ± 3.43 mg/dL, 77.28 ± 5.19 mg/dL and 82.27 ± 5.26 mg/dL respectively (Figure 2).

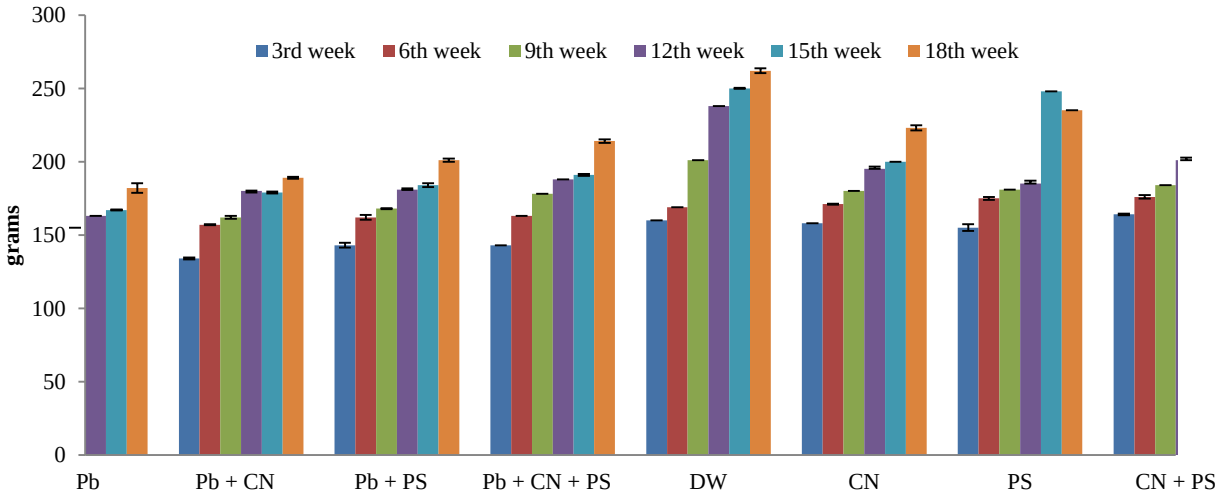

Figure 1: Mean body weight (g) of albino rats exposed to lead metal and the dietary protocol. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

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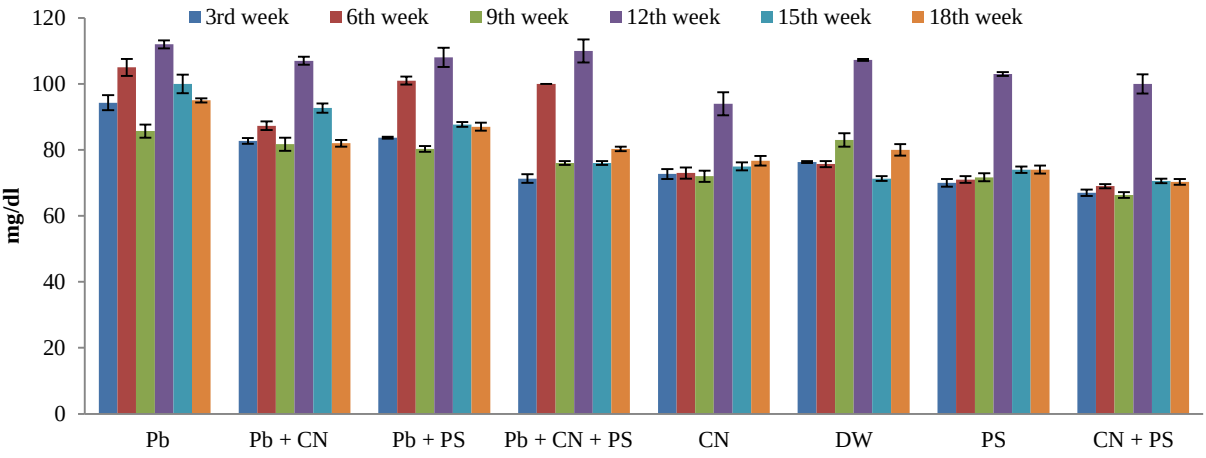

Figure 2: Mean values of total cholesterol of albino rats exposed to lead and the dietary protocols. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

Triglyceride

The lowest mean triglyceride value of 78.28 ± 8.55 mg/dL was recorded in the group of rats administered with both *Pisum sativum* extracts and *Cocos nucifera L* water only while the highest mean value of 140.43 ±10.60 mg/dL was recorded in the group of rats administered with lead salt. The mean triglyceride value for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* extracts and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* extracts were 119.23 ± 10.97 mg/dL, 124.05 ± 9.43 mg/dL and 99.67± 10.36 mg/dL respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* extracts and the control group were 88.78 ± 12.92 mg/dL, 84.83 ± 8.62 mg/dL and 106.28 ± 12.76 mg/dL respectively (Figure 3).

High density lipoprotein-cholesterol

The lowest mean high-density lipoprotein-cholesterol value of 29.22 ± 3.49 mg/dL was measured in the category of rats administered with lead metal while the highest mean value of 48.00 ± 1.20 mg/dL was measured in the category of rats administered with both *Pisum sativum* extracts and *Cocos nucifera L* water only. The mean high density lipoprotein-cholesterol value for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* extracts and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* extracts were 35.12 ± 1.95 mg/dL, 37.65± 3.03 mg/dL and 44.67± 2.51 mg/dL respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* extracts and the control group were 40.23 ± 1.73 mg/dL, 46.88± 1.95 mg/dL and 47.22± 2.86 mg/dL respectively (Figure 4).

Low Density lipoprotein-cholesterol (LDL-ch)

The lowest mean low-density lipoprotein-cholesterol value of 10.01 ± 3.76 mg/dL was measured in the category of rats administered with both *Pisum sativum* and *Cocos nucifera L* (coconut) water only while the highest mean value of 37.03 ± 5.15 mg/dL was measured in the category of rats administered with lead metal. The mean High Density lipoprotein-cholesterol value for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* were 29.25 ± 4.15mg/dL, 27.58± 3.69mg/dL and 19.50± 4.65 mg/dL respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* and the control group were 18.48 ± 3.99mg/dL, 13.53± 4.24 mg/dL and 13.40± 5.99 mg/dL respectively (Figure 5).

Coronary risk index

The lowest mean Coronary risk index value of 1.53 ± 0.10 was measured in the category of rats administered with both *Pisum sativum* extracts and *Cocos nucifera L* water only while the highest mean value of 3.30 ± 0.54 was measured in the category of rats administered with lead metal. The mean Coronary risk index value for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* extracts and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* extracts were 2.4 ± 0.26, 2.33± 0.22 and 1.97± 0.16 respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* extracts and the group that took only feed were 1.95 ± 0.18, 1.63± 0.11 and 1.82 ± 0.28 respectively (Figure 6).

High LDL/HDL-ch

The lowest mean LDL/HDL-ch value of 0.13 ± 0.03 was measured in the category of rats administered with both *Pisum sativum* extracts and *Cocos nucifera L* water only while the highest mean value of 1.43 ± 0.41 was measured in the category of rats administered with lead metal. The mean LDL/HDL-ch value for the group treated with both lead salt and *Cocos nucifera L* water, the group treated with both lead salt and *Pisum sativum* extracts and the group treated with lead salt, *Cocos nucifera L* water and *Pisum sativum* extracts were 0.63 ± 0.11, 0.72± 0.18 and 0.37± 0.12 respectively. The mean values of the groups administered only *Cocos nucifera L* water, *Pisum sativum* extracts and the feed group were 0.42 ± 0.08, 0.18± 0.07 and 0.20± 0.04 respectively (Figure 7).

From the results obtained, the mean body weightof the rats in Group A (154.33 ± 9.05 g) meaningfully ($P < 0.05$) reduced when related to the weight of rats in every other group, which ranged from 166.83 ± 8.18 g - 213.33± 17.59 g (Figure 1). The rats' weights increased as the weeks progressed in category administered with only lead nitrate (Group A). Studies done by Sun et al.[23]showed a relationship of lead exposure with body weight, resulting in dose-specific weight gain in adult Wistar rats. The findings in this study corresponded to Amjad et al.[24] research, who also stated an overall reduction in the body weight, when albino rats were exposed to 8 mg/kg lead acetate when compared with the control group. Possible explanations for the loss of body weight may be the decreased muscle mass and cachexia due to the oxidative stress induced by lead.[24]

Further study also revealed that the loss of body weight was due to loss of appetite and gastrointestinal disturbance.[25]The mean weight in the group of rats administered with lead nitrate combined with *Cocos nucifera* water and *Pisum sativum* extracts had an increase in weight compared with the group of rats exposed to lead nitrate only. This could be because of the amount of vitamin C present in *Cocos nucifera* water and *Pisum sativum* extracts.

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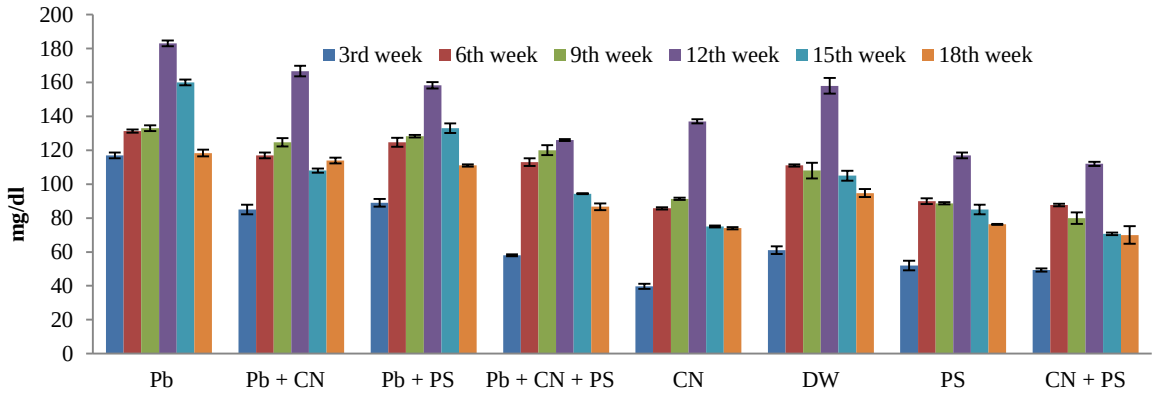

Figure 3: Mean values of triglyceride of albino rats exposed to lead and the dietary protocols. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

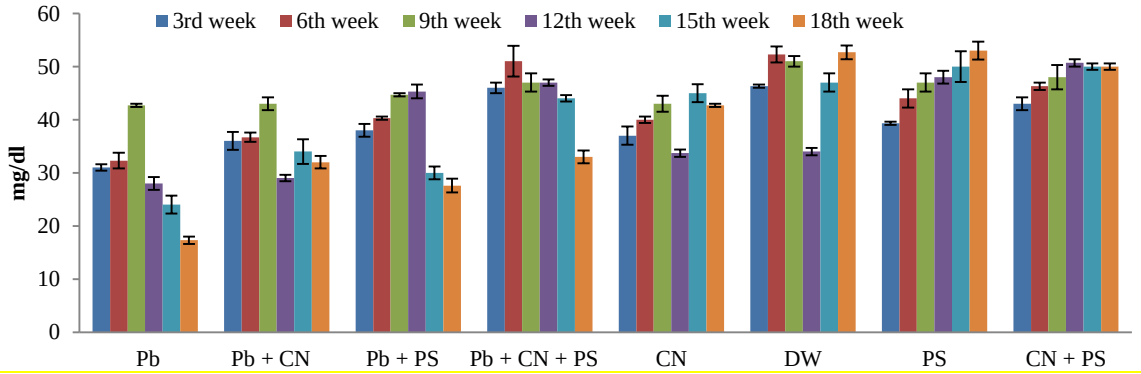

Figure 4: Mean values of high density lipoprotein-cholesterol of albino rats exposed to Lead and the dietary protocols. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

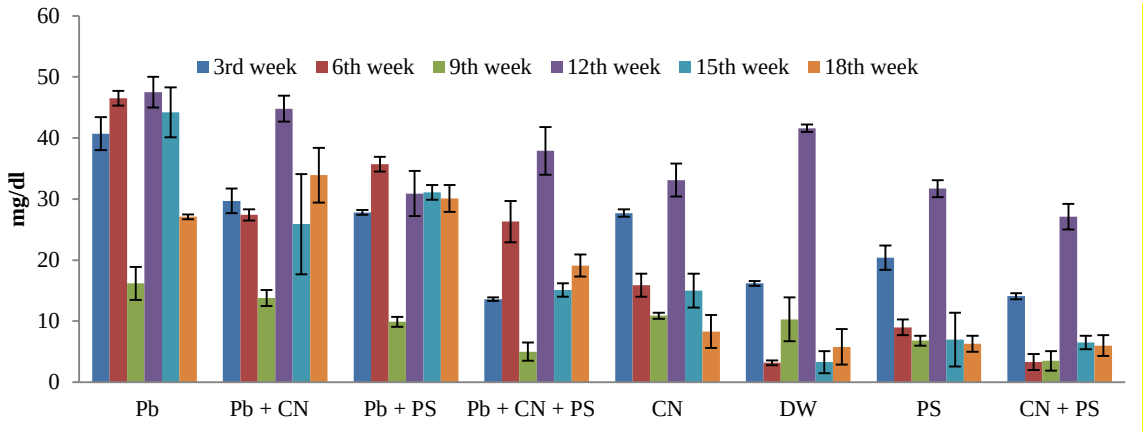

Figure 5: Mean values of low density lipoprotein-cholesterol of albino rats exposed to lead and the dietary protocols. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

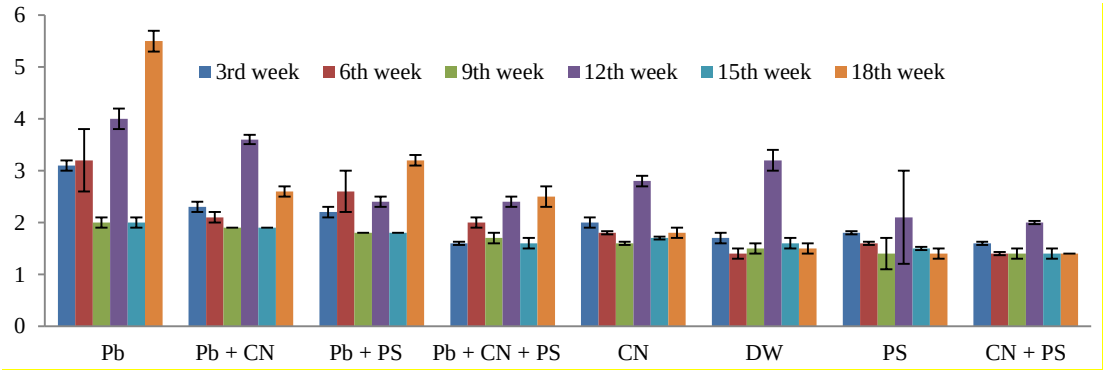

Figure 6: Mean values of coronary risk index of albino rats exposed to lead and the dietary protocols. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

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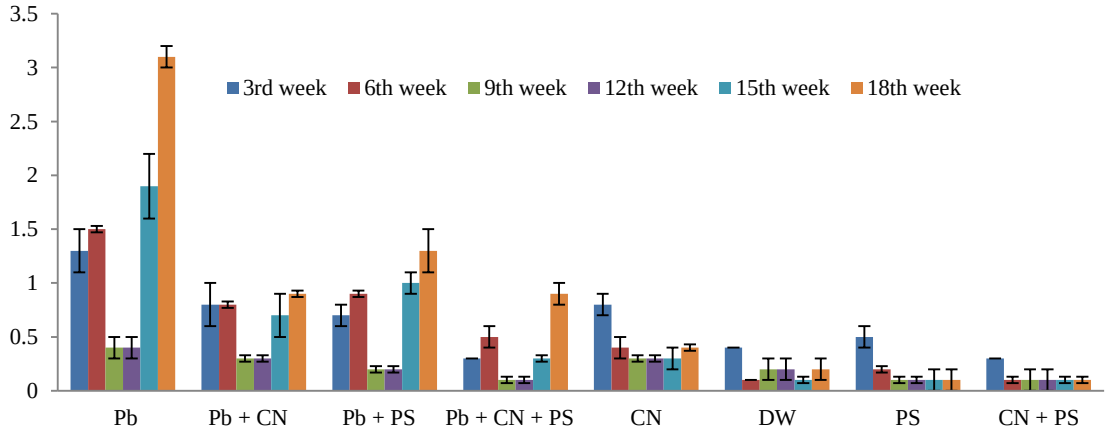

Figure 7: Mean values of low density lipoprotein-cholesterol/high density lipoprotein-cholesterol of albino rats exposed to Lead and the dietary protocols. Pb = lead, CN = *Cocos nucifera* water, PS = *Pisum sativum* extract, DW = distilled water

As it has been reported, vitamin C can protect body weight against lead metal.[25]The defensive mechanism of vitamin C counter to lead acetate can be credited to the antioxidant activities of vitamin C.[26]

In this investigation, administration of lead nitrate led to an increase in the mean values of total cholesterol (98.67 ± 3.74 mg/dL), triglyceride (140.43 ±10.60 mg/dL), low density lipoprotein-cholesterol (37.03 ± 5.15 mg/dL), LDL/HDL-ch (1.43 ± 0.41) and coronary risk index (3.30 ± 0.54). It also led to a reduction in the mean value of high -density lipoprotein-cholesterol (29.22 ± 3.49 mg/dL) of the rat model compared with the average values of the category of rats administered with only distilled water. The mean values of the group of rats administered with only distilled water : total cholesterol (82.27± 5.26 mg/dL), triglyceride (106.28± 12.76 mg/dL), low density lipoprotein-cholesterol (13.40± 5.99 mg/dL), LDL/HDL-ch (0.20± 0.04), coronary risk index (1.82± 0.28) and high density lipoprotein-cholesterol (47.22± 2.86 mg/dL) of the rat model as in displayed in figures 2-7. A similar result, which entails an increase in the TC, TG, LDL-ch, and a sharp drop in serum HDL-ch level, was also seen in rat blood after injection of Pb^{2+} as reported by Abdel-Moneim et al.[27] The relationship between lead exposure and high serum lipids might be the decreased removal of lipoproteins due to the alteration of their cell surface receptors and the inhibition of hepatic lipoprotein lipase activity.[28]Alvares, Kapelner[29]reported that lead could depress the activity of cytochrome P450, as a result restraining the biosynthesis of the bile acids, which is the major way for removal of cholesterol from the body. Reduction in the values of coronary risk index can be a strong pointer for forecasting the risk of coronary heart disease and atherosclerosis, and it became evident in the low values of the TC, LDL-ch or TG in the serum. Co-treatment with *Pisum sativum* extract and *Cocos nucifera* water, especially in the combined form, showed an ameliorating effect against lead-induced disorders in the lipid profile of the rats. When *Pisum sativum* extract and *Cocos nucifera* water were administered alongside with lead nitrate, there were significant reduction ($P < 0.05$) in the mean values of total cholesterol (from 98.67 ±3.74 mg/dL to 85.60± 6.38 mg/dL), triglyceride (from 140.43 ± 10.60 mg/dL to 99.67± 10.36 mg/dL), low density lipoprotein-cholesterol (from 37.03 ± 5.15 mg/dL to 19.50± 4.65 mg/dL), LDL/HDL-ch (from 1.43 ± 0.41 to 0.37± 0.12) and coronary risk index (from 3.30 ± 0.54 to 1.97± 0.16) when compared with the group of rats administered with only lead nitrate (Group A). Furthermore, there was an increase in the mean values of high -density lipoprotein-cholesterol (from 29.22 ± 3.49 mg/dL to 44.67± 2.51 mg/dL) when compared with the group of rats administered with only lead nitrate (Group A) as displayed in Figure 2-7. Oxidative stress can be prevented through dietary intake of antioxidants which can prevent cellular oxidation evolution. *Cocos nucifera* water and its active components shikimic acid has been reported by Manna, Khan[16]to reverse hydrogen peroxide-induced oxidative damage in hepatocytes, maybe via the obstruction of nuclear factor-κB(NF-κB), with the stimulation of the phosphorylated-phosphatidylinositol 3-kinase-Akt-glycogen synthase kinase 3 beta (p-PI3K/Akt/GSK3β) pathways and reduction of apoptosis by interfering the SAPK/JNK/Bax pathway. *Pisum sativum* protein, when broken-down, may produce peptides with bioactivities, plus angiotensin 1 -converting enzyme inhibitor activity and antioxidant activity and their phytochemicals (polyphenolics and saponins), mineral and vitamin contents contribute significantly in the mitigation of deficiency-connected diseases[30] and may inhibit hypercholesterolaemic or hypocholesterolaemic and anticarcinogenic activity.[31,32]

Conclusion

Pisum sativum extract and *Cocos nucifera* water, especially in the combined form, showed an ameliorating effect against lead-induced disorders in lipid profile and coronary risk index of the rat model used in this study. Therefore, it shows their potentials as a beneficial attenuating factor against lead toxicity due to the features of the antioxidant activities. However, further research is needed for more insight into the ameliorative potentials of *Pisum sativum* extract and *Cocos nucifera* water.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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