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**ORIGINAL ARTICLE**

# Effect of Folic Acid and Vitamin-C Administration on Paraoxonase and Arylesterase -1 Activities in Rats intoxicated with Lead

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**KEYWORDS**

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**ABSTRACT:** Paraoxonase and aryl-esterase-1 (PON-1) in serum of rats intoxicated with lead then administered Folic acid and Vitamin-C was investigated in forty male albino rats (160-190 g) randomly separated into 2 groups of 20 rats each namely: Lead acetate (60 mg kg $^{-1}$) and normal saline was given to each group for 6 weeks and there after administered Folic acid (500 µg kg $^{-1}$) and/or Vitamin-C (60 mg kg$^{-1}$) orally for 4 weeks. Blood sample was obtained at the end from each rat for biochemical assessment. Results obtained indicated significant ($p<0.05$) reduction in activities of arylesterase (60.51±8.52 U L$^{-1}$) and paraoxonase (74.95±3.63 U L$^{-1}$) of non-supplemented, rats intoxicated with lead compared to 121.51±7.34 and 98.19±2.25 U L$^{-1}$ respectively for the control groups. Similarly, the values of catalase (10.54±0.46 U mg$^{-1}$) and superoxide dismutase (4.93±0.66 U mg$^{-1}$), differ significantly ($p<0.05$) in the non-supplemented group intoxicated with lead as compared to 31.62±0.67 U mg$^{-1}$, and 28.46±1.54 U mg$^{-1}$ respectively for the control groups. Malonyldialdehyde level (0.58±0.29 nmol L$^{-1}$) decreased significantly ($p<0.05$) in the control group as compared to the 5.21±0.16 nmol L$^{-1}$ observed in the non-supplemented group intoxicated with lead. Significant ($p<0.05$) decrease was observed for high-density lipoprotein cholesterol level (41.08±0.48 mg L$^{-1}$) in the non-supplemented, rats intoxicated with lead as compared to the level (78.67±0.66 mg L$^{-1}$) noticed in the control group. Findings from this study deduced that sub-chronically lead intoxication may cause cardiovascular diseases as evident in the decreased activities of arylesterase and paraoxonase. However, administration of Folic acid and Vitamin-C to the rats intoxicated with lead improves the catalytic activities of PON-1, and this may mitigate lead-induced cardiovascular disease risk.

## INTRODUCTION

The existence and sustainability of life depends largely on sundry needs such as water, air and soil. These factors are indispensable integral components of the environment for survival of human life. However, the environment could be harmful to life by way of retarding human activities and posing health risk, if interrupted by man or a natural

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phenomenon. Consequently, the environment is often negatively affected with contaminants, and of which heavy metals including lead represent an important and most challenging contaminants to the environment [1].

Lead is a harmful heavy metal that posed public health threats to both plants and animals [2-5]. It has a long history of being mined for over 8000years and has been used for industrial applications by the Egyptians as early as 5000BC [6-7]. Anthropogenic activities such as mining is one peculiar way by which lead is spread and persist in our environment, particularly the case of lead poisoning in Zamfara and Niger State, Nigeria, in 2010 and 2015. Other ways include, industrial emission, car exhaust gases [8-10], milk and dairy products [1], raw material for food industries contaminated by lead from soils and water for irrigation is also a potential risk exposure [11,12]. Previously, it was reported that honey, a sweet and viscous food substance, collected from an industrial area had significant degree of lead among other heavy metals [13].

Children are the most vulnerable to environmental lead exposure and lead containing compounds [14-16], with the nervous system being the most susceptible, as it absorbs high fractions of lead compared to the nervous system of adults [17]. The consequence is decrease in learning ability, intelligence, permanent brain damage and even death among children [18], as well as cardiovascular diseases [15].

The history of PON-1 can be traced back to 1946, during which it was recognized as an enzyme capable of hydrolyzing organophosphates such as paraoxon (a metabolite of insecticide 'parathoin'), hence the name paraoxonase [19], and the active metabolites of some organophosphorus insecticides such as chlorpyrifosoxon, diazoxon and homocysteine thiolactone [20]. Lead has been shown to inhibit PON-1, a process that may render individuals more susceptible to atherosclerosis [21-22].

Lead toxicity is a highly explored and comprehensively published subject matter, and today, developing countries have the most serious lead pollution problems. For instance, is the case of lead poisoning in Zamfara and Niger States in Nigeria. Unfortunately, the use of Chelating agents like dimercaptosuccinic acid (DMSA), edetate calcium disodium ($Na_2CaEDTA$), and Penicillamine is very expensive, with severe adverse effects such as renal damage [23]. This calls for the need to offer cheaper and safer alternative chelating agents like vitamin C, which has been extensively studied with regard to lead intoxication, but most studies were tailored towards protective measure rather than post-treatment. This is because intoxication occurs before treatment and not otherwise.

## MATERIAL AND METHODS

### *Experimental animal*

In this study, 40 albino rats (male) that weigh between 160-190 g were used. Female rats were excluded to avoid interference of hormonal disturbances/changes such as hormonal interplay and changes in estrus cycle which could course interference in the experimental study. The rats were allowed to acclimatize for two weeks and during this period were fed *ad libitum*. Ethical Committee of Ahmadu Bello University, Zaria, Nigeria approves the experimental protocol for the study. The policy for the conduct of clinical and experimental studies described by the Basic & Clinical Pharmacology & Toxicology was adhered [24]. All etiquette was in conformism with the established procedures that are in agreement with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research. The rules and regulations in harmony with the Ethical Committee's instruction were firmly observed. Concerted efforts were made to minimise suffering. The criterion for anaesthesia was the lack of body or limb movement in reaction to a standardised tail clamping stimulus.

### *Experimental design*

The experimental rats were randomly allocated into 2 sets of 20 rats each namely: Lead acetate (60 mg $kg^{-1}$ body weight) and normal saline. They were exposed to equal quantity/quality of food and water. After 6 weeks, the 2 sets were further divided into 4 units of 5 groups containing 5 rats per group. The groups in the lead acetate and normal saline sets were further labelled group 1 to 4 and group 5 to

8 respectively (Table 1). All groups (except 1 and 8) were given folic acid (500 µg $kg^{-1}$ body weight) and/or vitamin-C (60 mg $kg^{-1}$ body weight) orally for another period of 4 weeks. All treatment was done orally using oral gavage.

**Table 1.** Grouping of Experimental Animal

| Group | Nature of Treatment | | | |
|---|---|---|---|---|
| | Normal saline | Lead acetate (60 mg $kg^{-1}$) | Vitamin-C (60 mg $kg^{-1}$) | Folic acid (500 µg $kg^{-1}$) |
| **1** | Yes | Yes | No | No |
| **2** | Yes | Yes | No | Yes |
| **3** | Yes | Yes | Yes | No |
| **4** | Yes | Yes | Yes | Yes |
| **5** | Yes | No | No | Yes |
| **6** | Yes | No | Yes | No |
| **7** | Yes | No | Yes | Yes |
| **8** | Yes | No | No | No |

At the completion of the experiment, the experimental animals were deprived of food overnight, thereafter they were sacrificed, followed by a collection of sample (blood).

***Collection of blood sample***

Sample of blood was collected in EDTA collection tubes, and then centrifuged at 1788×g for 20 minutes, and the supernatant obtained was kept at $4^0$C, prior to the analysis

***Determination of PON-1 activity***

The method of Eckerson *et al.*, [25] as described by Mogarekar and Chawhan [26] was used for the determination of serum PON-1.

***Determination of arylesterase activity***

The phenyl acetate was used as the synthetic substrate for the determination of arylesterase activity. Exactly 10 µl of the sample was added to the 500 µl of the substrate medium. The substrate medium contains 100 µl of 10 mM $L^{-1}$ of the substrate, 1 mM $L^{-1}$ $CaCl_2$ in 100 mM $L^{-1}$ tris buffer (pH 8.0). The production of phenol was determined spectrometrically after two minutes at 270 nm. The absorbance was taken after 20 seconds lag time and was monitored up to first one minute, and the difference was recorded as the absorbance. The serum level of arylesterase was calculated using the formula below, and the result obtained was expressed as U $L^{-1}$ of arylesterase activity.

$$\text{arylesterase activity (U L}^{-1}) = \left|(\Delta Abs) \times (\tfrac{1}{\varepsilon}) \times (\tfrac{tv}{sv}) \times (\tfrac{1}{d})\right|$$

Where:

$\Delta Abs$: is the change in absorbance per minute of sample minus change in absorbance of blank.

$\varepsilon$: is the molar extinction coefficient (1310 L $mol^{-1}cm^{-1}$)

tv: is the total reaction volume (0.51 ml)

sv: is the sample reaction volume (0.01 ml)

d : is the path length (1 cm)

***Determination of Paraoxonase activity***

Diethyl p-nitro phenyl phosphate (paraoxon) was used as the synthetic substrate for the determination of paraoxonase activity. Exactly 40 µl of the sample was added to the 500 µl of the substrate medium. The substrate medium contains 100 µl of 10 mM $L^{-1}$ of the substrate, 1.0 mM $L^{-1}$ $CaCl_2$ in 100 mM $L^{-1}$ tris buffer (pH 8.0). The generation of p-nitrophenol was determined spectrometrically after two minutes at 412 nm. The absorbance was taken after 20 seconds lag time and was monitored up to first one minute, and the difference was recorded as the absorbance. The serum level of paraoxonase was calculated using the formula below, and the result obtained was expressed as U $L^{-1}$ of paraoxonase activity.

$$\text{Paraoxonase activity } = \left[(\Delta Abs) \times (\frac{1}{\varepsilon}) \times (\frac{tv}{sv}) \times (\frac{1}{d})\right]$$

Where:

$\Delta Abs$: is the change in absorbance per minute of sample minus change in absorbance of blank.

$\varepsilon$: is the molar extinction coefficient (17000 L $mol^{-1}cm^{-1}$)

tv: is the total reaction volume (0.54ml)

sv: is the sample reaction volume (0.04ml)

d: is the path length (1cm)

***Serum lipid profile determination***

The serum lipid profiles, such as to TC, HDL-c, and TG were determined using standard diagnostic kits, while LDL-c was calculated using the formula below

$$LDLc = (TC–HDLc) – 0.2 \times TG$$

Where:

LDLc: Serum low-density lipoprotein cholesterol,

HDLc: Serum high-density lipoprotein cholesterol,

TC: Serum total cholesterol

TG: Serum triglycerides.

***Serum oxidative stress biomarkers determination***

***Estimation of malondialdehyde***

This was determined using the method of Placer *et al.,* [27] as described by Prakash *et al*., [28]. Malondialdehyde is the most extensively studied, and is used as a biochemical marker for the assessment of lipid peroxidation. The reaction is a function of the pink coloured complex between malondialdehyde and thiobarbituric acid reagent. Two tubes were set up for the blank and the test, and 0.5 ml of distilled water and 3.0 ml of thiobarbituric acid reagent were pipetted into the blank tube, while 0.75 ml of each sample and 3.0 ml thiobarbituric acid reagent were also pipetted into the test tubes. All samples in the test tubes were boiled in a water bath for 15 minutes. The tubes were allowed to cool, centrifuge for 10 minutes at 3000 rpm and the absorbance of supernatant was read at 535 nm.

$$malondialdehyde = \frac{absorbance\ of\ test \times\ total\ volume}{nonomolar\ extinction\ coefficient\ \times\ sample}$$

Where extinction coefficient = $1.56 \times 10^5$

***Determination of activities of catalase***

The activity of catalase (CAT) was determined using the method described by Aebi [29]. As catalase decomposes hydrogen peroxide ($H_2O_2$), the absorption decreases with time and from this decrease, catalase activity was measured at 240 nm. The analysis was carried out by adding 2 ml of the sample and 1 ml of hydrogen peroxide solution into the sample test tubes while 2 ml of the blank solution and 1 ml of hydrogen peroxide was added to the blank test tube. The change in the absorbance of test sample against blank at 240 nm was recorded every 15 seconds for a period of using UV – Visible Spectrophotometer.

$$\text{Concentratio} = \frac{\log\left(\frac{Abs_1}{Abs_2}\right) \times 0.2}{0.00693}$$

$Abs_1$: is absorbance at t = O second

$Abs_2$: is absorbance at t = 15 seconds

Where 0.25 and 0.00693 are constant

***Determination of activities of superoxide dismutase***

The superoxide dismutase (SOD) was determined by the method described by Fridovich [30]. The ability of the superoxide dismutase to inhibit auto oxidation of adrenaline at pH 10.20 forms the basis of this assay. Exactly 0.2 ml of the serum was added to 2.5 ml of 0.05 M carbonate buffer. The reaction was started by the addition of 0.3 Mm adrenaline. The absorbance was taken over 30 seconds up to 150 seconds at 450 nm.

Increase in absorbance per minute = (A2 – A1) / 2.5

% Inhibition = 100 – (Increase absorbance for sample / Increase absorbance for Blank) × 100

One unit of SOD activity is the quantity of SOD necessary to elicit 50 percent inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

***Statistical analysis***

For each parameter that was analyzed, the value of data obtained was presented as mean (n=5) ± standard deviation, and was subjected to analysis of variance (ANOVA) using (SPSS version 20.0). The significance level (P value <0.05) was calculated using Duncan multiple test range. The association between parameters was performed using Pearson's correlation test.

## RESULTS

Findings obtained from this study shown statistical significant ($p<0.05$) increase in aryl esterase levels (80.52±9.59, 79.09±9.88 U $L^{-1}$) in the groups intoxicated with lead and treated with folic acid or vitamin-C compared to the level (60.51±8.52 U $L^{-1}$) in groups intoxicated with lead without treatment (Figure 1). Furthermore, statistical significant ($p<0.05$) increase (107.20±5.33 U $L^{-1}$) in the serum level of this enzyme was also observed in the groups intoxicated with lead, treated with combined administration of folic acid and vitamin-C, as compared to the level observed in the group intoxicated with lead, supplemented with either folic acid or vitamin C. The control on the other hand showed insignificant ($p>0.05$) statistical difference in the serum level (121.51±7.34 U $L^{-1}$) of arylesterase as compared to the values (119.45±6.61, 119.12±7.54, and 121.44±8.29 U $L^{-1}$) observed in the non-lead intoxicated groups supplemented with Folic acid, vitamin C, and Folic Acid and vitamin C (Figure 1).

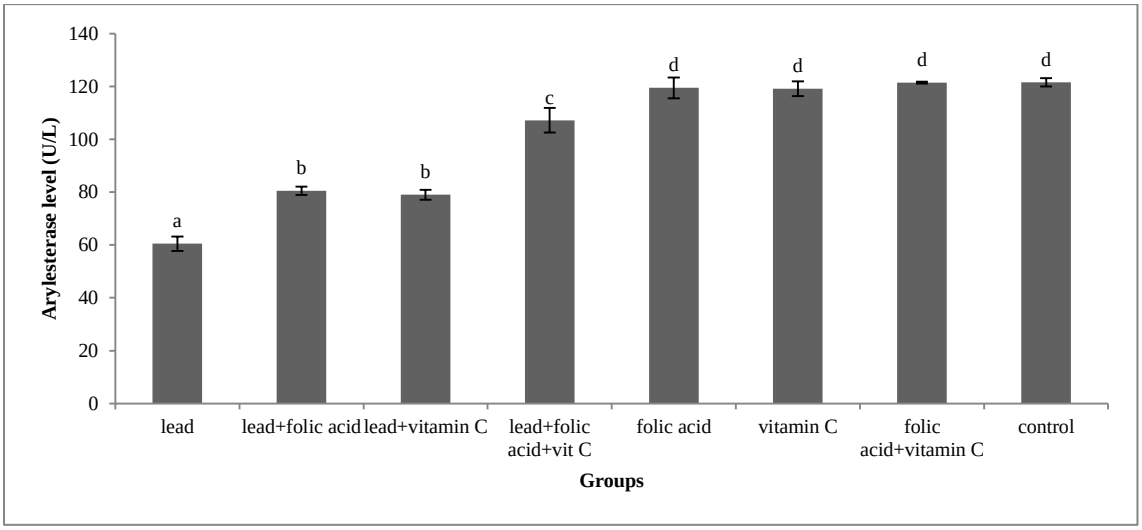


**Figure 1.** Serum arylesterase of Rats Intoxicated with Lead and Supplemented with Folic acid and/or Vitamin C. Bars with different alphabet differ significantly (P<0.05).

The analysis of the paraoxonase activity in the serum of the experimental rats as shown in Figure 2 revealed statistical significant ($p<0.05$) increase (92.11±2.39 U $L^{-1}$) of this enzyme in the group intoxicated with lead treated with folic acid and vitamin C administration, compared to the level (85.09±2.47 U $L^{-1}$, 84.66±3.09 U $L^{-1}$) observed in the groups treated with either folic acid or vitamin-C administration (Figure 2). Statistical significant difference ($p<0.05$) was also observed in the group intoxicated with lead, supplemented with folic acid and/or vitamin-C, as compared to the group intoxicated with lead without treatment (Figure 2).

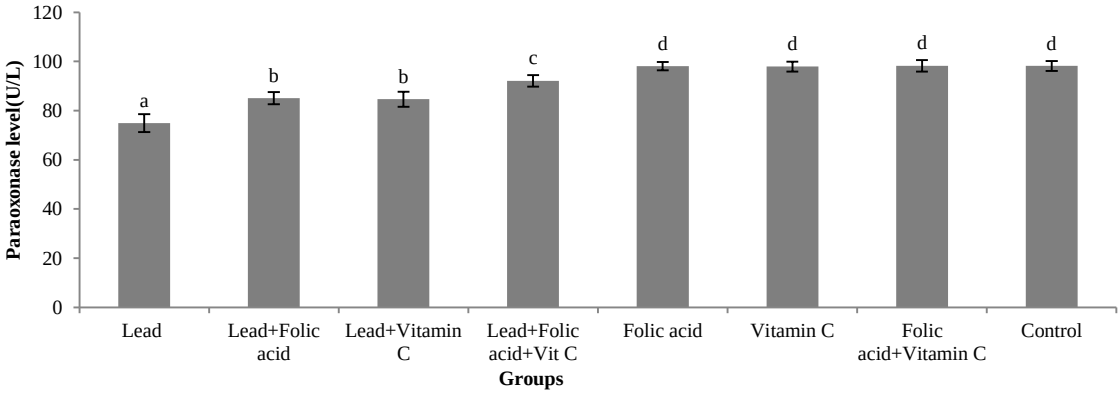


**Figure 2.** Serum paraoxonase of Rats Intoxicated with Lead and Supplemented with Folic acid and/or Vitamin C. Bars with different alphabet differ significantly (P<0.05).

The results obtained from the analysis of the serum lipid profile of rat intoxicated with lead, treated with folic acid and/or vitamin C administration is shown in Table 2. The result showed significant ($p<0.05$) increase in the level of cholesterol (404.90± 1.33 mg $dL^{-1}$) observed in the group intoxicated with lead with no treatment, compared to the experimental groups treated with folic acid and/or vitamin-C administration (Table 2). The triglyceride content (55.96 ± 1.62 mg $dL^{-1}$) observed in the control differs significantly ($p<0.05$) from the content (166.70 ± 1.44 mg $dL^{-1}$) observed in the non-supplemented group intoxicated with lead (Table 1). Similarly, the administration of both folic acid and vitamin-C to the group intoxicated with lead also differ in the triglyceride content (63.40 ± 0.64 mg $dL^{-1}$), compared with the level observed in the control (Table 1).

The serum level (330.54 ±1.54 mg $dL^{-1}$) of low-density lipoprotein cholesterol (LDL-c) increased significantly ($p<0.05$) in the group intoxicated with lead, compared with the levels (145.91 ± 1.26 mg $dL^{-1}$ and 162.18 ± 1.39 mg $dL^{-1}$) observed in the groups intoxicated with lead and treated with either folic acid or vitamin C administration (Table 2). In another observation, the serum content (41.08 ± 0.48 mg $dL^{-1}$) of high-density lipoprotein cholesterol, was significantly ($p<0.05$) lower in the lead administered group as compared to the content (78.67 ± 0.66 mg $dL^{-1}$) observed in the control (Table 2). Similarly, statistical significant ($p<0.05$) reduction was also observed in the HDL contents (57.98 ± 0.58 mg $dL^{-1}$ and 58.58 ± 0.84 mg $dL^{-1}$) of the rats intoxicated with lead, treated with folic or vitamin C administration, compared to the contents (78.39 ± 0.39 mg $dL^{-1}$ and 78.51 ± 0.42 mg $dL^{-1}$) observed in the non-lead intoxicated groups, supplemented with folic acid or vitamin C (Table 2).

**Table 2.** The Serum Lipid Profile **(**mg $dL^{-1}$**)** of Rats Intoxicated with Lead and Supplemented with Folic acid and/or Vitamin-C

| Groups | LDL-C | HDL-C | TG | TC |
|---|---|---|---|---|
| **Lead** | 330.54 ±1.54$^{a}$ | 41.08 ± 0.48$^{a}$ | 166.70 ± 1.44$^{a}$ | 404.90 ± 1.33$^{a}$ |
| **Lead+Fa** | 145.91 ± 1.26$^{b}$ | 57.98 ± 0.58$^{b}$ | 88.47 ± 1.09$^{b}$ | 221.59 ± 1.13$^{b}$ |
| **Lead+Vit C** | 162.18 ± 1.39$^{c}$ | 58.59 ± 0.84$^{b}$ | 102.12 ± 1.67$^{c}$ | 241.19 ± 0.44$^{c}$ |
| **Lead+Fa+VC** | 118.44 ± 1.45$^{d}$ | 71.63 ± 0.50$^{c}$ | 63.40 ± 0.64$^{d}$ | 202.75 ± 1.12$^{d}$ |
| **Folic acid** | 108.37 ± 1.22$^{e}$ | 78.39 ± 0.39$^{d}$ | 59.29 ± 0.55$^{e}$ | 198.62 ± 0.41$^{e}$ |
| **Vitamin C** | 108.97 ± 0.88$^{e}$ | 78.51 ± 0.40$^{d}$ | 55.34 ± 0.43$^{f}$ | 198.92 ± 0.46$^{e}$ |
| **Fa+Vit C** | 103.83 ± 0.21$^{e}$ | 79.05 ± 0.42$^{d}$ | 51.95 ± 1.15$^{g}$ | 193.27 ± 0.53$^{f}$ |
| **Control** | 108.86 ± 0.74$^{e}$ | 78.67 ± 0.66$^{d}$ | 55.96 ± 1.62$^{f}$ | 198.68 ± 0.51$^{e}$ |

Values are expressed as mean ± Standard deviation (n=5). Values down the group having different superscripts differ significantly (P≤0.05). LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein cholesterol, TG: Triglyceride, TC: total cholesterol, ApoB: Apolipoprotein B, Fa: Folic acid, Vit-C: Vitamin-C

The correlation results of arylesterase versus serum lipid profile, as depicted in Table 3, show insignificant moderate positive correlation (0.558) between high-density lipoprotein cholesterol versus catalytic concentrations of arylestrase in non-supplemented, rats intoxicated with lead. However, the relationship between arylesterase versus high-density lipoprotein in the co-supplemented (folic acid plus vitamin-C), non-lead intoxicated group, showed very strong significant positive correlation (0.960) (Table 3), while the correlation between paraoxonase versus high-density lipoprotein cholesterol showed an insignificant positive strong correlation (0.651) in the non-supplemented group intoxicated with lead (Table 4).

**Table 3**. Correlation between Arylesterase and Serum Lipid Profile of Rats Intoxicated with Lead and Supplemented with Folic acid and Vitamin C.

| Groups | LDL-Cholesterol | HDL Cholesterol | Total Cholesterol | Triacylglycerol |
|---|---|---|---|---|
| **Lead** | -0.300 (0.624) | 0.558 (0.333) | -0.324 (0.594) | -0.641 (0.244) |
| **Lead+Fa** | 0.376 (0.533) | 0.327 (0.592) | 0.147 (0.747) | 0.074 (0.841) |
| **Lead + Vit-C** | -0.124 (0.843) | -0.345 (0.570) | -0.275 (0.655) | -0.666 (0.220) |
| **Lead+Fa+Vit-C** | -0.592 (0.293) | 0.521 (0.368) | 0.406 (0.498) | 0.366 (0.544) |
| **Folic acid** | -0.461 (0.435) | 0.482 (0.411) | -0.487 (0.406) | -0.673 (0.213) |
| **Vitamin-C** | -0.814 (0.094) | 0.946 (0.015) | -0.748 (0.146) | -0.607 (0.278) |
| **Fa + Vit-C** | -0.054 (0.931) | 0.960 (0.010) | 0.126 (0.840) | -0.498 (0.402) |
| **Control** | 0.777 (0.122) | 0.457 (0.439) | 0.175 (0.778) | 0.312 (0.610) |

Correlation is a significant at $p \leq 0.05$. Figures in brackets indicate a significant level of correlations, while the non-brackets values are the correlation figures.0.00 indicate No correlation; 0.01 to 0.19 indicate very weak correlations; 0.20 to 0.39 indicate weak correlations; 0.40 to 0.59 indicate moderate correlations; 0.60 to 0.79 indicate strong correlations;0.80 to 0.99 indicate very strong correlations.LDL: Low-density lipoprotein cholesterol, HDL: High-density lipoprotein cholesterol; Fa: folic acid; Vit-C: Vitamin C

**Table 4**. Correlation between Paraoxonase and Serum Lipid Profile of Rats Intoxicated with Lead and Supplemented with Folic acid and Vitamin C

| Groups | LDL-Cholesterol | HDL Cholesterol | Total Cholesterol | Triacylglycerol |
|---|---|---|---|---|
| **Lead** | 0.028(0.091) | 0.651(0.234) | -0.242(0.695) | -0.800(0.104) |
| **Lead+Fa** | 0.142(0.818) | 0.134(0.789) | 0.322(0.601) | 0.139(0.812) |
| **Lead+Vit C** | -0.991(0.010) | 0.312(0.609) | -0.907(0.034) | -0.125(0.841) |
| **Lead+Fa+vitC** | -0.542(0.458) | 0.851(0.149) | -0.508(0.502) | -0.015(0.985) |
| **Folic acid** | -0.466(0.429) | -0.188(0.762) | -0.414(0.489) | -0.375(0.534) |
| **Vitamin C** | -0.135(0.828) | 0.185(0.766) | -0.143(0.819) | -0.294(0.632) |
| **Fa+Vit C** | -0.574(0.311) | 0.377(0.535) | -0.281(0.646) | -0.801(0.103) |
| **Control** | -0.320(0.600) | 0.180(0.772) | -0.013(0.984) | -0.331(0.587) |

LDL: Low-density lipoprotein cholesterol, HDL: High-density lipoprotein cholesterol. The correlation is significant at $p \leq 0.05$, Figures in brackets indicate significant level of correlations, while the non-brackets values are the correlation figures.0.00: No correlation; 0.01 to 0.19: Very weak correlations; 0.20 to 0.39: Weak correlations; 0.40 to 0.59: Moderate correlations; 0.60 to 0.79: Strong correlations; 0.80 to 0.99: Very strong correlations.

The results in Table 5 showed the serum levels of endogenous antioxidant enzymes (SOD and Catalase), and the level of malonyldialdehyde (MDA) of rats intoxicated with lead treated with folic acid and/or vitamin-C administration. The group intoxicated with lead, with no treatment, showed statistical significant ($p<0.05$) decrease in the serum level of catalase and superoxide dismutase (SOD), as compared to the levels observed in the groups intoxicated with lead, treated with folic acid and/or vitamin C administration (Table 5).

**Table 5**. Serum Oxidative Stress Status of Rats Intoxicated with Lead and Supplemented with Folic acid and/or Vitamin C

| Groups | CAT (U $mg^{-1}$ protein) | SOD (U $mg^{-1}$ protein) | MDA (nmol $L^{-1}$) |
|---|---|---|---|
| **Lead** | 10.54 ± 0.46a | 4.93 ± 0.66[a] | 5.21 ± 0.16[a] |
| **Lead+fa** | 19.18 ± 0.76[b] | 10.94 ± 1.13[b] | 3.02 ± 0.30[b] |
| **Lead+VC** | 24.18 ± 0.43[c] | 18.36 ± 1.04[c] | 2.90 ± 0.45[b] |
| **Lead+fa+VC** | 30.96 ± 0.47[d] | 27.55 ± 0.43[d] | 0.62 ± 0.04[c] |
| **Folic acid** | 31.30 ± 1.31[d] | 27.67 ± 0.83[d] | 0.55 ± 0.23[c] |
| **Vitamin C** | 31.72 ± 0.80[d] | 27.89 ± 1.88[d] | 0.53 ± 0.04[c] |
| **Fa+ VC** | 32.91 ± 1.52[d] | 28.61 ± 1.57[d] | 0.51 ± 0.30[c] |
| **Control** | 31.62 ± 0.67[d] | 28.46 ± 1.54[d] | 0.58 ± 0.29[c] |

Values are expressed as mean ± Standard deviation (n=5). Values down the group having different superscripts differ significantly. CAT: Catalase; SOD: Superoxide dismutase; MDA: Malonyldialdehyde

The correlation between serum arylesterase, paraoxonase versus serum oxidative stress markers revealed in Tables 6 and 6, showed insignificant moderate negative correlation (-0.445) between arylesterase versus malonyldialdehyde (MDA) in the non-supplemented group intoxicated with lead Table 6. In contrast, very weak negative correlations (-0.021 and -0.143) exist between paraoxonase versus malonyldialdehyde contents (Table 7). Although, insignificant strong positive correlation (0.615) exists between catalytic concentrations of paraoxonase versus catalase in the non-supplemented group intoxicated with lead (Table 7).

**Table 6**. Correlation between Arylesterase and Serum Oxidative stress markers in Rats Intoxicated with Lead and Supplemented with Folic acid and Vitamin-C.

| Groups | SOD | CAT | MDA |
|---|---|---|---|
| **Lead** | 0.026(0.671) | -0.169(0.786) | -0.445(0.452) |
| **Ld+Fa** | 0.798(0.106) | 0.251(0.684) | -0.067(0.914) |
| **Ld+Vit C** | 0.317(0.569) | -0.242(0.695) | 0.312(0.609) |
| **Ld+Fa+vitC** | 0.830(0.082) | -0.021(0.635) | 0.219(0.724) |
| **Folic acid** | 0.016(0.979) | -0.291(0.635) | 0.055(0.870) |
| **Vitamin C** | -0.641(0.198) | 0.698(0.190) | 0.201(0.746) |
| **Fa+Vit C** | -0.689(0.198) | -0.122(0.845) | 0.133(0.831) |
| **Control** | 0.262(0.670) | -0.366(0.544) | -0.258(0.675) |

Correlation is significant at $p<0.05$, Figures in brackets indicate significant level of correlations, while the non-brackets values are the correlation figures. CAT: Catalase, SOD: Superoxide dismutase, MDA: Malonyldialdehyde, Fa: Folic acid, Vit-C: Vitamin-C. 0.00: No correlation; 0.01 to 0.19: Very weak correlations; 0.20 to 0.39: Weak correlations; 0.40 to 0.59: Moderate correlations; 0.60 to 0.79: Strong correlations; 0.80 to 0.99: Very strong correlations.

**Table 7**. Correlation between Paraoxonase and Serum Oxidative Stress Markers in Rats Intoxicated with Lead and Supplemented with Folic acid and Vitamin C.

| Groups | SOD | CAT | MDA |
|---|---|---|---|
| **Lead** | -0.257(0.677) | 0.615(0.270) | -0.143(0.819) |
| **Lead+Fa** | 0.356(0.557) | 0.677(0.210) | -0.086(0.891) |
| **Lead+Vit C** | -0.774(0.125) | 0.798(0.105) | -0.099(0.874) |
| **Lead+Fa+vitC** | 0.015(0.980) | 0.083(0.895) | -0.644(0.241) |
| **Folic acid** | 0.396(0.509) | 0.846(0.071) | 0.257(0.676) |
| **Vitamin C** | 0.606(0.279) | 0.165(0.791) | 0.354(0.534) |
| **Fa+Vit C** | 0.515(0.375) | 0.903(0.036) | 0.240(0.645) |
| **Control** | 0.559(0.327) | 0.154(0.805) | 0.361(0.528) |

Correlation is significant at $p \leq 0.05$, Figures in brackets indicate significant level of correlations, while the non-brackets values are the correlation figures.CAT: Catalase, SOD: Superoxide dismutase, MDA: Malonyldialdehyde, Fa: Folic acid, Vit-C: Vitamin-C.0.00: No correlation; 0.01 to 0.19: Very weak correlations; 0.20 to 0.39: Weak correlations; 0.40 to 0.59: Moderate correlations; 0.60 to 0.79: Strong correlations; 0.80 to 0.99: Very strong correlations.

## DISCUSSION

Lead is a ubiquitous metal in the environment, and its adverse effects are well studied in human and animal models. The decreased level of PON-1 in the non-supplemented group intoxicated with lead deduced that, lead has the ability to mitigate the catalytic activity of PON-1. Permongpaiboon *et al*., [31] revealed a similar observation that exposure to low levels of lead decreased serum concentration of PON-1, and pose imbalance in pro-oxidant and antioxidants status, leading to oxidative damage in lead-exposed workers. The Noticeable increase of PON-1 in the folic acid and vitamin-C supplemented group, implies that synergistic supplementation of both vitamins tremendously increased the activity of PON-1 in the rats intoxicated with lead, and this was affirmed in the work of Gursu *et al.*, [32], that combined administration of both vitamins (folic acid and vitamin C) increased the serum level of PON-1. On the contrary, their study was based on stress-induced decrease in the level of PON-1. The decreased levels of PON-1 could be a potential risk factor for cardiovascular related problems [33], possibly due to its ability to diminish the oxidation of low-density lipoprotein (OX – LDL), thus offering protection against the development of atherosclerosis [26] and other cardiovascular related problems. Oxidized low-density lipoprotein cholesterol (OX-LDLc) occurs when LDL-c react with free radicals and the product (OX-LDLc) goes directly into inner lining of arteries that supplies blood to every part of the body, thus blocking blood supply. It is obvious that lead-induced oxidative stress and increased LDL-c might immensely result to elevated OX-LDL-c level. This may be the first study revealing increase in the serum level of PON-1 of lead-administered rats treated with combined administration of folic acid and vitamin-C. Although vitamin-C with other vitamins such as vitamin-E (at higher doses), has also been shown to improve PON-1activity [34], not lead toxicity related.

The administration of Lead to the experimental rats induced hyperlipidaemia (Table 2), and our findings are similar to the observation revealed in the work of Kamal *et al*., [33]. They observed increased levels of triglycerides, low-density lipoprotein cholesterol (LDL-c) and decrease in the level of high-density lipoprotein cholesterol (HDL-c). These lipoproteins are bio-vehicles that function in the transport of lipids around the body, and also allow fats to be taken up by receptor-mediated endocytosis [35]. Hyperlipidaemia is characterised by an increase in serum levels of total cholesterol, triglycerides, low-density lipoprotein and a decrease in the serum levels of high-density lipoprotein (HDL). As observed in this study, folic acid or vitamin-C administration could not greatly reverse lead-induced hyperlipidaemia in the experimental rats. This observation is contrary to previous report that vitamin-C

administration at a lower dose, nearly reverse lead-induced hyperlipidaemia [36]. Increased serum level of LDL-c might elevate the rates of cholesterol accumulation within the walls of the arteries, and decreased levels of HDL-c can hamper elimination of accumulated cholesterol in the arterial walls. This signify lead-induced hypercholesterolaemia as shown in Table 2, could be ascribed to increased level of LDL-c and decreased level of HDL-c. Contrary to our view, Gajawat *et al*., [37] attributed lead-induced hypercholesterolaemia to disruption of cell membrane resulting to the release of cholesterol into the circulation. On the other hand, Kojima *et al*., [38] attributed lead-induced hypercholesterolaemia to changes in some key enzymes such as 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA), and suppression of cholesterol catabolic enzyme such as 7α-hydroxylase. However folic acid and/or vitamin-C administration lowers the levels of cholesterol, triglycerides and low-density lipoprotein cholesterol (Table 2). More so, lead-induced hepatic damage could be responsible for hyperlipidemia, as the liver is the major site for the metabolism and storage of folate. Folate deficiency is common in many liver diseases, which results to elevated level of homocysteine [39]. Increase homocysteine overexpresses HMG-COA reductase (a key enzyme in the cholesterol synthesis pathway), through the activation of several transcription factor, thus increasing cholesterol biosynthesis [40].

Folic acid is one of the targeted nutrients by lead, resulting in accumulation of demethylated methionine (homocysteine) and a more stable homocysteine thiolactone, which apparently impairs the functionality of HDL-c, and promotes oxidation of LDL-c [41], that has more atherosclerosis implication than the native LDL-c. The PON-1, of which its activity was lowered by lead intoxication as evident in this study, isanti-homocysteine thiolactone and OX-LDL-c formation. So the need for folic acid administration is key to addressing lead-induced all forms of cardiovascular diseases.

The moderate correlation between aryl-esterase versus HDL-c of the group intoxicated with lead with no treatment cannot best explain the relationship between the two variables. However, co-administration of folic acid and vitamin-C in the non-lead intoxicated group, significantly improves the catalytic concentration of aryl-esterase that in turn elevates serum level of high-density lipoprotein cholesterol (HDL-c). On the other hand, the downhill correlation between triacylglycerol versus aryl-esterase in the non-supplemented group intoxicated with lead implies lead-induced decrease in the catalytic concentration of aryl-esterase may alter (increase) the metabolic synthesis of triacylglycerol. As such, laboratory assessment of aryl-esterase might be a useful diagnostic biomarker to predict the level of triacylglycerol in cardiovascular disease risk patients. The insignificant positive correlation between paraoxonase versus HDL-c implies lead-induced metabolic decrease in the catalytic activity of paraoxonase may be depend on the metabolic decrease in the serum level of high-density lipoprotein cholesterol level. In contrast, a study revealed significant positive correlation between HDL-c versus paraoxonase, while the LDL-c is independently related to paraoxonase in patients with chronic kidney disease (CKD) on haemodialysis (HD) [42].

Oxidative stress is one of the most extensively studied health implications with respect to lead toxicity over the years. The decreased level of SOD, CAT and increased level of MDA observed in this study is in line with previous research findings that lead administration to the experimental rats resulted in decrease in the serum level of CAT and SOD, and increase in the serum level of MDA [43]. Catalase is heme containing antioxidant enzyme, which catalyses the reduction of hydrogen peroxide to water and oxygen, while superoxide dismutase (SOD) keeps the superoxide radicals at low levels. Thus, both offer protection against damage by free radicals [44]. The increased level of catalase and superoxide dismutase (SOD), by folic acid administration observed in this study, is an indication that folic acid is a vitamin with antioxidants properties. Unlike folic acid that has not been extensively studied with respect to lead-induced oxidative stress, vitamin-C has been extensively studied, and has been shown to strengthen the anti-oxidative defenses and decrease oxidative stress [44].

Information on the combined administration of folic acid and vitamin-C on lead-induced oxidative stress is either

scrimp or not available. However, combinations of vitamin-C with other vitamins have also yielded promising results. For example, Wang *et al*., [45] revealed that vitamin-C and thiamine in combined form lessen the liver damage mediated by lead-induced oxidative stress. Ebuehi *et al*., [9] also revealed that lead administration induced a significant increase in the MDA level of the experimental rats. However, such an increase was ameliorated by oral administration of vitamin-C and tocopherol (vitamin E). Interestingly, this may be the first time that oral combined administration of folic acid and vitamin-C, in fact at lower doses, significantly decreased the serum level of MDA in lead-induced oxidative stress (Table 5).

The moderate negative correlation between aryl-esterase versus serum MDA contents was not substantial enough to establish dependent or independent relationship between the variables. By implication, the effects of lead on serum MDA levels, may or may not lead to metabolic increase or decrease in the catalytic concentration of aryl-esterase (Table 4). However, study by Ramana [46] revealed insignificant strong negative correlation between paraoxonase versus serum MDA levels, although the study was based on the relationship between MDA versus paraoxonase among pregnant women with preeclampsia and normal pregnancy. Increased malonyldialdehyde could result to oxidation of low-density lipoprotein cholesterol, which can be prevented by PON-1 enzyme. Ox-LDL-c is the key initial step in atherosclerosis [47-48].

## CONCLUSIONS

The combined administration of folic acid and vitamin-C to rats intoxicated with lead improves the activities of PON-1 and other biochemical indices analysed in this study. The elevation of PON-1 level, due to the combined administration of folic acid and vitamin-C could increase serum HDL cholesterol and may reduce cardiovascular disease risk. Further studies should focus on the effect of combined administration of folic acid and vitamin-C on the activities of aryl-esterase and paraoxonase of rats intoxicated with lead at molecular level.

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### *Conflict of interest*

The authors declare that there is no conflict of interest.

### *Author's contribution*

Ibrahim Abdulwaliyu and Stanley I.R. Okoduwa got the concept and design of the study. Ibrahim Abdulwaliyu, Shefiat O. Arekemase and Aliyu Muhammad carried out the acquisition of data and analysis. Sani Ibrahim, Elewechi Onyike participated in the interpretation of data. Project supervision was by Sani Ibrahim, Elewechi Onyike. Ibrahim Abdulwaliyu, Shefiat O. Arekemase, participated in Drafting of manuscript. Revision and editing for intellectual content was by Stanley I.R. Okoduwa and Aliyu Muhammad. All the authors gave a final approval of the revised manuscript for submission and publication.

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Data will be made available upon request.

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