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Haematological Parameters of Albino Rats Exposed to Lead Metal: Alleviating Effect of *Cocos nucifera* l. Water and *Pisum sativum* Extract

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Abstract
Lead is among the most persistent environmental contaminants that adversely affect health of organisms. Measuring lead level in blood is a commonly accepted and verifiable biomarker for lead exposure. Hence, this study evaluates the effect of lead poisoning on haematological parameters in forty-eight wistar albino rats and the alleviating potency of Cocos nucifera water and Pisum sativum extract. The results showed a significant reduction in the mean values of white blood cells, red blood cells, haemoglobin content, hematocrit percent, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocytes, red blood cell distribution width except in blood platelets count in the group of rats exposed to lead nitrate when compare to the control group. However these values increased in the group of rat administered with both lead nitrate and Cocos nucifera water or both lead nitrate and Pisum sativum extract only. Much improvement was seen in the mean values of WBC, RBC, haemoglobin content, HCt%, MCV, MCH, and MCHC in the group of rat administered with lead nitrate, Cocos nucifera water and Pisum sativum extract. These findings suggest that, Cocus nucifera water and Pisum sativum extract could alleviate the effect of lead toxicity in the haematological parameters of albino rats.
Keywords: Toxin abatement Coconut water, Green pea, Lead, *Rattus norvegicus*

Introduction
Lead is amid the most prevalent environmental contaminants that badly affects human and animal healt [1]. Oxidative stress which results from the accumulation of free radicals and reactive oxygen species beyond the normal capacity of the body's antioxidant system to neutralize it, is the common molecular mechanism implicated in lead poisoning [2, 3]. To prevent these oxidative challenges, the body must be supplemented with exogenous antioxidants and regular exercise [4]. Measuring blood lead is the most commonly accepted and verifiable biomarker for lead exposure [5]. Lead interferes with heme biosynthesis by altering the activity of three enzymes (δ-aminolevulinic acid synthetase (δ-ALAS), δ-aminolevulinic acid dehydratase (δ-ALAD) and ferrochelatase) [6]. The anaemia induced by lead is microcytic and hypochromic and results primarily from both inhibition of heme and globin synthesis and shortening of the erythrocyte lifespan [7, 8]. The work of Pagrut *et al*. [1] on the haematological changes in lead intoxication in mice revealed that, there were a significant decline in the total erythrocyte count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration of the mice following exposure of lead acetate. Researchers have called for natural products to get rid of lead from tissues as some chemical substances reported to expel lead from tissues, could cause nephrotoxicity, hepatotoxicity and neurotoxicity in experimental animal studies [9]. Vegetables, fruits and other edible plants are important dietary sources of vitamins and essential metals which can decrease the risks of Pb toxicity [9]. *Cocos nucifera* (L.) (Arecaceae) is commonly called the "coconut tree" and is the most naturally widespread fruit plant on Earth [10] Scientific evidence that support the position of *Cocos nucifera* water and *Pisum sativum* in health and medicinal application has been on increase in recent times [11, 12]. They are reported to contain large amount of vitamins and minerals [13] which can play important roles in protecting, alleviation or prevention of heavy metal toxicity because of their antioxidant properties [11, 13]. Therefore, this present study aims at examining the alleviating potency of *Cocos nucifera* water and *Pisum sativum* on haematological parameters changes of wistar albino rats induced by lead nitrate.

Materials and Methods
Materials
Experimental animals
Albino rat (*Rattus norvegicus*) of both sexes of approximately body weight of 154 ± 5 g were purchased from the breeding unit of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University

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of Benin, Benin City. 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. Animal care was performed according to the guidelines of the National Research Council and the American Association of Accreditation for Lab Animal Care [14]. The choice of using albino rats for this study is because albino rats are considered to have some degree of similarity in organic constitution with human and they serve as model for understanding the entry, distribution, transportation, biotransformation, toxicology, abatement and elimination of heavy metals in man.

Procurement of lead nitrate

Lead nitrate ($Pb(NO_3)_2$) with a laboratory reagent grade was procured from Paten Chemicals in Benin, Nigeria. Lead nitrate is selected for this work because it is one of the few common soluble lead compounds that readily dissolve in water to give a clear, colourless solution. This can make the rats drink lead solution unreservedly [15].

Procurement of dietary protocol

Cocos nucifera water and *Pisum sativum* extract are the selected dietary protocol for this work and they are obtained from traditional non-industrialized area in Evbuotubu village, and vegetable market respectively in Benin City. *Cocos nucifera* water and *Pisum sativum* have numerous medicinal properties laden with antioxidative properties, hence chosen for this work [11, 13].

Methods

Experimental procedure

A total of forty-eight (48) healthy albino rats (*Rattus norvegicus*) of average weight of approximately 154 g (The rats were weighed alive using a ohaus scale crop weighing balance with a sensitivity of 0.1 g) were distributed randomly into 8 groups with 6 rats each.

Group A- Received 2 ml of 4 g/kg bwt of lead nitrate

Group B - Received 2 ml of 4 mg/kg bwt of lead salt + 2 ml of 15 mg/kg bwt of *Cocos nucifera* water alone

Group C- Received 2 ml of 4 g/kg bwt of lead nitrate + 2 ml of 15 mg/kg bwt of *Pisum sativum* extract

Group D- Receives 2 ml of 4 mg/kg bwt of lead salt + 2 ml of 15 mg/kg bwt of *Cocos nucifera* water + 2 ml of 15 mg/kg bwt of *Pisum sativum* extract

Group E- 2 ml of 15 mg/kg bwt of *Cocos nucifera* water

Group F- 2 ml of 15 mg/kg bwt of *Pisum sativum* extract

Group G- 2 ml of 15 mg/kg bwt of *Cocos nucifera* water + 2 ml of 15 mg/kg bwt of *Pisum sativum* extract

Group H- Receives distilled water

Each group was administered twice weekly using gavage needle and this lasted for a period of 18 weeks. At the end of each 3 weeks period, one (1) rat from each group was sacrificed using chloroform as anaesthetics through inhalation for the needed blood and this were subsequently analyzed for their respective haematological parameters changes.

Collection of samples

Blood samples were collected from the heart of dissected albino rats using 5ml disposable sterile syringes and were transferred into tubes containing potassium EDTA anticoagulant for complete blood count (CBC) analysis. The sample bottles were then kept in ice briefly and were transferred to the haematological laboratory unit of the University of Benin Teaching Hospital (UBTH), where they were analyzed.

Haematological parameters

The following blood parameters were analyzed: White blood cells (WBC), Red Blood Cells (RBC), blood platelets count, haemoglobin content, hematocrit percent (HCt%), Mean corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Lymphocytes, Red Blood Cell distribution Width (RDW), were obtained from Complete Blood Count analysis by Mythic Haematological Five Park Automated Analyzer (2014 Model made by Sysmex) Machine.

Statistical analysis

Data collected from the study were analyzed using general descriptive statistics, one way analysis of variance (ANOVA) at 95% probability level of significant. If significant differences were found, Duncan's multiple range tests was used to compare the different experimental groups. Computer software Statistical Package for Social Scientists (SPSS) and Microsoft Excel were used for the statistical analyses.

Results

Red blood cell count

The lowest mean value recorded for RBC was 5.48 ± 0.55 ($\times 10^6$µl). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 9.93 ± 0.07 ($\times 10^6$µl) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 1).

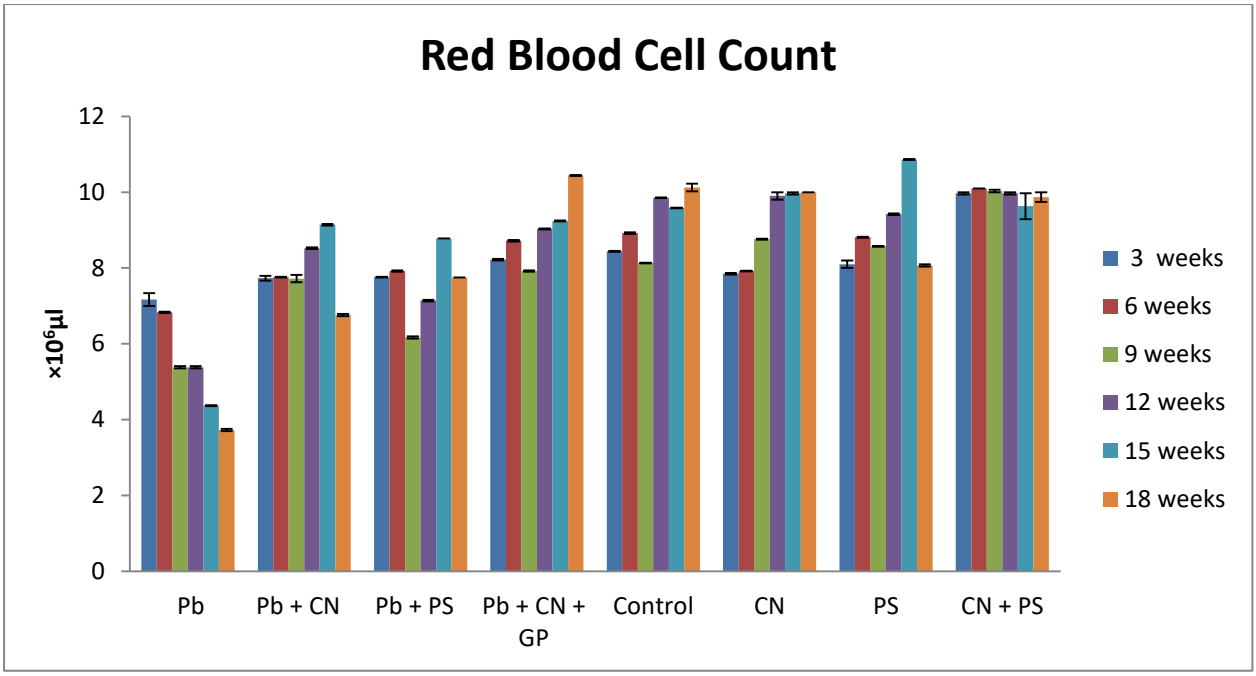

Figure 1: red blood cell count of albino rats exposed to lead metal and dietary protocols

Key: Pb = lead only, Pb + CN = lead salt and *Cocos nucifera* water, Pb + PS = lead salt and *Pisum sativum* extract, Pb + CN + PS = lead salt, *Cocos nucifera* water and *Pisum sativum* extract, CN = *Cocos nucifera* water only, PS = *Pisum sativum* extract only, CN + PS = *Cocos nucifera* water and *Pisum sativum* extract

Haemoglobin

The lowest mean value recorded for HGB was 10.33 ± 0.92 (g/dl). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 16.57 ± 0.08 (g/dl) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 2).

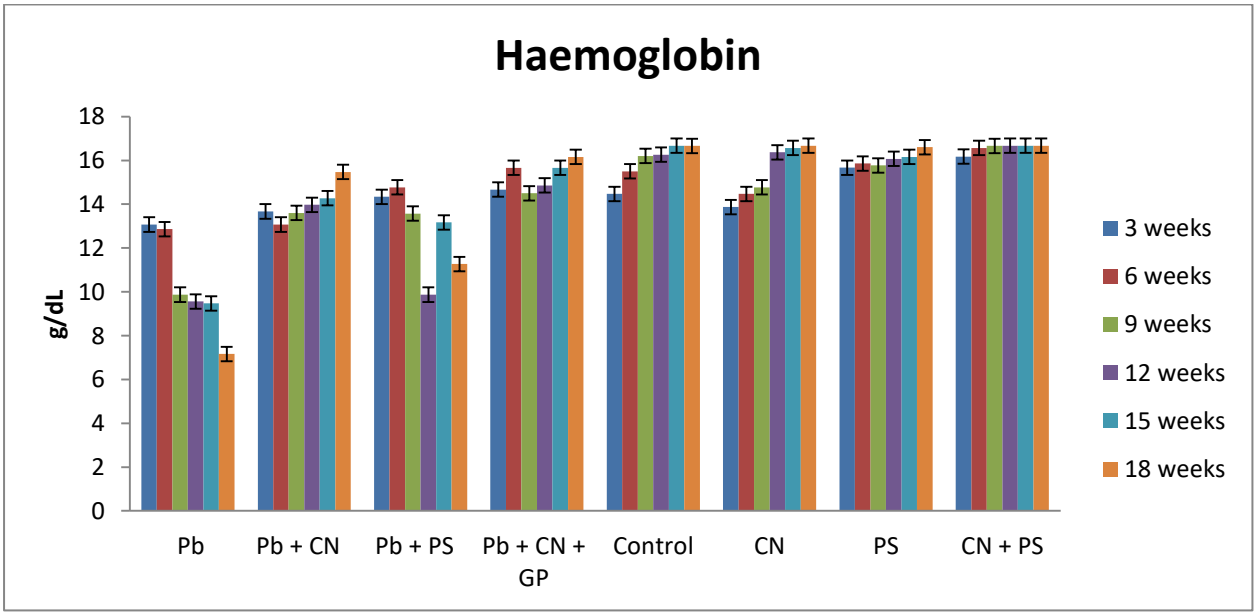

Figure 2: haemoglobin of albino rats exposed to lead metal and dietary protocols

Mean corpuscular volume

The lowest mean value recorded for MCV was 48.14 ± 4.32 (μm^3). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 68.77 ± 4.52 (μm^3) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 3).

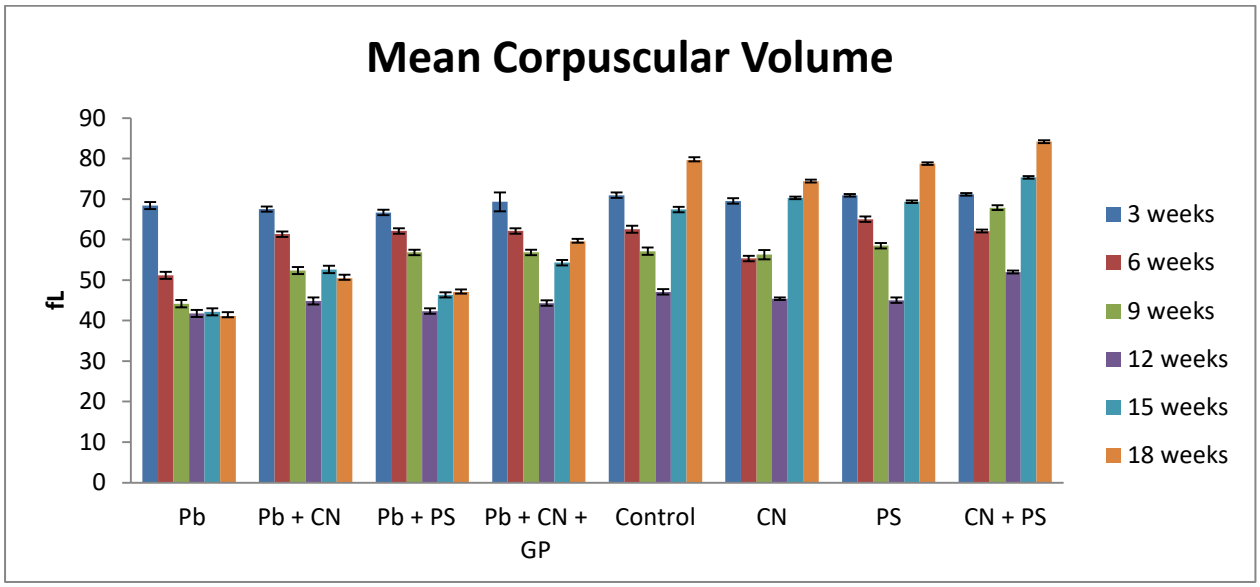

Figure 3: mean corpuscular volume of albino rats exposed to lead metal and dietary protocols

Mean corpuscular haemoglobin

The lowest mean value recorded for MCH was 13.34 ± 1.16 (pg). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 26.62 ± 0.83 (pg) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 4).

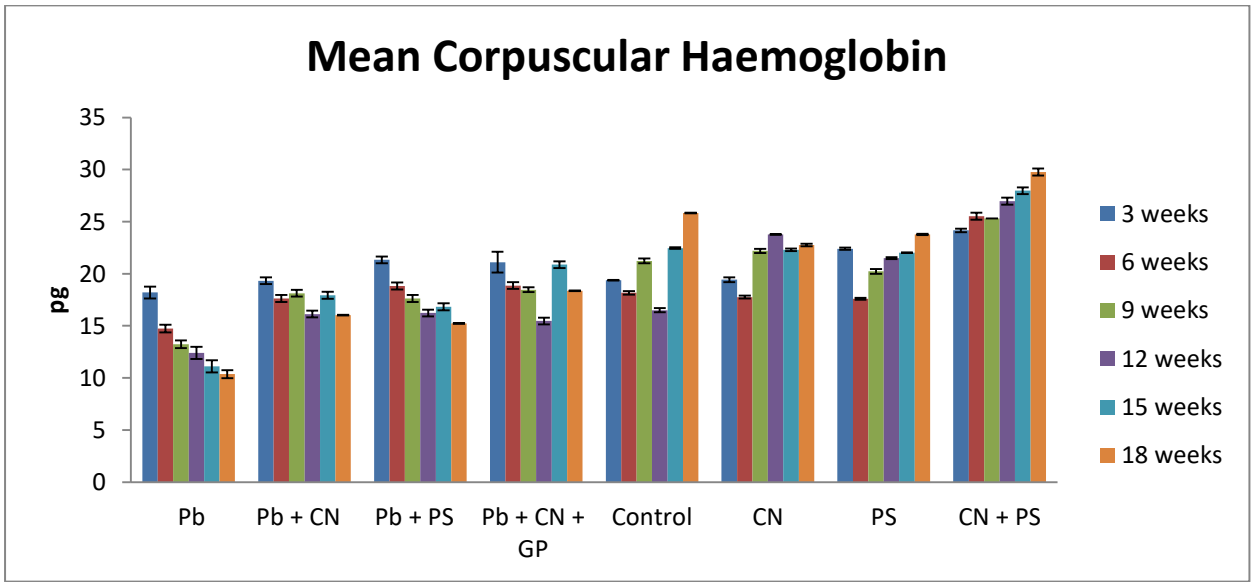

Figure 4: mean corpuscular haemoglobin of albino rats exposed to lead metal and dietary protocols

Mean corpuscular haemoglobin concentration

The lowest mean value recorded for MCHC was 24.76 ± 1.33 (g/dl). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 35.08 ± 0.6 (g/dl) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 5).

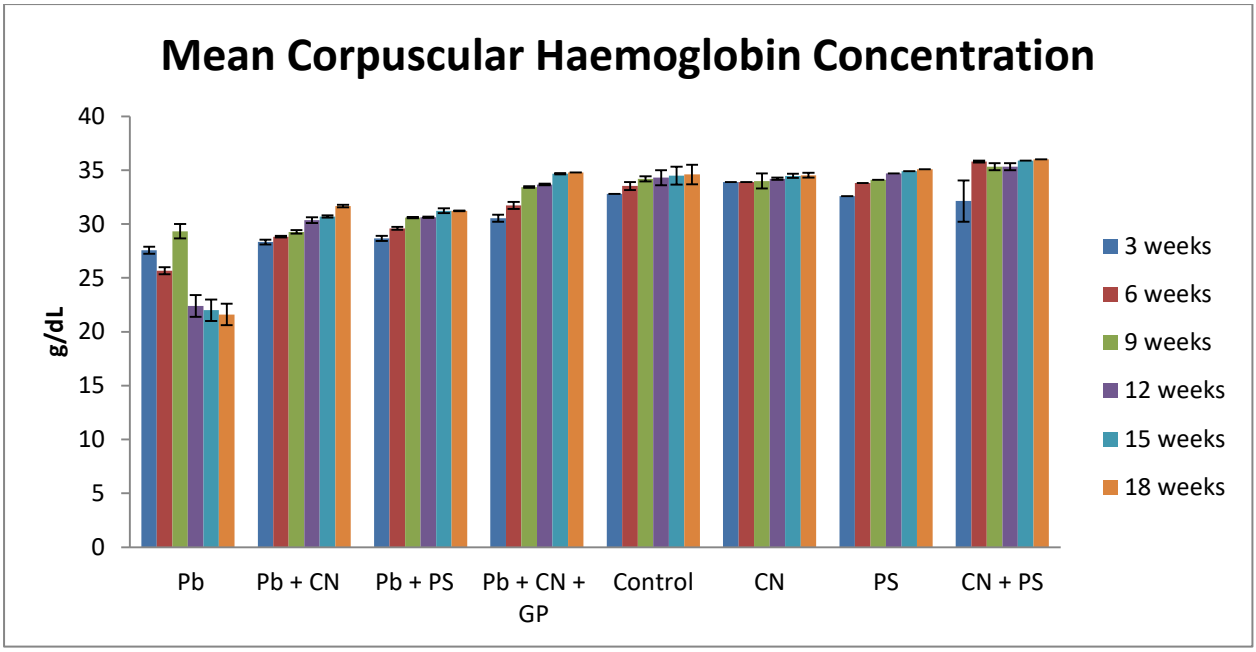

Figure 5: mean corpuscular haemoglobin concentration of albino rats exposed to lead metal and dietary protocols

Red blood distribution width

The lowest mean value recorded for RDW was 9.4 ± 0.13 (%). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 16.27 ± 0.27 (%) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 6).

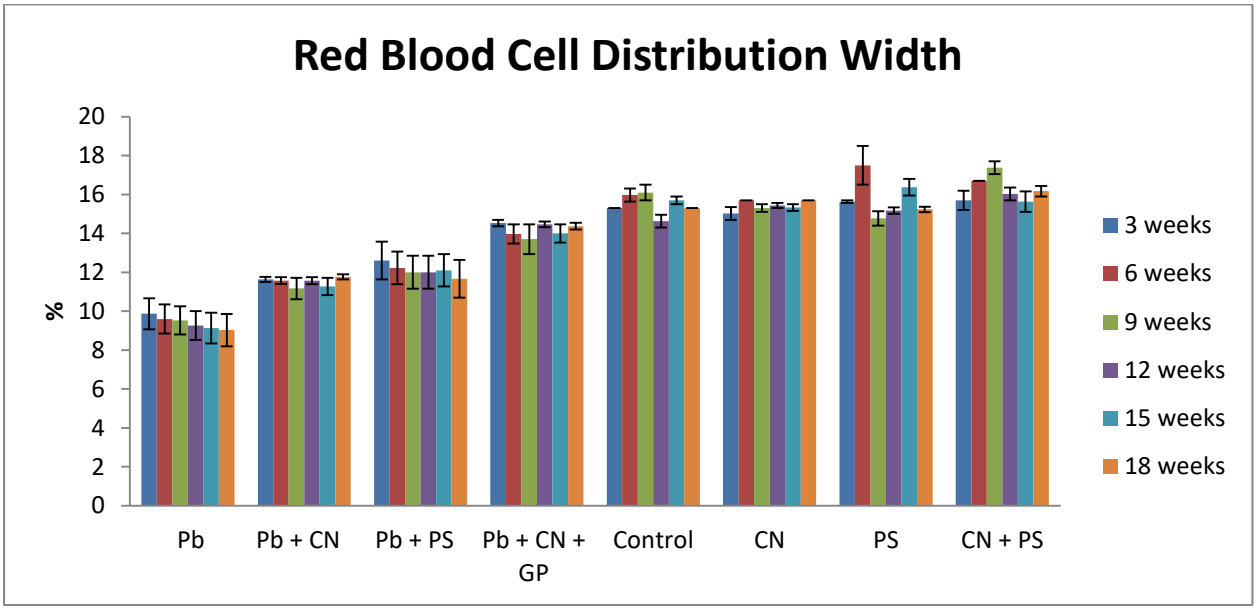

Figure 6: red blood distribution width of albino rats exposed to lead metal and dietary protocols

Haematocrit

The lowest mean value recorded for HCT was 29.22 ± 0.93 (%). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 55.74 ± 2.25 (%) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 7).

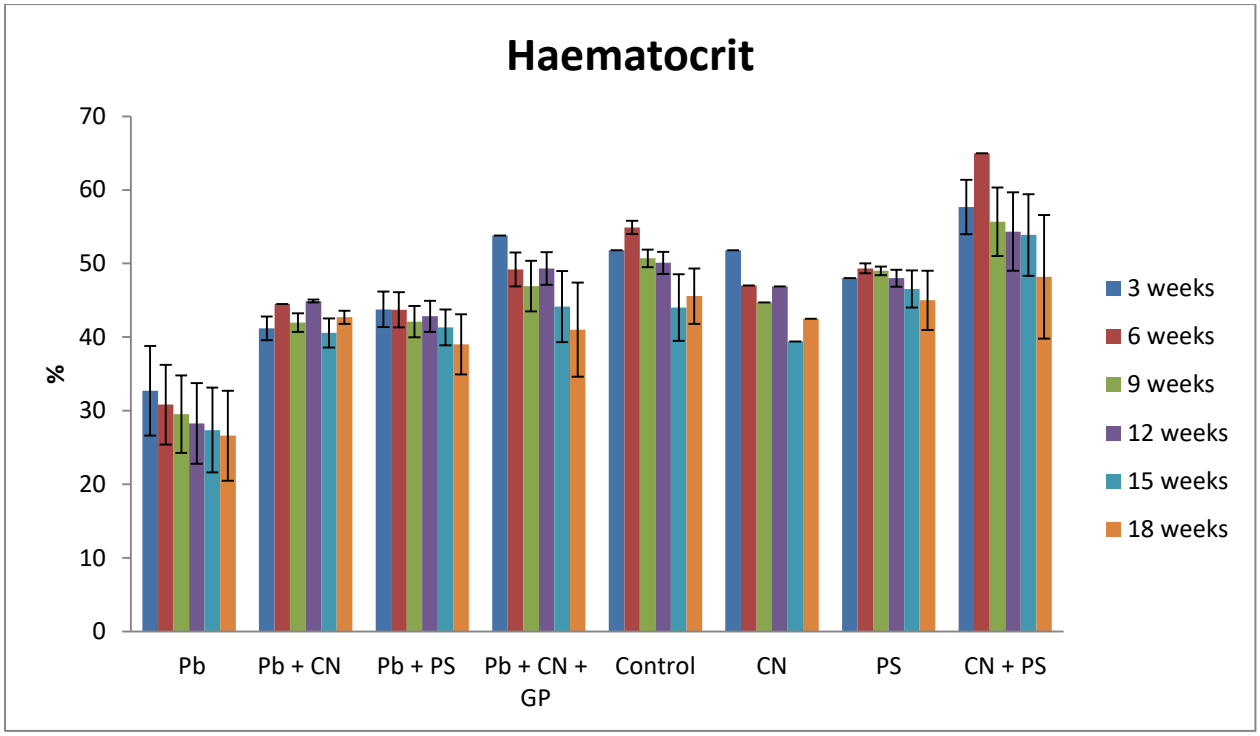

Figure 7: haematocrit of albino rats exposed to lead metal and dietary protocols

White blood cell count

The lowest mean value recorded for WBC was 5.42±0.47 ($\times 10^3$µl). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 10.84 ± 0.35 ($\times 10^3$µl) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 8).

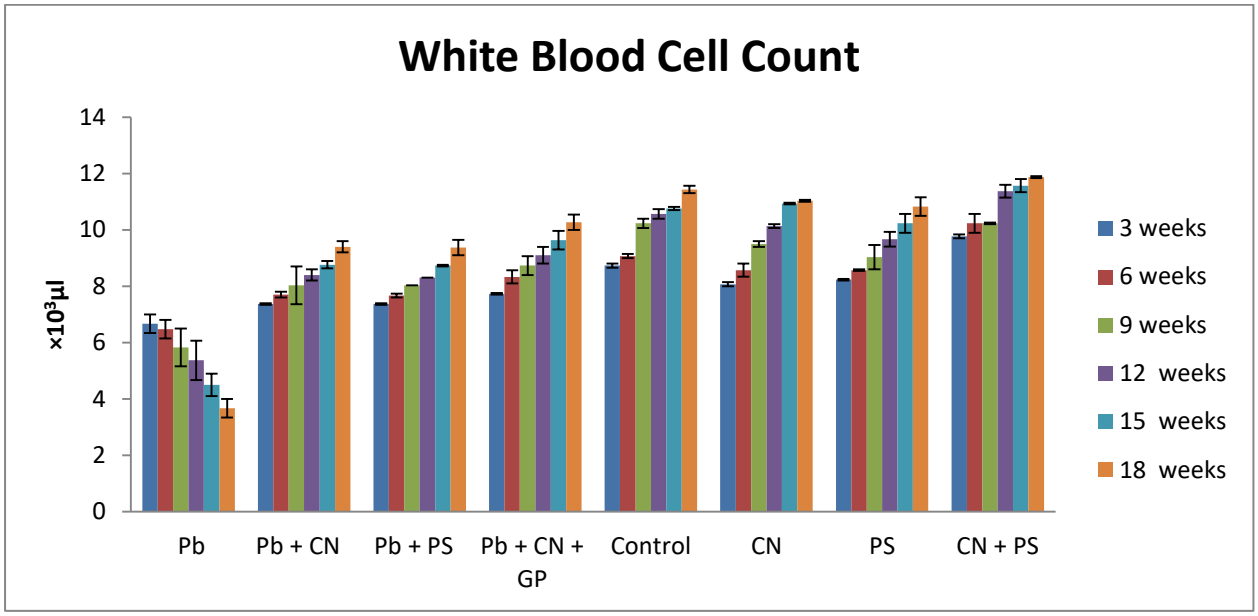

Figure 8: white blood cell count of albino rats exposed to lead metal and dietary protocols

Lymphocytes

The lowest mean value recorded for LYM was 20.95 ± 0.77 (%). This value was recorded in the group of rat exposed to lead metal while the highest mean value of 47.29 ± 0.66 (%) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were higher than the mean values in the group of rat exposed to lead metal (Figure 9).

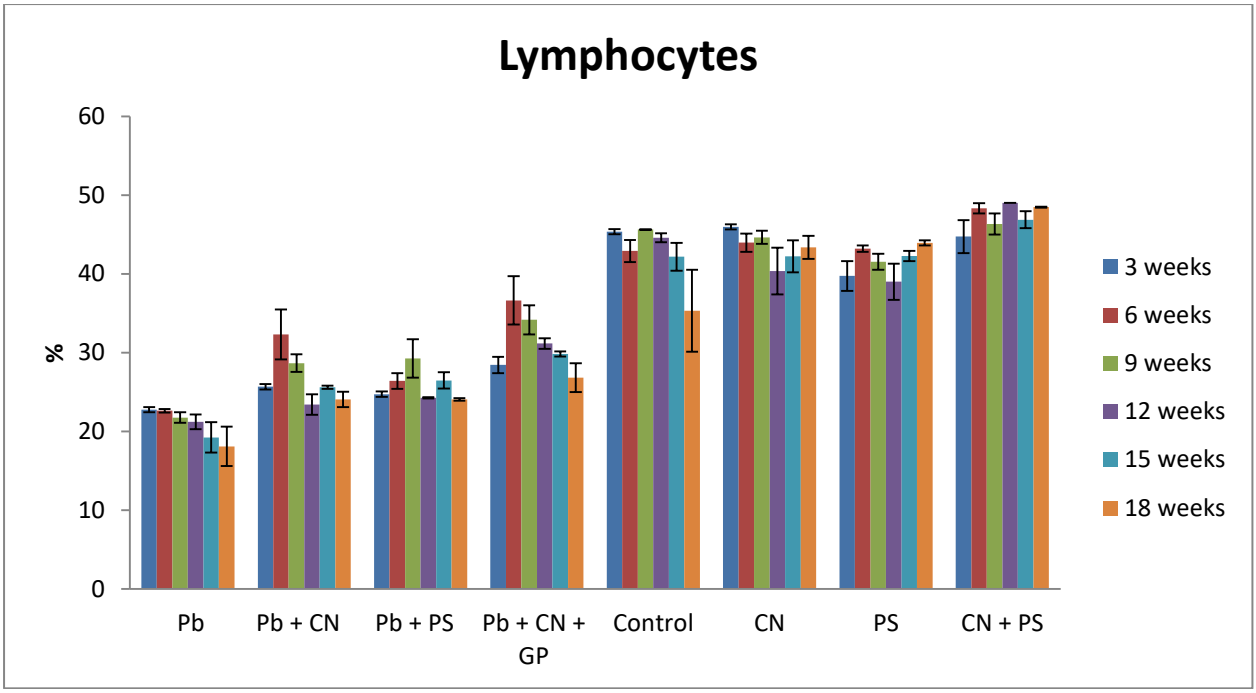

Figure 9: lymphocytes of albino rats exposed to lead metal and dietary protocols

Platelets

The highest mean value recorded for PLT was 578.62 ± 13.48 ($\times 10^3$µl). This value was recorded in the group of rat exposed to lead metal while the lowest mean value of 355.55 ± 8.75 ($\times 10^3$µl) was observed in the group of rats fed *Cocos nucifera* water and *Pisum sativum* extract. However, all other values were lower than the mean values in the group of rat exposed to lead metal (Figure 10).

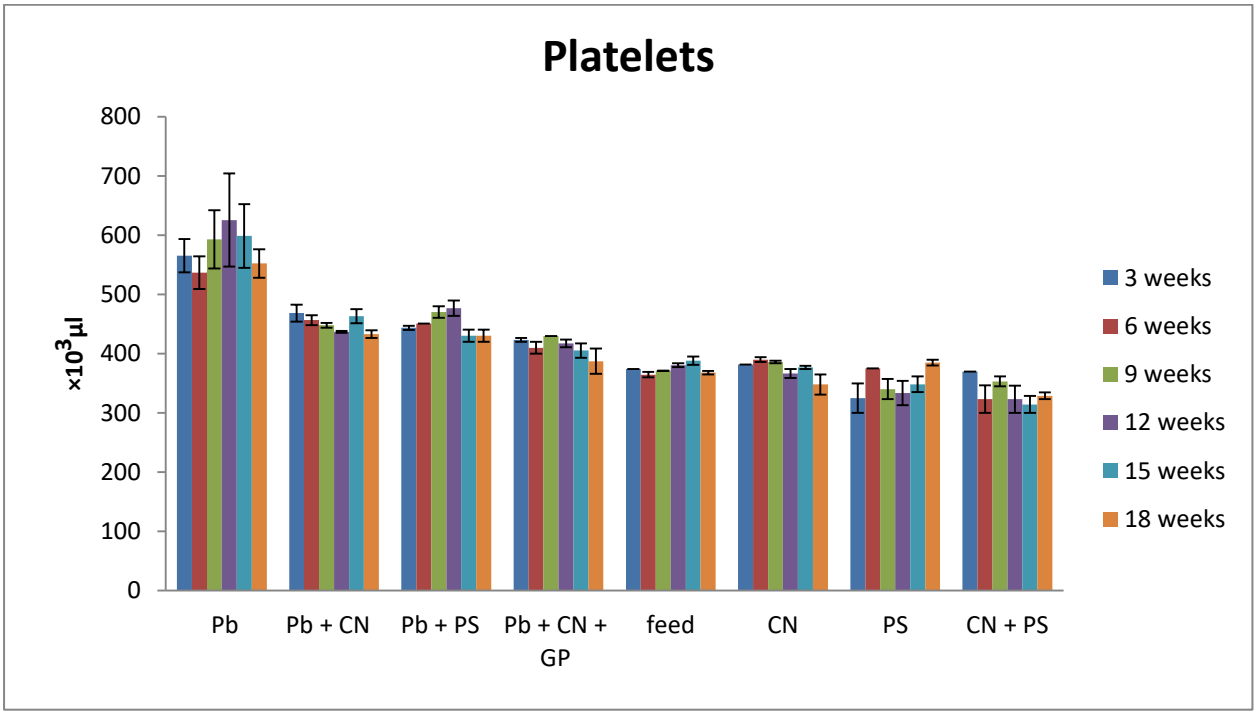

Figure 10: platelets of albino rats exposed to lead metal and dietary protocols

Discussion

The results of this study revealed that there were significant differences ($P<0.05$) between the mean values of the haematological parameters in albino rats exposed to lead metal compared to the mean values in the control group. There were reduction in the mean values of WBC, RBC, haemoglobin content, HCt%, MCV, MCH, MCHC, Lymphocytes and RDW (except in Platelets) in the group of rats exposed to lead only when compared

with the other groups. These reductions suggested that there is an etiology relationship between acute renal failure (ARF) and anemia through different suggested mechanisms such as bone marrow cell destruction and decrease or delay in mitosis [16]. The current data showed that hemoglobin content, MCV and hematocrit value were significantly decreased in parallel to the decrease in RBC count in experimental animals with lead nitrate exposure at doses of 2 ml of 4 g/kg bwt of albino rats. The decrease in RBC could be that lead could affect the rat erythrocyte membrane and decrease their mobility and also lead may induce oxidative stress in RBCs [1]. Similar decreases in hematological parameters in albino rats were reported [1, 17] in their work. This decrease in hematological parameters in albino rats may coincide with higher blood lead levels (BLLs) and the threshold of blood lead level is 50 µg/dl as reported by [9]. Lead may inhibit the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway [2]. This finding is in line with Hsieh *et al*. [18] which suggested that lead may induce anemia both by interfering with heme-biosynthesis and by diminishing RBC survival [19]. The reduction in WBC could reflect on the alteration in the immune function. Iliyasu *et al*. [20] reported from their studies, that there were also non-significant increase in white blood cell and lymphocytes count in rats exposed to lead acetate. The increase in platelet cells count in this study shows that lead influence on PLT parameters is exposure duration-dependent and that chronic lead exposure, unlike acute exposure, may be associated with increased megakaryocytopoiesis and PLT turnover [21].

Treatment of albino rats with *Cocos nucifera* water or *Pisum sativum* extract resulted in some improvement in the WBC, RBC, haemoglobin content, HCt%, MCV, MCH, MCHC, lymphocytes and RDW value especially in the combined form when compared to group of albino rats exposed to lead metal. These improvements are as a result of the fact that *Cocos nucifera* water and *Pisum sativum* extract are laden with some important antioxidants which can play important roles in protecting, alleviation or prevention of Pb toxicity [11, 13]. *Cocos nucifera* water and *Pisum sativum* extract are important dietary sources of vitamins and essential metals which at sufficient levels can promote the levels of the vitamins and essential metals in the human body, which in turn can decrease the risks of Pb toxicity [9]. Some of these components in *Cocos nucifera* water are cytokinins, folate also known vitamin B9, vitamin C, iron and other valuable antioxidants Enagbonma [22, 10] while *Pisum sativum* is loaded with vitamin A, B1, B6, C, K, Fe and Zn ([23]. It could be concluded form this study that, *Cocus nucifera* water and *Pisum sativum* extract can alleviate the effect of lead toxicity in the haematological parameters of albino rats therefore, this study support the role of *Cocus nucifera* water and *Pisum sativum* extract in medicinal application.

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