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Synthetic and Natural Vitamin C Modulation of Leaded Paint-Induced Nephrotoxicity of Automobile Painters in Ile-Ife, Nigeria.

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Abstract:-

- ***Introduction:***

Lead (Pb) occupational exposure in painters has been documented to be contacted via additives in paints due to its many important properties and vitamin C has been the most widely studied when it comes to Pb-induced oxidative stress.

- ***Aim:***

This study aimed at the use of freshly squeezed orange-juice due to its accessibility in investigating the modulating role of synthetic and natural vitamin-C on leaded paint-induced nephrotoxicity of automobile painters.

- ***Study Design and Methods:***

Sixty (60) male automobile painters were consecutively selected and divided equally into 2 groups. Vitamin-C and orange juice were administered daily to painters for 4 weeks at dosage levels of 200 and 184 mg/day respectively. Thirty (30) male non-painters constituted the control group. Orange juice vitamin-C content was assessed by titrimetric method and synthetic vitamin-C served as the standard drug. Renal biomarkers and reduced glutathione (GSH) were done by Colorimetry. Urine aminolevulinic acid (ALA) and Pb were assessed by ELISA technique and atomic absorption spectrophotometry respectively. Phytochemical screenings (quantitative/qualitative) and proximate analysis were done using standard methods. Data were analyzed using Pearson's correlation coefficient and One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for pairwise comparison. Statistical significance was $p< 0.05$.

- ***Results:***

Baseline results at 0-week of orange juice administered group showed a significantly ($P<0.05$) higher serum Pb, urea and creatinine compared to non-painters. Also, their urine baseline results at 0-week showed a significantly ($P<0.05$) higher levels of ALA and GSH compared to non-painters. Orange juice administration at 4 weeks showed significant ($P<0.05$) reductions in concentrations of lead, urea, and creatinine in serum, decreased concentrations of GSH and ALA in urine but increased urine Pb compared to baseline. However, compared with baseline, after 4 weeks of vitamin-C supplementation, serum Pb, urine GSH, and urine ALA were significantly ($P<0.05$) reduced and urine Pb significantly ($P<0.05$) increased. A positive correlation was observed at 2-weeks of taking orange-juice between serum lead and urine ALA ($r= 0.703$) and GSH ($r= 0.913$) but 4-week positive correlation between urea and urine GSH ($r= 1.000$). A negative correlation was observed at 2-week of taking vitamin-C between serum creatinine and urine lead ($r= -0.857$) while 4-week a negative correlation was observed between urine GSH and urine lead ($r= -0.743$). Presence of tannin, phenol, saponin, alkaloid, and flavonoid was detected in orange juice.

- ***Conclusion:***

Orange juice administration conferred significant amelioration to renal and lead toxicity biomarkers by 4 weeks. The presence of phytochemicals suggests why orange juice may be a viable alternative in amelioration of toxic effects of leaded paint among automobile painters.

Keywords:- *Orange Juice, Vitamin-C, Renal Markers, Lead Toxicity Biomarkers, Automobile Painters, Paints.*

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I. INTRODUCTION

Environmental exposure to heavy metals is a continuing source of problem throughout the world [1]. In Nigeria, the growing rate of industrialization is not only leading to contamination and deterioration of the environment [2] but also to increase mortality and morbidity among human subjects. Paint, a liquid composition that is applied to a substrate in a thin layer to form a solid film, is used to provide color or improve texture. Chemical compounds used in paint products include pigments, extenders, binders, additives, and solvents (toluene, xylene, ketones, alcohols, esters, and glycol ethers), as well as different toxic metals [3].

Pb has been identified in paints produced by different manufacturers. It is the metal that is most consistently incorporated to paints due to its many important properties. Lead is added to paint to prevent corrosion, hasten drying, intensify durability, and sustain a fresh appearance. Yet lead incorporation to paint is a major cause of health and environmental hazards [4]. While inclusion of lead to paint has been phased out in the United States and the United Kingdom due to regulations prohibiting its use [5], in some countries addition of lead to paints use for domestic purposes still continues. Lead compounds that are commonly used in paints as primers, pigments and driers, have been reported to be absorbed after inhalation, oral or dermal exposure [6]. Patterns and rates of particle deposition are highly dependent on size and ventilation rate. Lead toxicity depends not only upon the absorbed dose, but also on the route and duration of exposure, i.e. acute or chronic. Significant contact with lead sets in motion excessive free radical generation that leads to oxidative stress, tissue damage and various disorders [7]. Evidence for metal toxicity from paint exposure has been offered by Hugo *et al*. [8]. They observed that paint-manufacture workers are potentially exposed to the chemicals present in paint products although the patterns and levels of exposure to individual agents may differ from those of painters. The clinical effects of lead toxicity cannot be dissociated from its fate in the human body, as disturbances in oxidative status can cause significant adverse effect [9]. Absorbed metals are distributed to the organs and, in the case of lead, are concentrated in the bone, liver, kidney, etc. Elimination of metals varies from several days to several years [10,11]. One of the mechanisms involved in the early stage of lead induced oxidative stress is the inhibition of δ-aminolevulinic acid dehydratase (ALAD), an important enzyme in heme biosynthesis which leads to the accumulation of δ-aminolevulinic acid (ALA) [12]. Lead also induces oxidative stress. Moreover, its effect on the enzymes of antioxidant defence system such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) is inhibitory [12].

Vitamin C or L-ascorbic acid is an essential nutrient and a potential antioxidant for humans. It is made internally by almost all organisms, with humans being a notable exception [13]. Citrus fruits and green leafy vegetables are sources of vitamin C in plants. Kidney along with liver from an animal source also serve as the main sources of vitamin C [14]. Vitamin C (ascorbic acid), a di-acid ($AscH_2$) with two ionisable hydroxyl groups, is a very important and powerful antioxidant that works in aqueous environments inside the body [15]. Transport of dehydroascorbic acid (Asc), an oxidized form of vitamin C, occurs by means of glucose transporters, while that of its reduced form is mediated by sodium-dependent amino acid transporters [16]. A study done by Chang *et al.* [17] showed the protective effect of ascorbic acid on oxidative stress, in the hippocampus of lead-exposed suckling rats. This study was aimed at investigating the ameliorative effects of synthetic and natural vitamin C on nephrotoxic biomarkers of leaded-paint exposure in automobile painters.

II. MATERIAL AND METHODS

A. Participants

Sixty (60) male automobile painters, who had been in the profession for at least 10 years, were consecutively recruited from different workshops for the study. Thirty (30) (age and sex-matched) male participants that served as controls (non-painters) were recruited from members of staff of Obafemi Awolowo University Teaching Hospital Complex. All participants were weight-matched, with weight of control, painters in synthetic vitamin C group and painters in orange group being 69.977 ± 6.72 kg; 70.365± 6.82 kg and 70.382± 6.90 kg respectively.

- *Study Location*

The study was conducted at Ile-Ife and its environs in Osun state. The male automobile painters samples were recruited from different workshops while controls subjects (non-painters) were the staff of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC).

- *Study Design*

This was an experimental study. The sixty participating male automobile painters were subdivided into 2 groups, consisting of 30 participants each. Group 1 and Group 2 were administered through the oral route with synthetic vitamin C and natural vitamin C (orange juice) for a period of 4 weeks at dose level of 200 mg/day and 184 mg/day respectively before meal in the morning.

B. Sources of Natural and Synthetic Vitamin C

Orange balls (*Citrus x sinensis*) were obtained from local market in Ile-Ife community and the fresh orange obtained was freshly squeezed, processed at Food Analytical Laboratory in the Department of Food Science and Technology at Obafemi Awolowo University, Ile-Ife. Synthetic vitamin C tablets were bought from accredited Pharmacy shop in Ile-Ife as a standard drug used in this study.

C. Qualitative Data

The questionnaire of different sections namely; demography [Information regarding age, gender, education, nature of occupation- part/full time]; work pattern and job description; and knowledge on occupational hazard and

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safety were examined (Table 7 and 8). All data obtained was treated with strict confidentiality.

D. Sample Collection

Approximately 8 mL of blood was collected from the ante-cubital vein of each of the participants into an anti-coagulant free bottle, centrifuged at 2,500 rpm for 10 minutes and serum stored at -20 0C. Ten milliliters (10 mL) of urine samples were collected into universal bottles in their respective workshops and stored at 4 0C prior the analyses.

E. Analytical Methods

➢ *Estimation of Ascorbic Acid in the Orange Samples*

- *Principle:*

Accurate 1 mL of freshly prepared orange sample solution was transferred and then diluted to 200 mL with distilled water. Then 10 mL of this solution was put into conical flask. To this flask 5.0 ml of potassium iodide (KI) solution (0.2 M), 2.5 mL of hydrochloric acid HCl (1.0 M) and few drops of starch solution were added. The solution was then titrated against potassium iodate (KIO_3) solution (0.015 M) from the burette until the appearance of blue-black colour which indicated the end point of the reaction. The titration was repeated three times for the orange sample. The results were recorded, tabulated and calculated for ascorbic acid determination for each samples [18].

➢ *Estimation of Metals (Lead, Phosphorus, Potassium, Sodium)*

- *Principle:*

The atoms (and ions) of an element (Serum/urine levels of lead, orange juice phosphorus, potassium and sodium), when aspirated into the AAS with specific wavelength of light provided, vaporized and absorbed light of the same wavelength by the atoms and the electrons in the atom move from the ground state to an excited state. The amount energy at the characteristics wavelength absorbed is proportional to the concentration element in the sample. (Wavelength at lead-283.3nm, potassium-766.5nm, sodium-589 nm, phosphorus- 450nm) [19].

➢ *Sample Preparation Procedures*

- *Sample Digestion:*

1mL of the thawed serum sample/urine/orange juice was added to a clean test tube , then diluted to 10 mL with 0.1N hydrochloric acid .The diluted serum sample was left for 24 hours to precipitate in order to liberate the trace metals from protein conjugates. A Bucket centrifuge of Brand name: xiangtian, Model Number: 800-1 (Jiangsu, China) was used to spin at 4000 rpm for 5 minutes to remove cellular debris. The supernatant was decanted into a plain tube.

➢ *Estimation of Serum Creatinine*

- *Principle:*

Creatinine in alkaline medium reacts with picric acid to form a red tautomer of creatinine picrate, the intensity of which is measured at 520 nm [20].

➢ *Estimation of Serum Urea*

- *Principle:*

Urea in serum is hydrolysed to ammonia in the presence of urease. Ammonia generated reacted with phenol in the presence of hypochlorite to form indophenol which gives a blue coloured compound in alkali. Nitro-prusside acts as a catalyst increasing the rate of reaction and the intensity of which is measured at 540 nm. [21].

➢ *Estimation of Urine Aminolevulinic Acid*

- *Principle:*

Urobilinogen, other Ehrlich-positive substances, and some substances which interfere with the coupling reaction were separated from ALA by extracting weakly acidified urine with n-butanol. ALA was converted to its pyrrole by reaction with ethyl acetoacetate at pH 6.8. The pyrrole reacted with Ehrlich's reagent to form a red colour, which was extracted with chloroform and wavelength was measured at 556 nm, leaving other Ehrlich-positive substances in the water phase [22].

➢ *Estimation of Urine Reduced Glutathione (GSH)*

- *Principle:*

The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of relatively stable (yellow) colour when 5', 5'-dithiobis- (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm which was read in a spectrophotometer. [23].

➢ *Phytochemical Screening*

- *Quantitative Screening of Phytochemicals:*

✓ *Working Principle of an HPLC:*

The liquid phase is pumped onto the column packed with the stationary phase at a constant rate. The analysis sample is injected into the carrier stream before entering the column. The sample components are selectively retained upon reaching the column on the basis of physico-chemical interactions between the molecules of the analyte and the stationary step. Based on the operating conditions, the mobile process that moves at a steady pace elutes the components. Techniques of detection are used to detect and measure the eluted components. Saponins, alkaloids, flavonoids, tannins and phenolics were done using High

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Power Liquid Chromatography according to Oladimeji and Valan [24].

- *Qualitative Screening of Phytochemicals:*

✓ *Test for Saponins (Frothing Test):*

Saponins were identified according to the method described by Banso and Adeyemo [25]. This was done by mixing 0.5mL of the juice in a test tube containing 3mL of hot distilled water, following by continuous vigorously shaking (1 min) to observe for persistent foaming.

✓ *Test for Flavonoids (Cyanidine Test):*

This was done according to the method of Stankovic [26]. Five hundred microliters (0.5mL) of juice was mixed with 2mL methanol and 1mL of concentrated sulphuric acid added. A spatula was used to add a powder of magnesium chloride ($MgCl_2$) and the mixture observed for 1 min for effervescence and also observed for a brick red colouration.

✓ *Test for Tannins (Ferric Chloride Test):*

This was done according to the method of Banso and Adeyemo [25]. 500 microliters (0.5mL) of juice was added to a test tube containing 20mL of boiled distilled water and then heated for an hour. Five drops of ferric chloride were added and the tube was allowed to stand for colour development. A blue-black colouration indicated the presence of tannins.

✓ *Test for Alkaloids (Wagner's Test):*

This was done according to the method of Joshi *et al.* [27]. One millilitre (1mL) of juice was stirred with 0.4mL of 1% HCl in a water bath for 5 min and filtered. Two grams (2g) of Potassium iodide and 1.27g of iodine were dissolved in 5mL of distilled water and the solution was diluted to 100 mL with distilled water. Two drops of this iodine solution were added to the filtrate; a brown coloured precipitate indicated the presence of alkaloids.

✓ *Test for Phenol:*

To 1 mL of each sample, one drop of 5% $FeCl_3$ (w/v) was added. This was observed for 10 min for the formation of a greenish precipitate [28].

F. Proximate Analysis:

The estimation of the various food parameters namely moisture content, total ash, crude fat, crude fiber, crude protein and total carbohydrate on dry matter basis were carried out according to standard procedures using dried powdered sample according to AOAC [28].

G. Statistical Analysis

The data obtained were analysed with statistical package of social sciences (SPSS) of version 22. Data were expressed as Mean ± S.D. (Standard deviation) for control and the test groups. Differences between the means of the control group and each of the test groups were determined using independent Student *t-test* while repeated measure Analysis of variance (ANOVA) was used to compare the means of each test groups (0, 2, 4 weeks).

Pearson product moment correlation coefficients were used to determine the level of association between continuous variables. Significant differences were taken at $P<0.05$.

III. RESULTS

Table 1 Nutritive Contents of Freshly Squeezed Orange used in this Study in Ile-Ife

Proximate Analysis	**Concentrations (%)**		
Moisture	87.84		
Protein	1.49		
Ash	1.40		
Fat	0.15		
Fiber	2.07		
Carbohydrates	7.05		
Phytochemical Screening	**Concentration (mg/100g)**	**Qualitative observation**	**Inference**
Tannin	0.61	green black colour	+
Phenol	0.43	greenish precipitate	+
Flavonoids	0.73	yellow colour persisted	+
Saponin	0.20	yellow emulsion formed	+
Alkaloids	3.12	creamy/reddish brown precipitate	++
Anti-nutrients	**Concentration**		
Oxalate	4.50 mg/g		
Phytate	20.60 mg/g		
Cyanide	1.02 mg/kg		
Minerals and vitamin	**Concentration**		
Phosphorus	0.00 mg/L		

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Potassium	2,044.00 mg/L
Sodium	355.00 mg/L
Vitamin C mg/100g	46 mg/100g

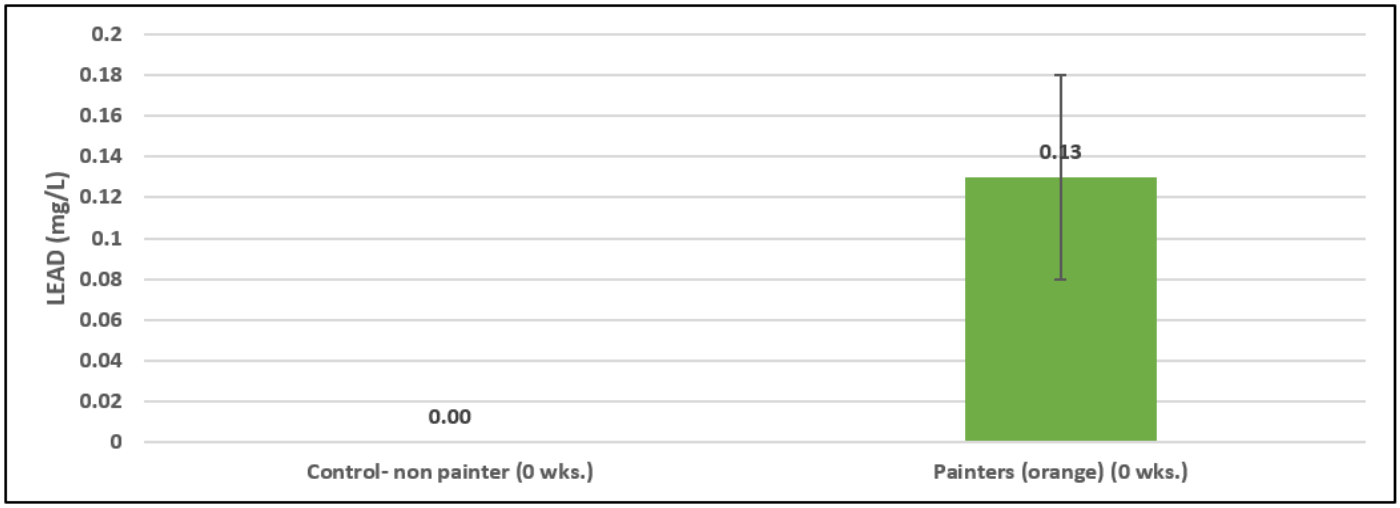

Fig 1a: Levels of serum lead of automobile painters (orange juice) compared to control group at 0 week. *Each value is an expression of mean ± SEM.* (P-value = 0.044). Lead was undetectable in control group compared to a value of 0.13 ± 0.05 mg/L in automobile painters.

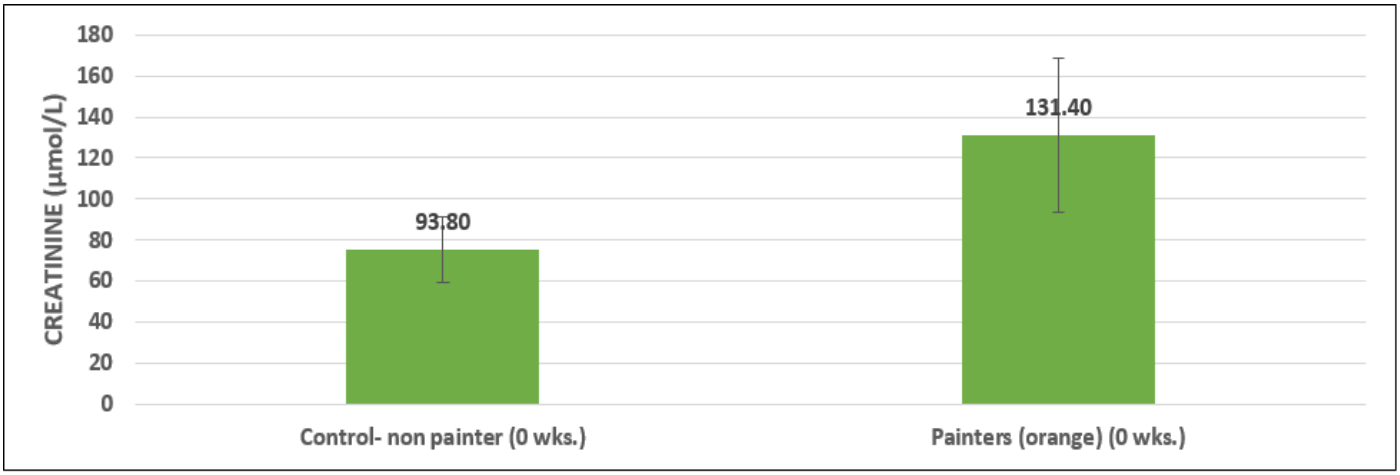

Fig 1b: Levels of serum creatinine of automobile painters (orange juice) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value < 0.001)

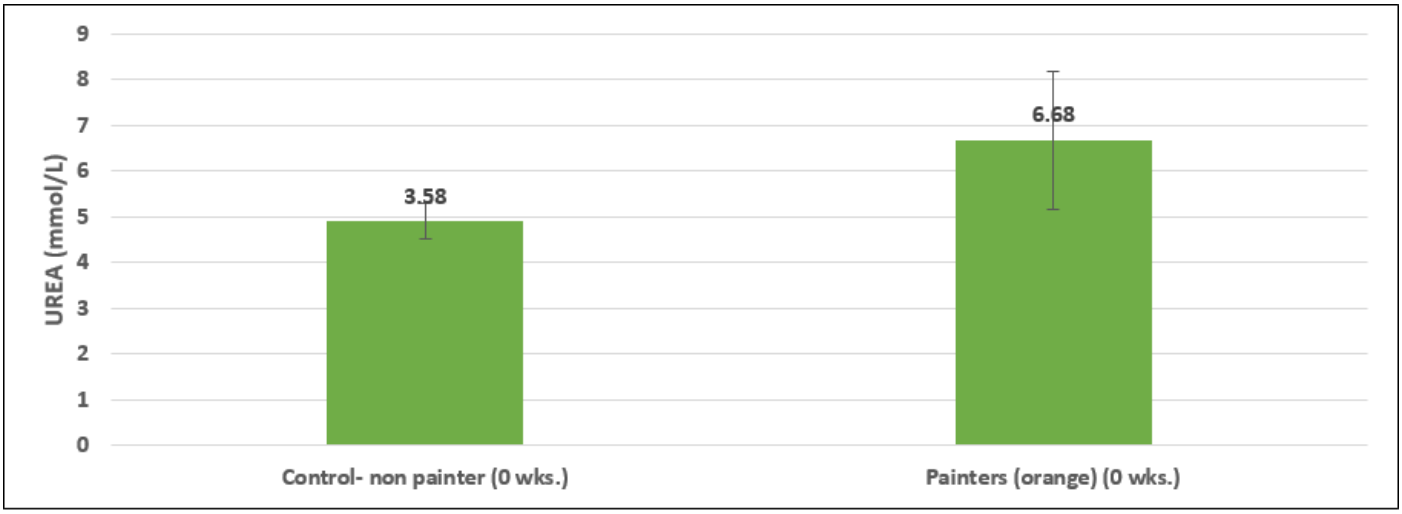

Fig 1c: Levels of serum levels of urea of automobile painters (orange juice) and control group at 0 week. Each value is an expression of mean ± SEM. (P-value < 0.001)

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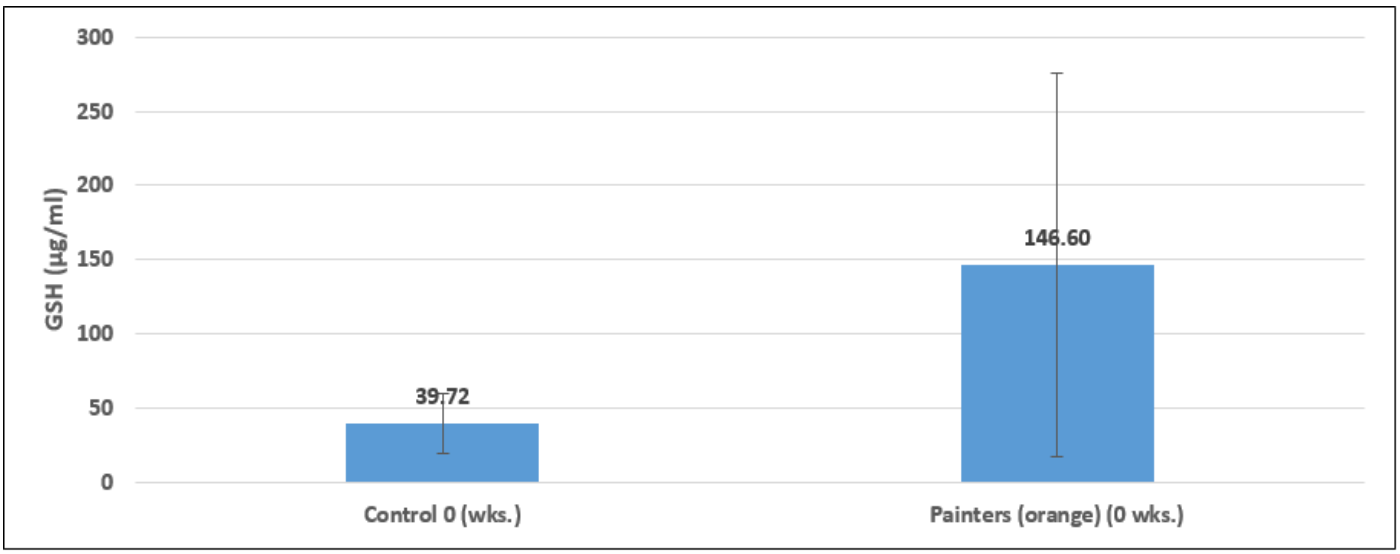

Fig 2a: Levels of urine reduced glutathione (GSH) of automobile painters (orange juice) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value = 0.019)

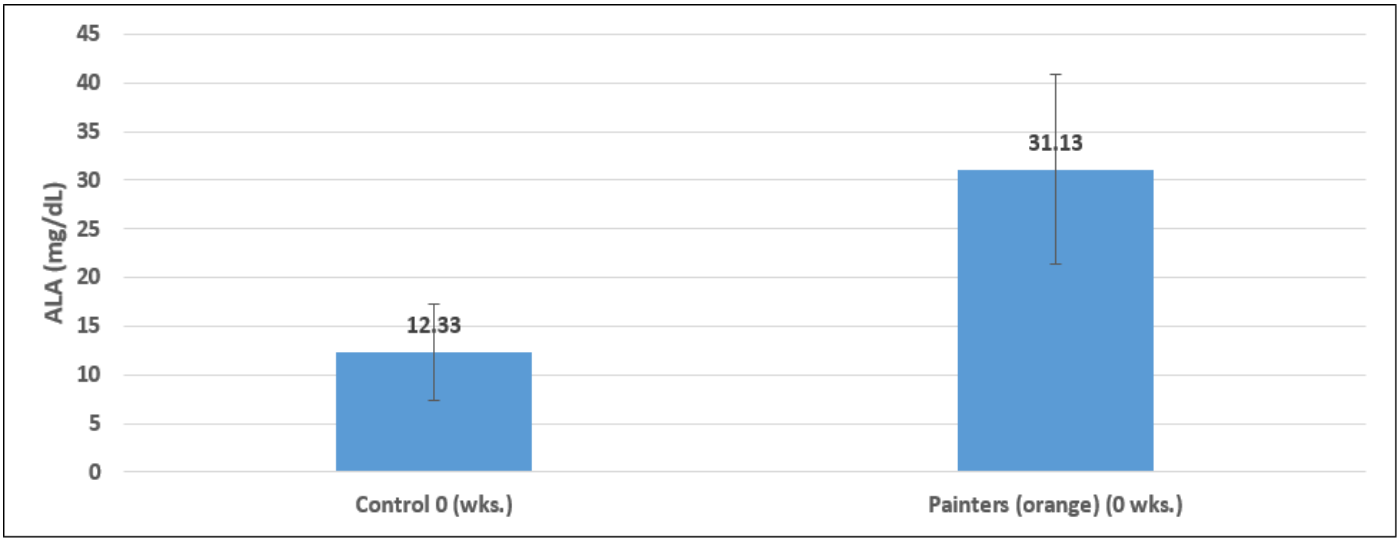

Fig 2b: Levels of urine aminolevulinic acid (ALA) of automobile painters (orange juice) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value < 0.001)

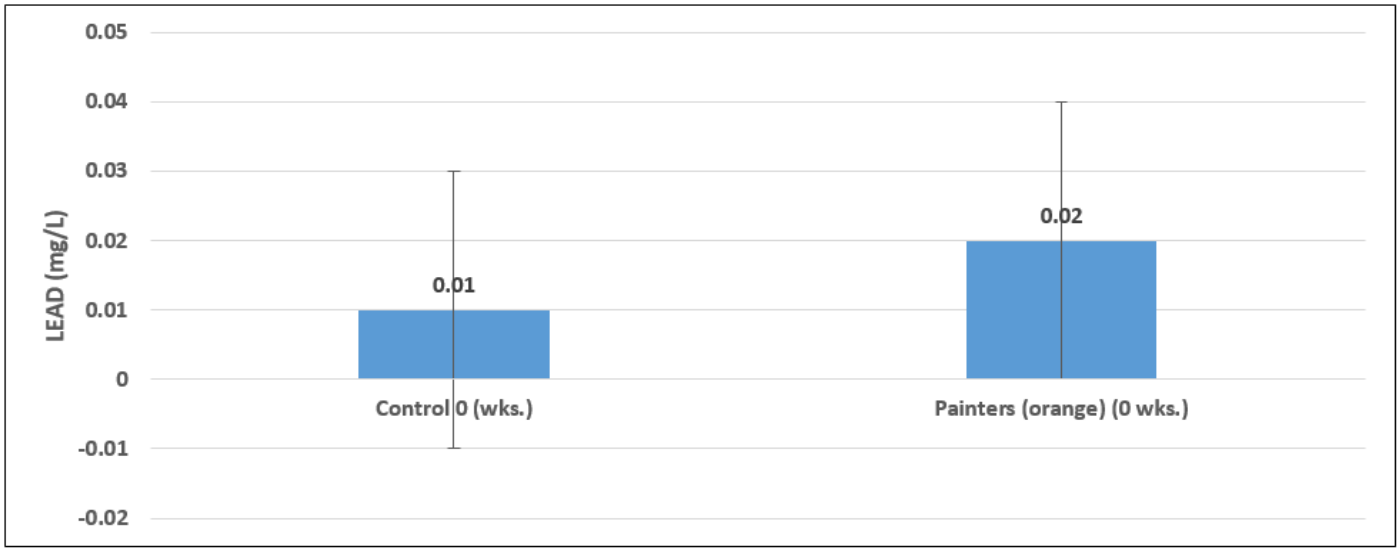

Fig 2c: Levels of urine lead of automobile painters (orange juice) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value > 0.05)

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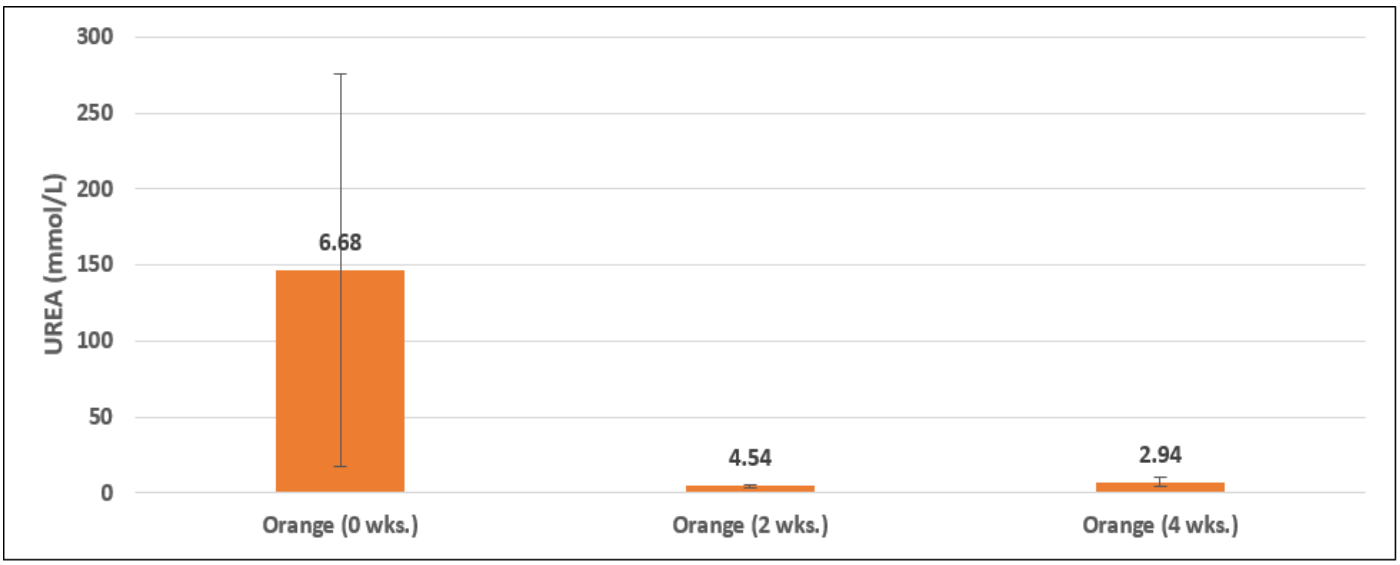

Fig 3a: Levels of serum urea in automobile painters (orange juice) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value <0.001)

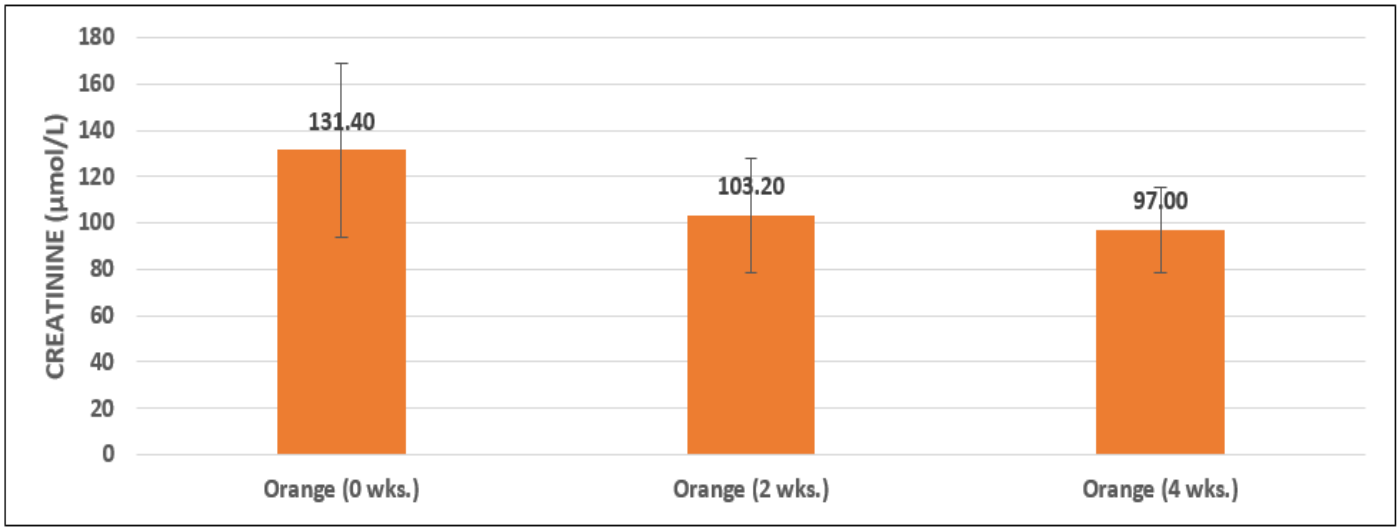

Fig 3b: Levels of serum creatinine in automobile painters (orange juice) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value = 0.025).

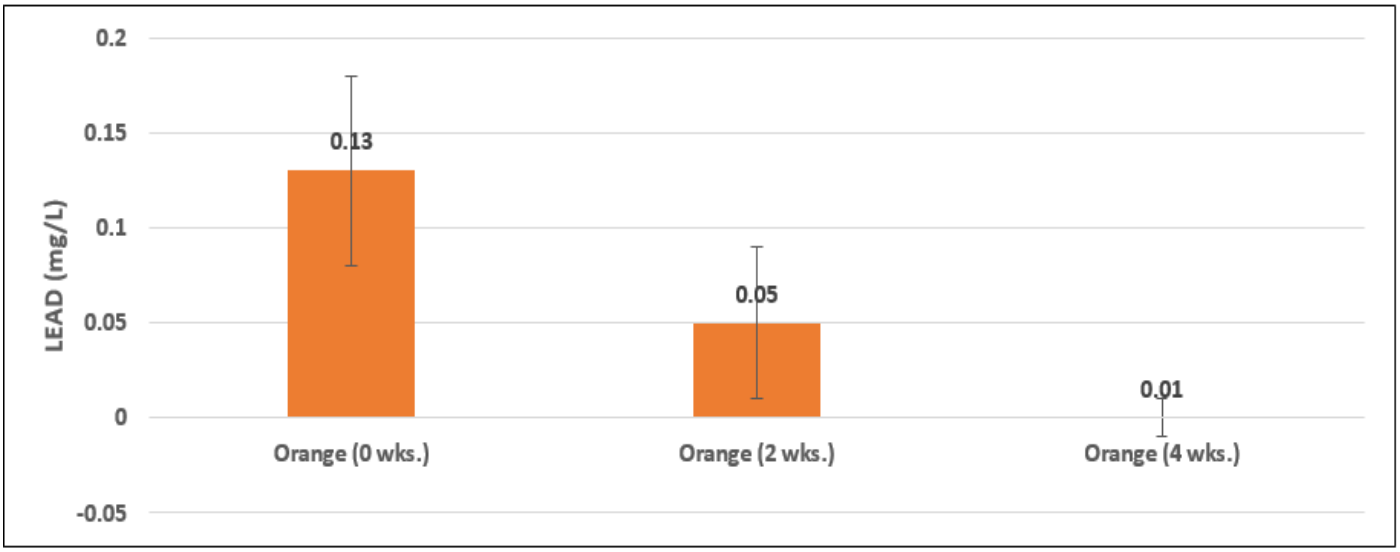

Fig 3c: levels of serum lead in automobile painters (orange juice) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value < 0.001)

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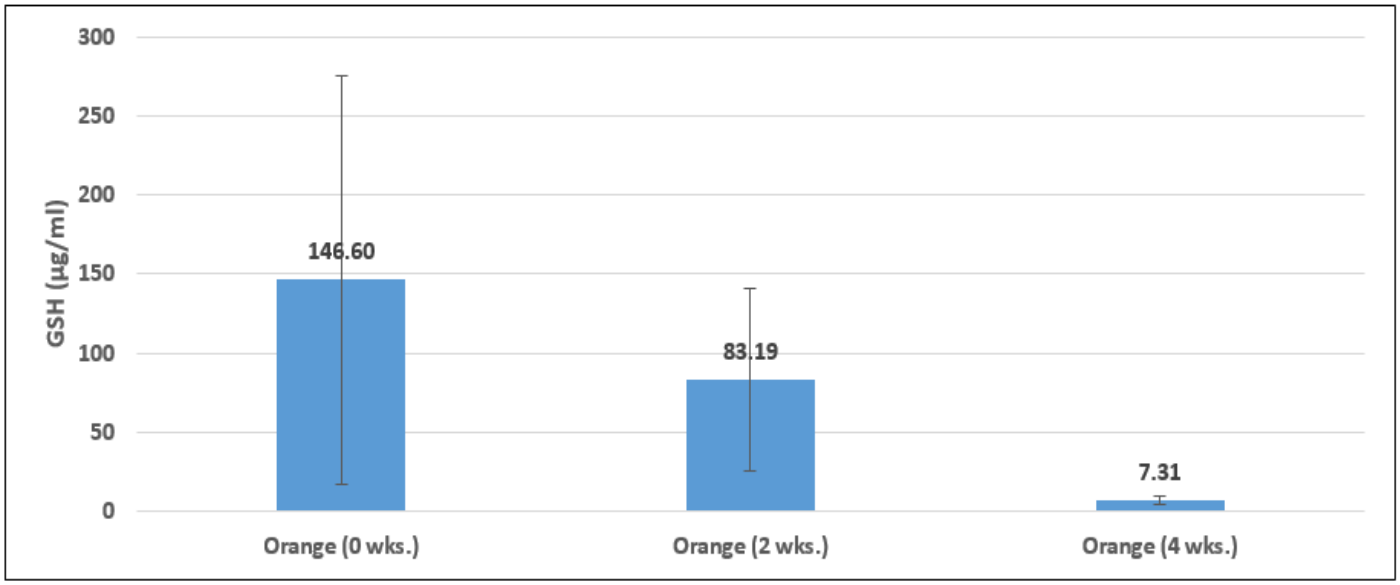

Fig 4a: Levels of urine reduced glutathione (GSH) of automobile painters (orange juice) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value = 0.003).

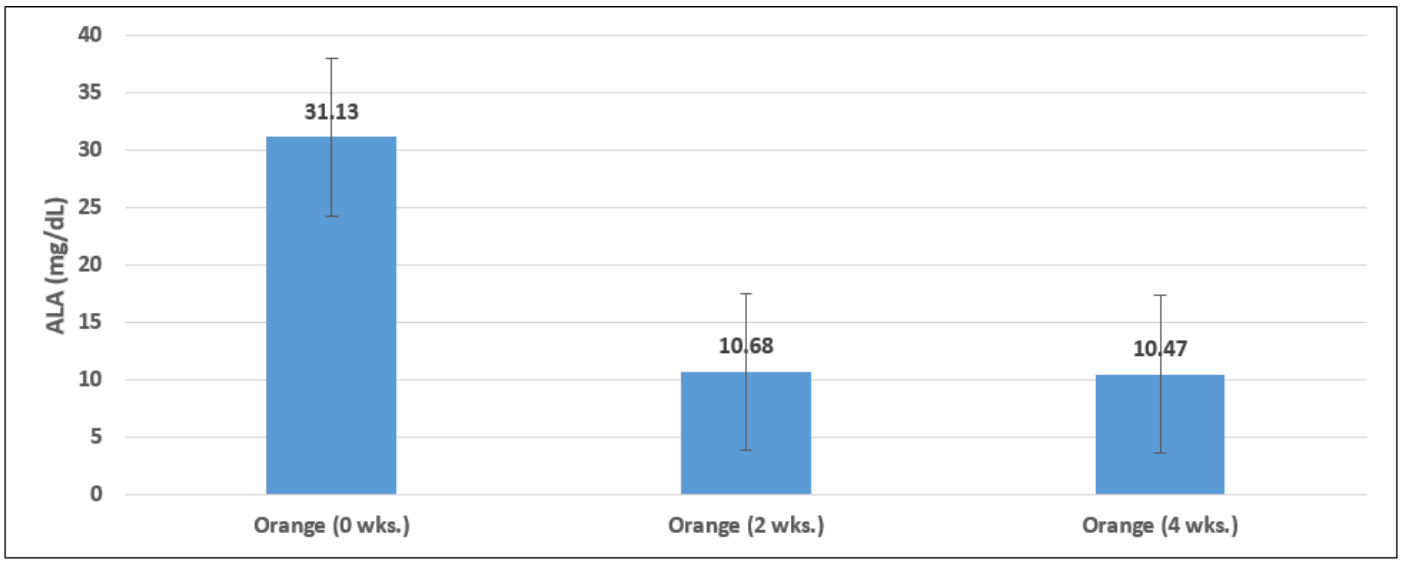

Fig 4b: Levels of urine aminolevulinic acid (ALA) of automobile painters (orange juice) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value < 0.001).

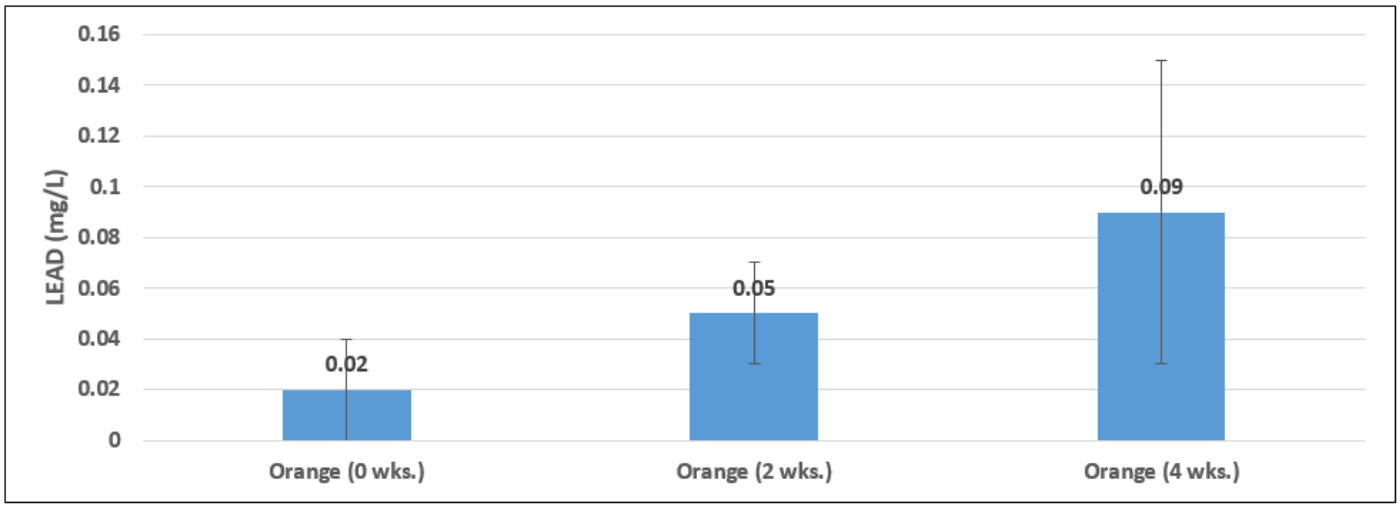

Fig 4c: Levels of urine lead of automobile painters (orange juice) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value = 0.001).

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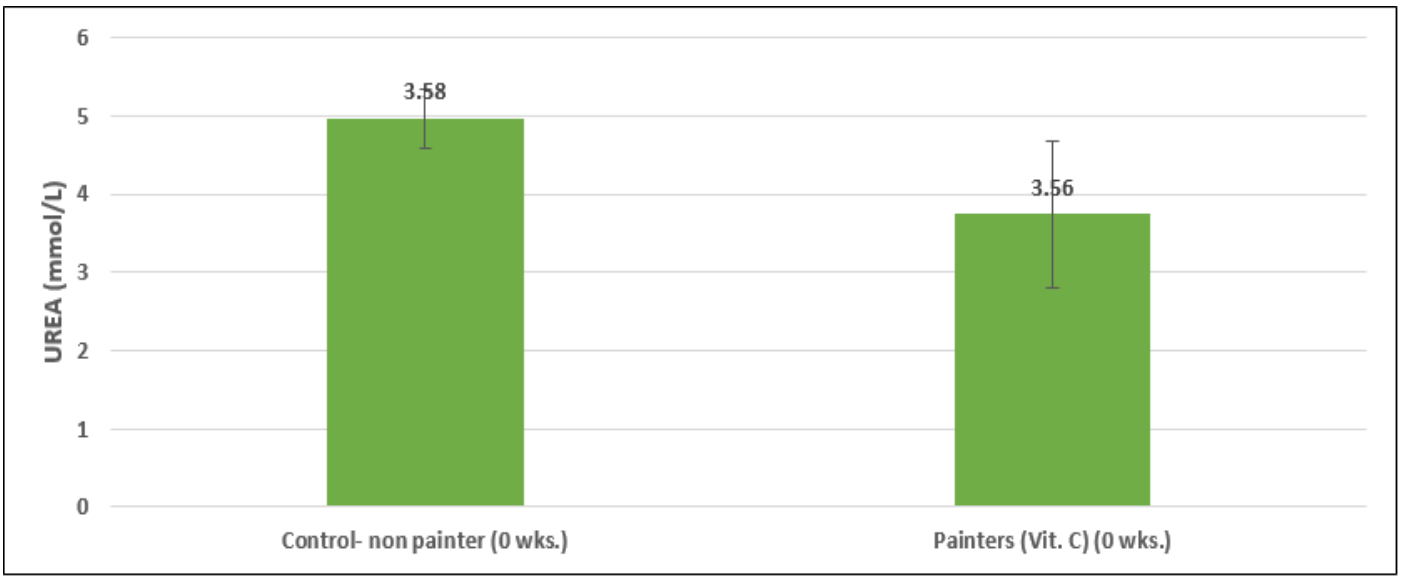

Fig 5a: Levels of serum urea of automobile painters (synthetic vitamin C) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value > 0.05).

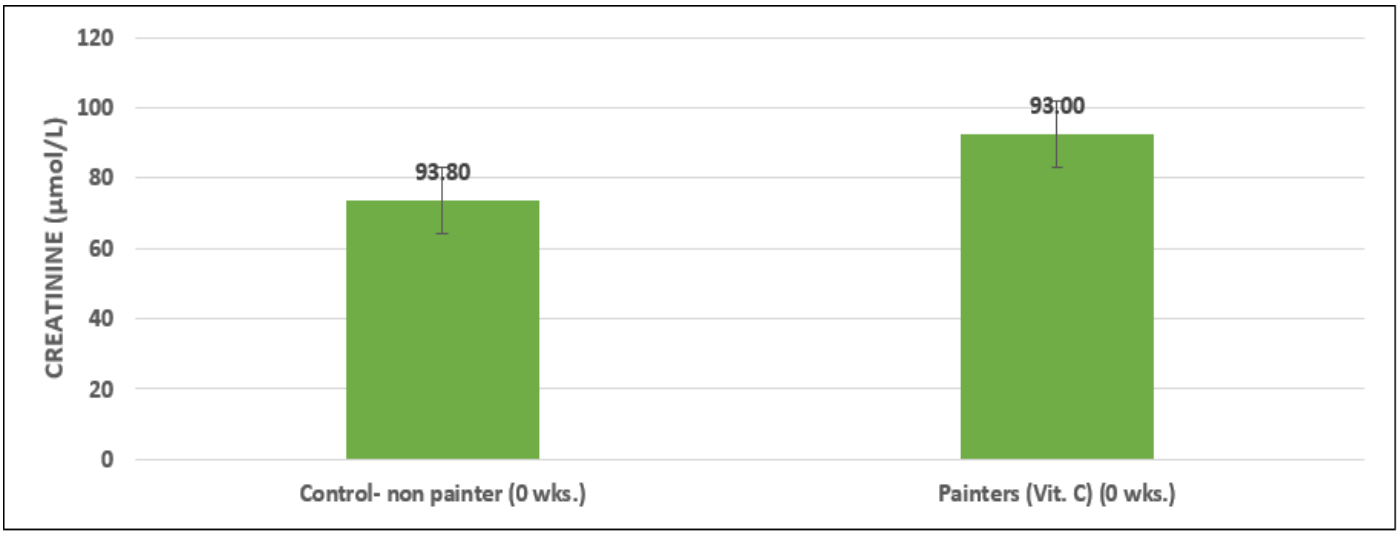

Fig 5b: Levels of serum creatinine of automobile painters (synthetic vitamin C) compared to control group at 0 week . Each value is an expression of mean ± SEM. (P-value > 0.05).

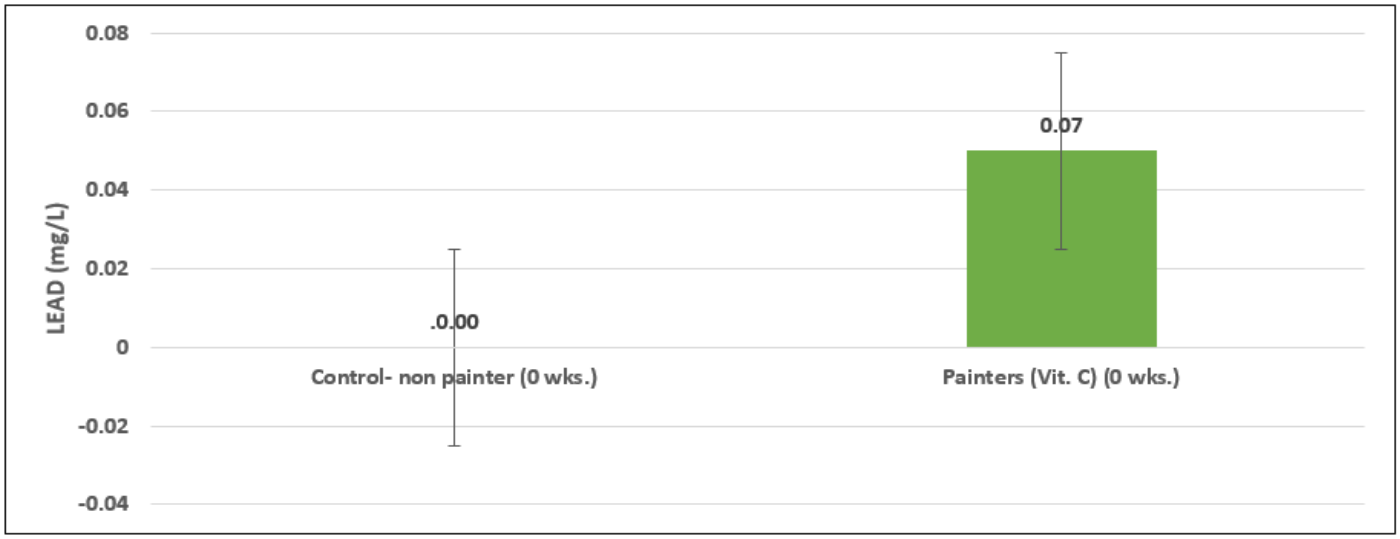

Fig 5c: Levels of serum lead of automobile painters (synthetic vitamin C) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value = 0.044)

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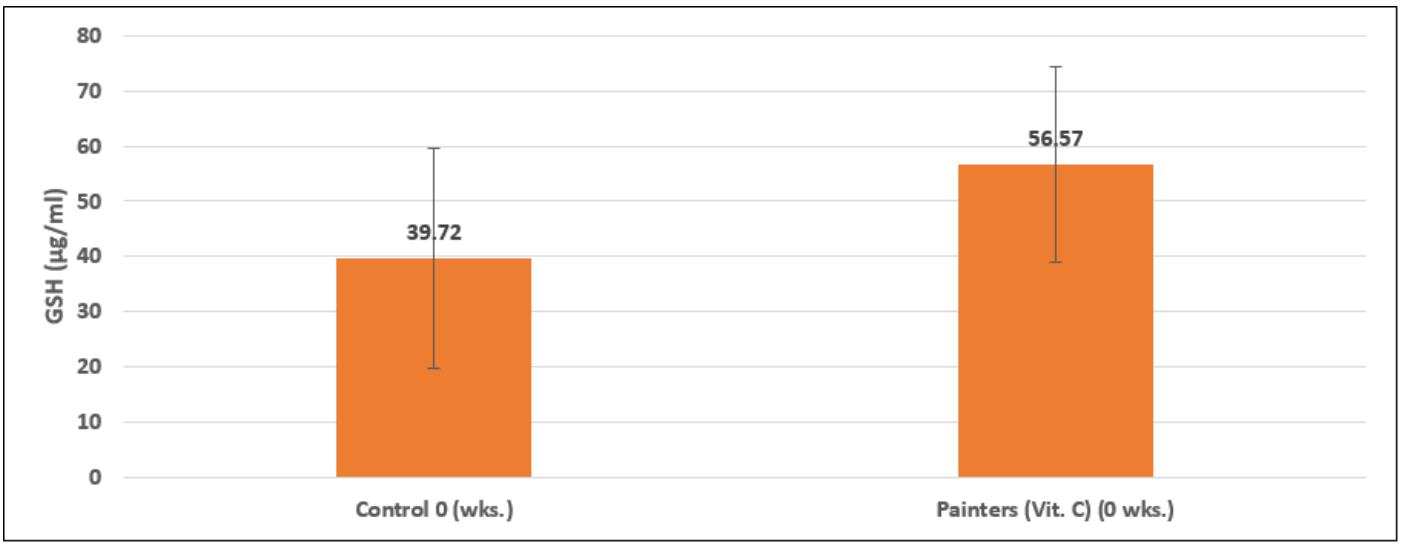

Fig 6a: Levels of urine reduced glutathione of automobile painters (synthetic vitamin C) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value > 0.05).

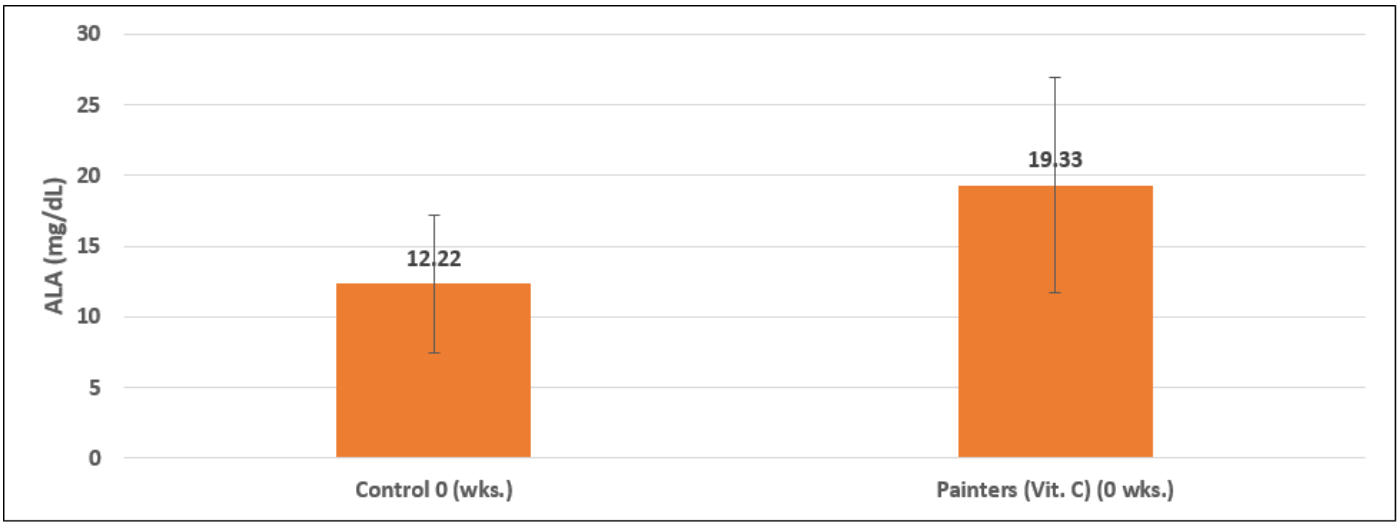

Fig 6b: Levels of urine aminolevulinic acid (ALA) of automobile painters (synthetic vitamin C) compared to control group at 0 week. Each value is an expression of mean ± SEM. (P-value > 0.05).

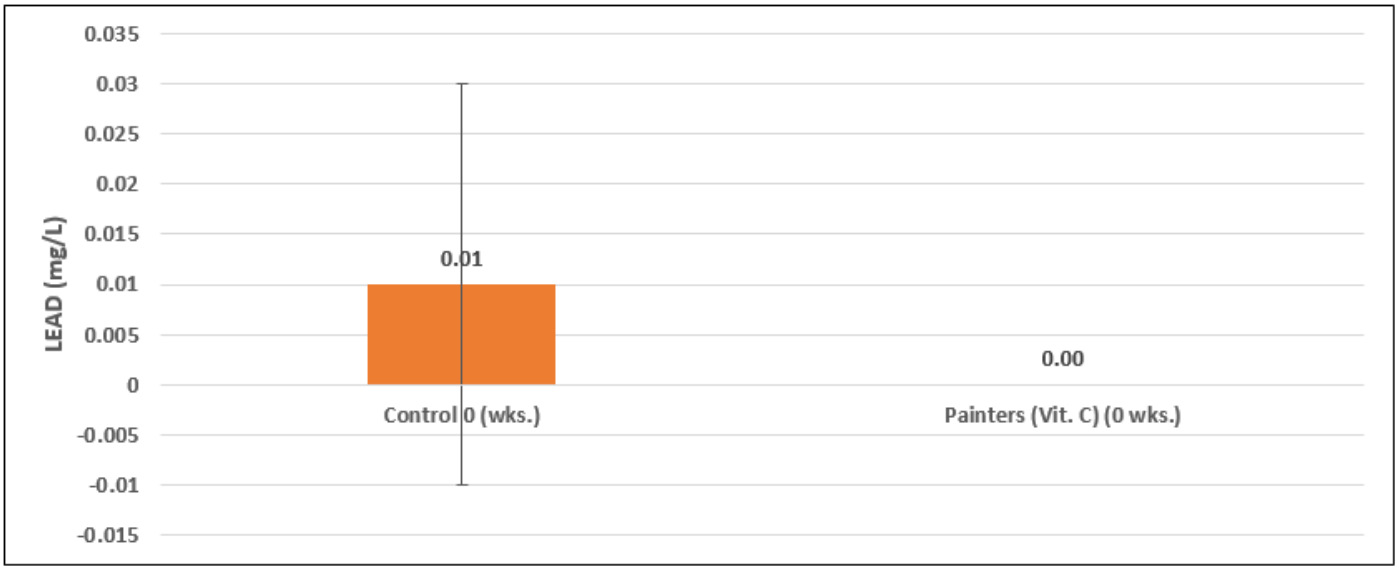

Fig 6c: Levels of urine lead of automobile painters (synthetic vitamin C) and control group at 0 week. Each value is an expression of mean ± SEM. (P-value > 0.05)

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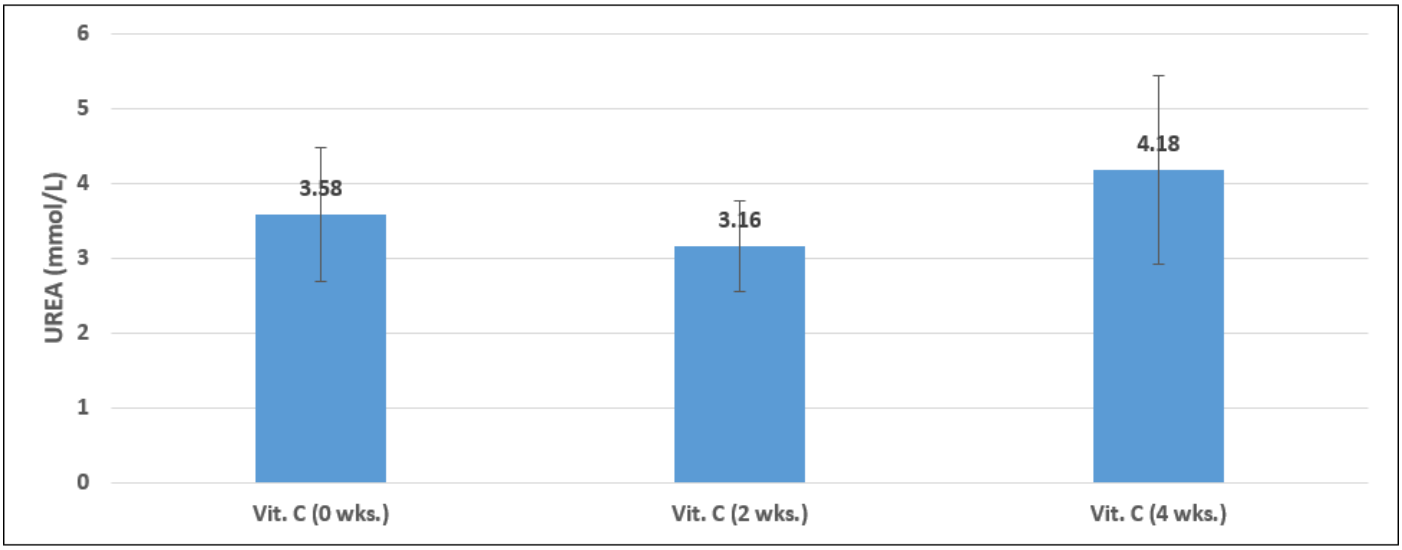

Fig 7a: Levels of serum urea in automobile painters (synthetic vitamin C) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value >0.05).

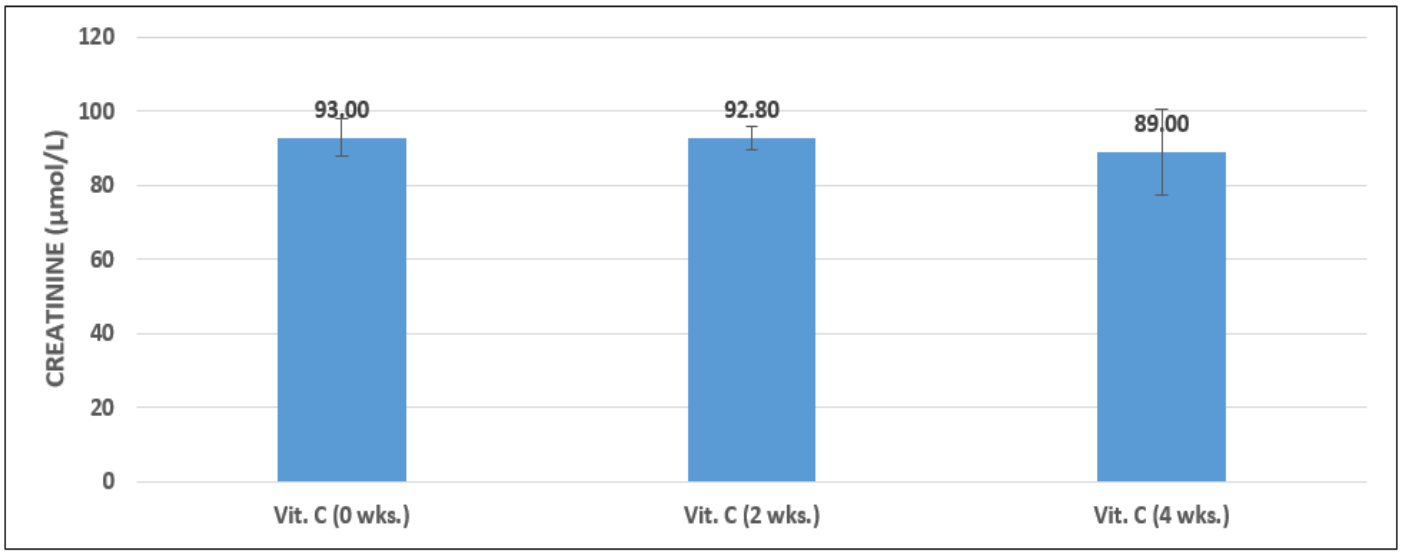

Fig 7b: Levels of serum creatinine in automobile painters (synthetic vitamin C) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value >0.05).

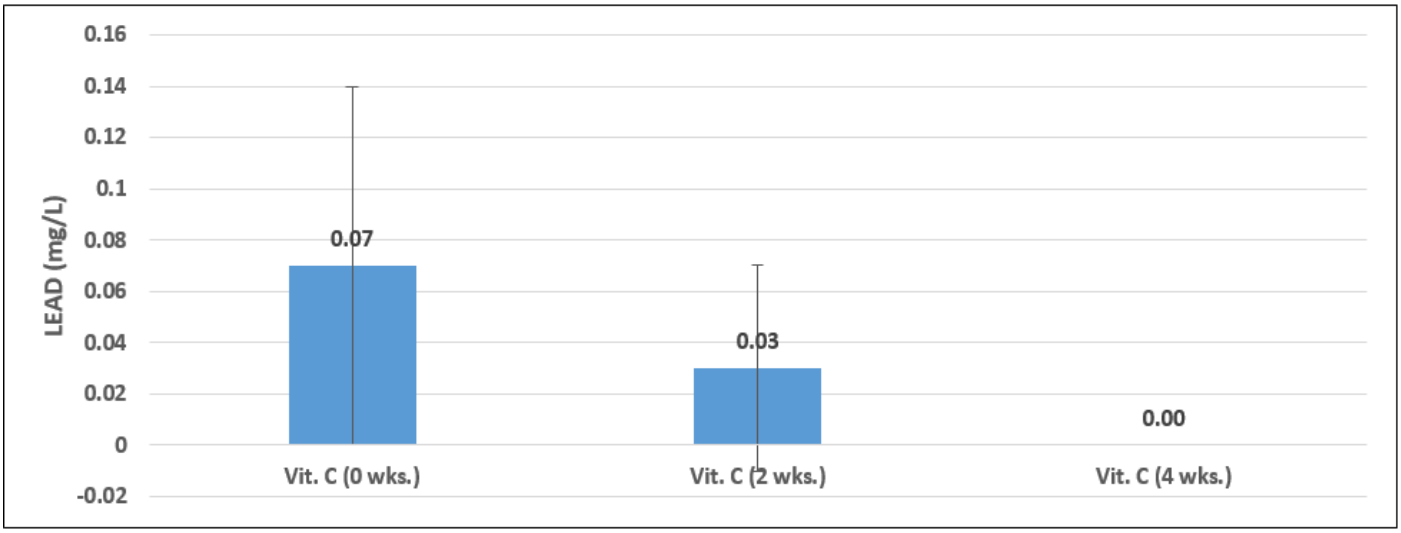

Fig 7c: Levels of serum lead in automobile painters (synthetic vitamin C) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value = 0.010).

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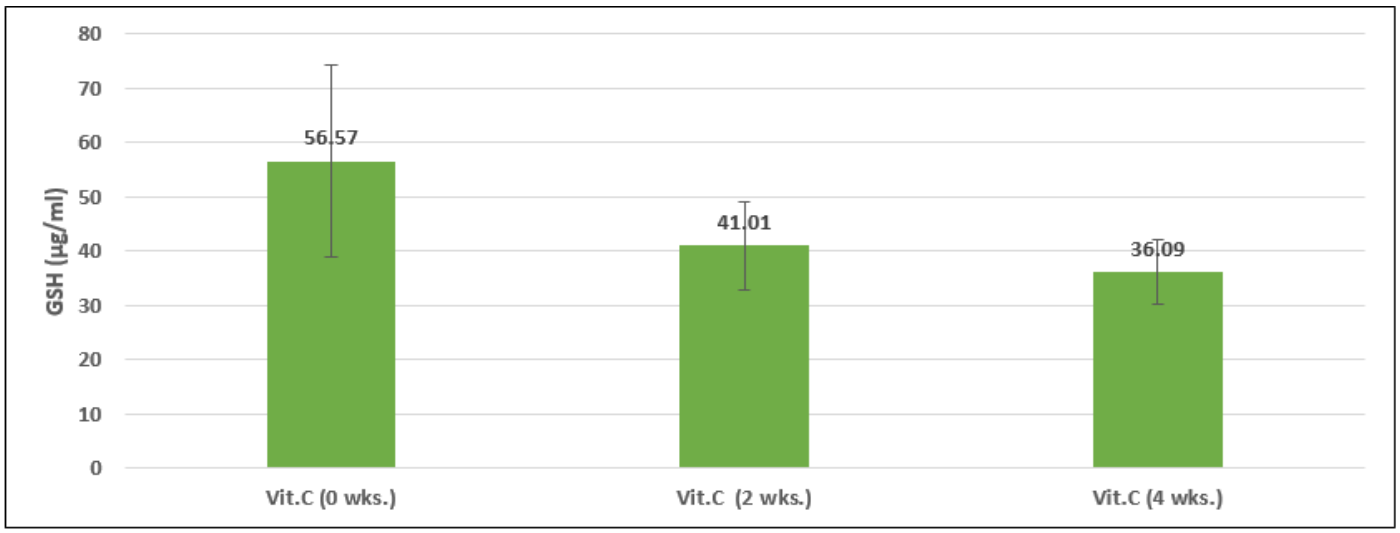

Fig 8a: Levels of urine reduced glutathione (GSH) of automobile painters (synthetic vitamin C) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value = 0.002).

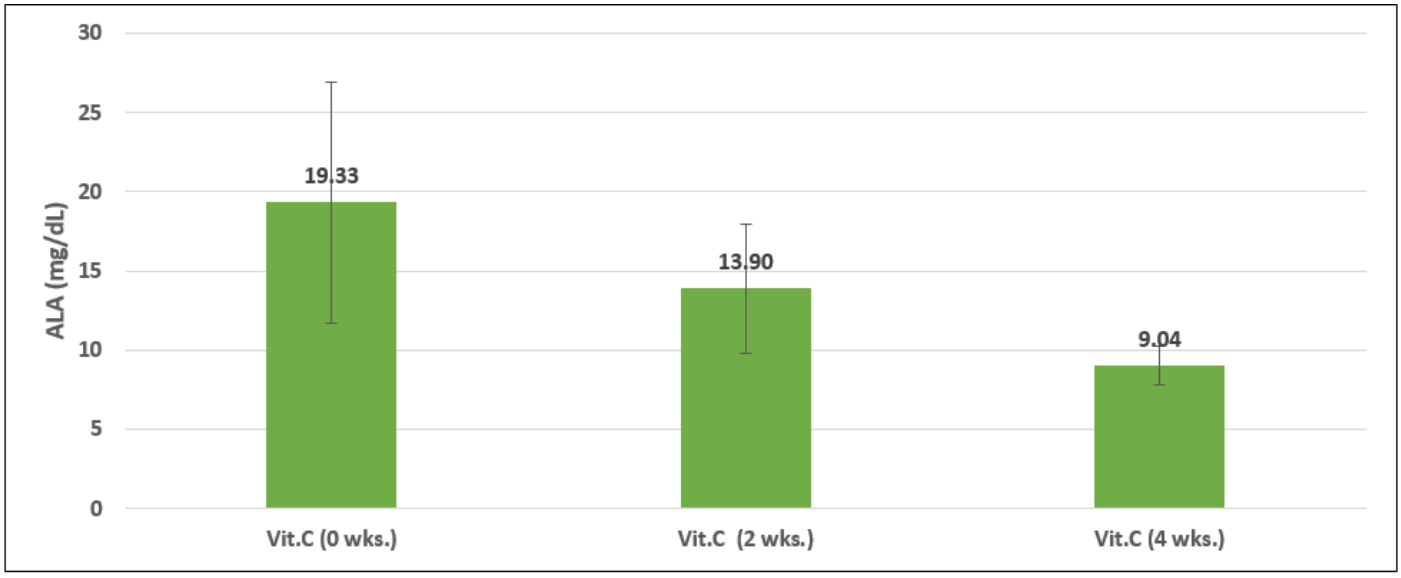

Fig 8b: Levels of urine aminolevulinic acid (ALA) of automobile painters (synthetic vitamin C) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value < 0.001).

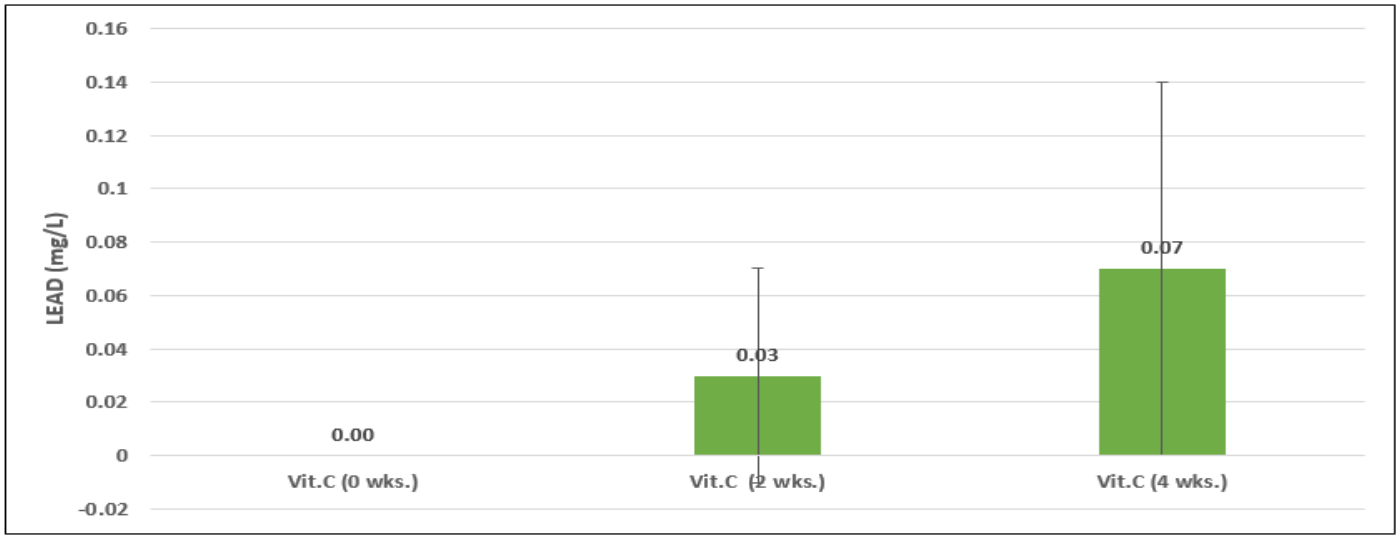

Fig 8c: Levels of urine lead of automobile painters (synthetic vitamin C) at 0, 2 and 4 weeks. Each value is an expression of mean ± SEM. (P-value = 0.013).

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Table 2: Correlation of Parameters of Control Baseline at 0 Week

Correlation @0 week	Serum Pb (mg/L)	Serum Ur (mmol/L)	Serum Cr (µmol/L)	Urine ALA (mg/24hrs)	Urine GSH(µg/mL)	Urine Pb(µg/dL)
Serum Pb						
r	1	-	-	-	-	-
P-value		-	-	-	-	-
Serum Ur						
r	-	1	-0.361	0.898	-0.421	0.018
P-value	-		0.305 (NS)	0.000 (S)	0.226 (NS)	0.961 (NS)
Serum Cr.						
r	-	-0.361	1	-0.431	0.648	-0.584
P-value	-	0.305 (NS)		0.214 (NS)	0.043 (S)	0.077 (NS)
Urine ALA						
r	-	0.898	-0.431	1	-0.328	-0.395
P-value	-	0.000 (S)	0.214 (NS)		0.354 (NS)	0.258 (NS)
Urine GSH						
r	-	-0.421	0.648	-0.328	1	-0.351
P-value	-	0.226 (NS)	0.043 (S)	0.354 (NS)		0.319 (NS)
Urine Pb						
r	-	0.018	-0.584	-0.395	-0.351	1
P-value	-	0.961 (NS)	0.077 (NS)	0.258 (NS)	0.319 (NS)	

r* correlation significant at the 0.05 level Pb- lead Cr-creatinine Ur- urea
GSH- reduced glutathione ALA- aminolevulinic acid

Table 2 showed the correlation of parameters of control baseline of non-painters at 0 week on serum level of (lead, creatinine and urea) and urine (lead, reduced glutathione and aminolevulinic acid) with respective values of their correlation and p-value. There were significant positive correlations on urine aminolevulinic acid and serum urea (r = 0.898, p = 0.000) and positive correlations on urine reduced glutathione and serum creatinine (r = 0.648, p = 0.043).

Table 3: Correlation of parameters on orange juice group at 2 week

Correlation @2 week orange	Serum Pb (mg/L)	Serum Ur (mmol/L)	Serum Cr (umol/L)	Urine ALA (mg/24hrs)	Urine GSH(µg/mL)	Urine Pb(µg/dL)
Serum Pb						
r	1	-0.312	0.691	0.703	0.913	0.636
P-value		0.380 (NS)	0.027 (S)	0.023 (S)	0.000 (S)	0.048 (S)
Serum Ur						
r	-0.312	1	-0.395	-0.406	-0.648	-0.403
P-value	0.380 (NS)		0.256 (NS)	0.245 (NS)	0.043 (S)	0.248 (NS)
Serum Cr.						
r	0.691	-0.395	1	0.999	0.796	0.996
P-value	0.027 (S)	0.256 (NS)		0.000 (S)	0.006 (S)	0.000 (S)
Urine ALA						
r	0.703	-0.406	0.999	1	0.806	0.991
P-value	0.023 (S)	0.245 (NS)	0.000 (S)		0.005 (S)	0.000 (S)
Urine GSH						
r	0.913	-0.648	0.796	0.806	1	0.765
P-value	0.000 (S)	0.043 (S)	0.006 (S)	0.005 (S)		0.010 (S)
Urine Pb						
r	0.636	-0.403	0.996	0.991	0.765	1
P-value	0.048(S)	0.248 (NS)	0.000 (S)	0.000 (S)	0.010 (S)	

r* correlation significant at the 0.05 level Pb- lead Cr- creatinine Ur- urea
GSH- reduced glutathione ALA- aminolevulinic acid

Table 3 showed the correlation of parameters of orange juice group at 2 week treatment on serum level of (lead, creatinine and urea) and urine (lead, reduced glutathione and aminolevulinic acid) with respective values of their correlation and p-value. There were significant positive correlations on the following: serum lead versus creatinine (r = 0.691, p = 0.027), ALA (r = 0.903, p = 0.023) and urine GSH (r = 0.913, p = 0.000), creatinine versus urine ALA (r = 0.999, p = 0.000) and urine GSH (r = 0.796, p = 0.006), urine ALA versus urine GSH (r= 0.806, p=0.005).

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Table 4: Correlation of Parameters on Orange Juice Group at 4 Week

Correlation @4 week orange	Serum Pb (mg/L)	Serum Ur (mmol/L)	Serum Cr (µmol/L)	Urine ALA (mg/24hrs)	Urine GSH(µg/mL)	Urine Pb(µg/dL)
Serum Pb						
r	1	0.061	-0.206	-0.941	0.061	0.057
P-value		0.899 (NS)	0.567 (NS)	0.000 (S)	0.866 (NS)	0.875 (NS)
Serum Ur						
r	0.061	1	0.128	0.065	1.000	0.110
P-value	0.899 (NS)		0.724 (NS)	0.858 (NS)	0.000 (S)	0.763 (NS)
Serum Cr.						
r	-0.206	0.128	1	0.511	0.128	0.964
P-value	0.567 (NS)	0.724 (NS)		0.131 (NS)	0.724 (NS)	0.000 (S)
Urine ALA						
r	-0.941	0.065	0.511	1	0.065	0.267
P-value	0.000 (S)	0.858 (NS)	0.131 (NS)		0.858 (NS)	0.455 (NS)
Urine GSH						
r	0.061	1.000	0.128	0.065	1	0.110
P-value	0.866 (NS)	0.000 (S)	0.724 (NS)	0.858 (NS)		0.763 (NS)
Urine Pb						
r	0.057	0.110	0.964	0.267	0.110	1
P-value	0.875 (NS)	0.763 (NS)	0.000 (S)	0.455 (NS)	0.763 (NS)	

r* correlation significant at the 0.05 level Pb- lead Cr- creatinine Ur- urea
GSH- reduced glutathione ALA- aminolevulinic acid

Table 4 showed the correlation of parameters of orange juice group at 4 week on serum level of (lead, creatinine and urea) and urine (lead, reduced glutathione and aminolevulinic acid) with respective values of their correlation and p-value. There was significant correlations on urea versus urine GSH (r = 1.000, p = 0.000).

Table 5: Correlation of Parameters on Synthetic Vitamin C Group at 2 Week

Correlation @2 week Vitamin C	Serum Pb (mg/L)	Serum Ur (mmol/L)	Serum Cr (µmol/L)	Urine ALA (mg/24hrs)	Urine GSH(µg/mL)	Urine Pb(µg/dL)
Serum Pb						
r	1	0.680	-0.850	-0.154	-0.298	1.000
P-value		0.031 (S)	0.002(S)	0.671 (NS)	0.403 (NS)	0.000 (S)
Serum Ur						
r	0.680	1	-0.539	0.009	0.397	0.672
P-value	0.031 (S)		0.108 (NS)	0.980 (NS)	0.255 (NS)	0.033 (S)
Serum Cr.						
r	-0.850	-0.539	1	-0.339	0.550	-0.857
P-value	0.002(S)	0.108 (NS)		0.338 (NS)	0.100 (NS)	0.002 (S)
Urine ALA						
r	-0.154	0.009	-0.339	1	-0.234	-0.143
P-value	0.671 (NS)	0.980 (NS)	0.338 (NS)		0.514 (NS)	0.694 (NS)
Urine GSH						
r	-0.298	0.397	0.550	-0.234	1	-0.313
P-value	0.403 (NS)	0.255 (NS)	0.100 (NS)	0.514 (NS)		0.378 (NS)
Urine Pb						
r	1.000	0.672	-0.857	-0.143	-0.313	1
P-value	0.000 (S)	0.033 (S)	0.002 (S)	0.694(NS)	0.378 (NS)	

r* correlation significant at the 0.05 level
Pb- lead Cr- creatinine Ur- urea GSH- reduced glutathione ALA- aminolevulinic acid

Table 5 showed the correlation of parameters of synthetic vitamin c group at 2 week on serum level of (lead, creatinine and urea) and urine (lead, reduced glutathione and aminolevulinic acid) with respective values of their correlation and p-value. There were significant correlations on the following: serum lead versus urea (r = 0.680, p = 0.031), creatinine versus urine lead (r= -0.857. p= 0.002).

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Table 6: Correlation of Parameters on Synthetic Vitamin C Group at 4 Week

Correlation @4 week Vitamin C	Serum Pb (mg/L)	Serum Ur (mmol/L)	Serum Cr (µmol/L)	Urine ALA (mg/24hrs)	Urine GSH(µg/mL)	Urine Pb(µg/dL)
Serum Pb						
r	1	-0.285	-0.968	-0.173	0.350	-0.434
P-value		0.425 (NS)	0.000 (S)	0.632 (NS)	0.321 (NS)	0.210 (NS)
Serum Ur						
r	-0.285	1	0.374	-0.605	0.780	-0.487
P-value	0.425 (NS)		0.287 (NS)	0.064 (NS)	0.008 (S)	0.153 (NS)
Serum Cr.						
r	-0.968	0.374	1	-0.054	-0.261	0.492
P-value	0.000 (S)	0.287 (NS)		0.881 (NS)	0.466 (NS)	0.149 (NS)
Urine ALA						
r	-0.173	-0.605	-0.054	1	-0.599	0.158
P-value	0.632 (NS)	0.064 (NS)	0.881 (NS)		0.067 (NS)	0.662 (NS)
Urine GSH						
r	0.350	0.780	-0.261	-0.599	1	-0.743
P-value	0.321 (NS)	0.008 (S)	0.466 (NS)	0.067 (NS)		0.014 (S)
Urine Pb						
r	-0.434	-0.487	0.492	0.158	-0.743	1
P-value	0.210 (NS)	0.153 (NS)	0.149 (NS)	0.662 (NS)	0.014 (S)	

r* correlation significant at the 0.05 level Pb- lead Cr- creatinine Ur- urea
GSH- reduced glutathione ALA- aminolevulinic acid

Table 6 showed the correlation of parameters of synthetic vitamin c group at 4 week on serum level of (lead, creatinine and urea) and urine (lead, reduced glutathione and aminolevulinic acid) with respective values of their correlation and p-value. There were significant correlations on the following: urine GSH versus urea ($r = 0.780$, $p = 0.008$) and urine lead ($r = -0.743$, $p = 0.014$).

Table 7: Absolute and Relative Frequency of Socio-Demographic Characteristics of Nigerian Painters in Ile-Ife

Socio-demography	Count (%) Orange group	Count (%) Synthetic vitamin C group
Age		
18-30	6 (20%)	5 (17%)
30-60	20 (67%)	22 (73%)
>60	4 (13%)	3 (10%)
Highest Educational status/attainment		
None	- (0%)	- (0%)
Primary	- (0%)	1(3%)
Secondary	25 (83%)	27 (90%)
Tertiary	5 (17%)	2 (7%)
Occupational status/work pattern		
Full-time	30 (100%)	30 (100%)
Part-time	-	-
Marital status		
Single	3 (10%)	2 (7%)
Married	20 (67%)	25 (83%)
Divorced	4 (!3%)	3 (10%)
Widowed	3 (10%)	0
Training type during apprenticeship		
None	-	-
Informal setting	24 (80%)	27 (90%)
Formal setting e.g. Technical school	6 (20%)	3 (10%)
Years of experience as painter		
10-15	2 (7%)	3 (10%)
16-20	16 (53%)	18 (60%)
25-30	10 (33%)	4 (13%)
>30	2 (7%)	5 (17%)
Average working hours per week		
< 8 hours	-	-
9-16 hours	-	-
17-24 hours	3 (10%)	2 (7%)
25-32 hours	3 (10%)	4 (13%)
33-40 hours	24 (80%)	24 (80%)

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Table 8: Absolute and Relative Frequency about Knowledge on Safety and Occupational Hazards of Nigerian Painters in Ile-Ife

Information on safety and job hazards	Count (%) Orange group	Count (%) Synthetic vitamin C group
Knowledge on Pb exposure from painting as occupation		
Yes	3 (10%)	5 (17%)
No	27 (90%)	25 (83%)
Received any training on safety and hazard prevention		
Yes	2	3 (10%)
No	28 (0%)	27 (90%)
Work in a well aerated environment		
Yes	30 (100%)	30 (100%)
No	-	-
Manifestation of any health risk associated with the job		
Yes	24 (80%)	26 ((87%)
No	6 (20%)	4 (13%)
Type of ailments/clinical conditions manifested		
Headache	7 (23%)	5 (17%)
Fever	8 (27%)	5 (17%)
Vomiting	-	-
Hypertension	12 (40%)	14 (47%)
Diabetes Mellitus	-	-
Stroke	-	-
Respiratory problem	3 (10%)	6 (19%)
Others:	-	-
Use personal protective equipment		
Yes	30 (100%)	30 (100%)
No	-	
The type of personal protective equipment used frequently		
Overall	20 (66%)	14 (47%)
Goggles	-	1 (3%)
Boots	-	-
Nose mask	5 (17%)	8 (27%)
Gloves	5 (17%)	7 (23%)
The type of personal protective equipment used occasionally		
Overall	-	-
Goggles	18 (60%)	14 (47%)
Boots	12 (40%)	16 (53%)
Nose mask	-	-
Gloves	-	
Type of paints commonly used		
Acrylic	-	-
Urathane	-	-
Metalic	-	-
Vinyl	-	-
Others Autobase	30 (100%)	30 (100%)

IV. DISCUSSION

This study revealed few phytochemical screening on freshly squeezed orange juice: alkaloid (3.12 mg/100g), flavonoids (0.73 mg/100g), tannins (0.61 mg/100g), phenol (0.43 mg/100g), saponin (0.20 mg/100g), proximate contents revealed moisture content of 87.84%, carbohydrate 7.05%, fiber 2.07%, protein 1.49%, ash 1.40% and fat 0.15%. Generally, the juices were rich in carbohydrate and moisture, but low in protein, fibre, fat and ash and this was in agreement to finding of Tiencheu *et al.* [29]. Vitamin c was found to be 46 mg/100g, mineral contents showed no detection of phosphorus , potassium found to be high as 2,044 mg/L while low sodium content of 355 mg/L, Anti-

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nutrients such as oxalate (4.50 mg/g), phytate (20.00 mg/g) and cyanide (1.02 mg/kg) were detected in this study (Table1).

In this study, the baseline results showed significant higher concentrations of serum Pb, creatinine and urea at 0 week especially among automobile painters in orange juice group compared to control of non-painters. The presence of Pb in painters might be as a result of their occupational activities related to exposure according to Khoshakhlagh *et al.* [30]. Pb deposition in tissue/organs such as kidney is commonly reported and supports renal alterations in painters. The alteration noticed in serum creatinine and urea as renal biomarkers used in this study might be as a result of impaired renal status of individual. That resulted from Pb-induced excessive free radical generation and oxidative renal damage [7]. This is similar to a study that an acute lead exposure induced renal damage which can occur in the absence of acute intoxication, even the occult lead nephropathy may not be recognized [31]. The result showed significant higher levels of urine ALA and GSH of automobile painters to be placed on orange juice compared to non-painter control subjects. Blood lead levels and inhibition of delta-aminolevulinic acid dehydratase (ALAD) activity are considered biomarkers of lead exposure and lead toxicity respectively [32,33]. ALAD, an octameric zinc-containing enzyme, catalyzes the condensation of two molecules of 5-aminolevulinic acid (ALA) into one molecule of monopyrrole porphobilinogen (PBG). Inhibition of ALAD activity produces increased urinary excretion of ALA. This result agreed to a finding that urinary ALA increases as delta-aminolevulinic acid dehydratase is inhibited by toxic level of plasma Pb [33]. Inhibition of ALAD resulting in the accumulation of ALA, is even detectable in the plasma and urine, at blood levels of less than 10 µg/L [34]. The urine lead in orange juice supplementation at 0 week showed non-significant higher Pb level compared to control subject of non-painters signifying that part of the Pb absorbed was filtered by the glomerulus. This result was similar to a significant report by Awodele *et al.* [35] that mean Pb urine concentration in urine samples from paint factory workers was higher and twice that found in non-factory workers.

The result showed a significant reduction in serum concentration of Pb and urea at 2 weeks of taking oral orange juice compared to baseline. A better reduction in serum concentration of Pb, creatinine and urea at 4 weeks of taking oral orange juice compared to baseline. This also showed the binding capacity of orange juice in alleviating lead induced free radicals and detoxifying the renal markers for elimination via the kidney. Urea and creatinine are known to be waste product of amino acid metabolism which is removed by the kidney [36]. The result showed an improvement of renal function, as suggested by the reduction of renal biomarkers (urea and creatinine) at 2 weeks which was more pronounced at 4 weeks. Ascorbic acid lowered serum Pb level probably due to its role as a Pb chelator, and explains the improved renal function at 2 and 4 weeks of orange juice treatment [37]. Ascorbic acid through its antioxidant property has been reported to ameliorate the renal histopathological presentation by reversing Pb-induced oxidative damage [37]. The protective effect of natural vitamin C in orange juice on Pb-induced toxicity was displayed, as urine lead and ALA levels were significantly increased and lowered at 4 weeks respectively compared with 0 week, results that are in agreement with serum lead levels. As serum Pb decreased from 0 - 4 weeks, urinary lead level progressively increased, and decreased serum Pb also resulted in decreased effect of Pb on ALAD with resulting low urine ALA level. Also, significant reduced urine GSH in automobile painters at exactly 4 weeks agreed to a finding that intracellular GSH is involved in the defence against the Pb-induced toxicity by preventing loss of cell viability according to Perez *et al*. [38]. Both vitamin C and glutathione are inter-connected; vitamin C helps to reprocess glutathione by converting oxidized glutathione to its reduced form, thereby potentiating the antioxidant effects of each other. Studies have shown that edible parts of plants (e.g. citrus) and sometimes in inedible parts have phenolic compounds capable of exhibiting multiple biological effects [39]. Aside phenolics, studies such as those of Escobedo-Avellaneda *et al*. [40], Chanson-Rolle *et al.* [41], Obasi *et al.* [42], Bala and Bashar [43] established the nutritive content, photochemical and antioxidant values of orange juice. According to Nowak et al. [44], vitamin C-rich orange juice is a good source of polyphenols, and that the synergistic effects of polyphenols on vitamin C seem to define the antioxidant properties of orange juice. The results of phytochemical and proximate analysis of orange juice were presented in Table 1.

In this study, the baseline results showed a significant increase in serum Pb while the creatinine and urea concentrations as renal markers were not significant at 0 week among automobile painters in synthetic vitamin c group compared to control of non-painters. Wan *et al.* [45] identified that lead-exposed workers with the ALAD1-2 genotype were associated with slight increase in blood lead level compared with those with the ALAD1-1 genotype that showed marked increase. This suggests many of the painters in the synthetic vitamin C group may be of ALAD1-2 genotype but those in orange juice group of ALAD1-1 genotype with serum Pb levels of the two groups being 0.067±0.072 and 0.134±0.050 mg/L respectively. The report of high lead level that does not correspond to altered urea and creatinine levels may be linked also with differences in genetic polymorphism of enzymes relating to Pb metabolism. Wan *et al*. [45] also established that genetic polymorphism affected renal marker- creatinine in lead-exposed states. According to them, lead-exposed workers with ALAD1-2 genotype showed minimal renal alterations compared to those with the ALAD1-1 genotype. They concluded that the ALAD-2 protein might modify the kinetics of lead in blood at toxic exposure and protect against hematopoietic, hepatic and renal toxicity from lead. It is puzzling that non-significantly higher urinary GSH in painters [compared with control] co-existed with normal renal markers, suggesting that the cause of urinary GSH loss was not due to impaired glomerular function. That significantly higher serum lead level in painters co-occurred

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with non-significantly lower urinary lead levels requires further investigation.

The result showed a significant decrease in serum Pb at 4 weeks supplementation of synthetic Vitamin C (200 mg daily). This also agreed to a study that administration of 250-500 mg of vitamin C daily to children was effective in removing free radicals and in treating Pb-induced health problems [46]. Similar study by Tandon *et al.* [47] stipulated that Pb-poisoned patients administered 250 mg of vitamin C two times daily for a month resulted in a reduction in blood Pb levels and an increased in blood ALAD activity. Vitamin C (ascorbic acid) is the most widely studied antioxidant capable of removing free radicals by its ability to bind and remove lead has been reported to be highly effective in alleviating lead toxicity [17]. This is further supported by previous results of this study in which plasma lead contents of painters administered with orange juice were significantly lowered at 4 weeks of administration compared with basal level. The result of synthetic vitamin c supplementation was justified in urine level which showed significant increase in urine Pb, decrease in ALA and reduced GSH in automobile painters at 4 weeks. Urine ALA and GSH have been known as biomarkers of lead toxicity and decreased level appeared to be the effect of vitamin C on lead binding in improving the antioxidant status and breakdown of ALA for haemoglobin synthesis. This finding in relation to haemoglobin synthesis is similar to a study according to Dosedel *et al.* [48]. In fact, studies have found that taking vitamin C supplements increased glutathione levels in white blood cells in healthy adults taking 500-1,000 mg of vitamin C daily for 13 weeks, leading to an 18% increase of glutathione in white blood cells [49]. A similar study by Ghanwat *et al.* [50] suggested that supplementation of 500 mg vitamin C daily for 1 month is sufficient enough to suppress the action of free radicals generated due to lead toxicity.

The control baseline at 0 week of non-painters showed a significant positive correlation between serum urea and urine aminolevulinic acid, serum creatinine and urine reduced glutathione (GSH). This has demonstrated normal renal and biochemical markers of lead toxicity relationship. A significant positive correlation was observed in serum lead, creatinine, urine ALA and urine GSH of automobile painters on orange juice at 2 weeks. The observation noticed in 2-week treatment showed improved lead toxicity markers. This report agreed to a finding that negative correlations between blood lead concentration and ALAD activity reported, even at low levels of lead in blood [51,52,53]. A better positive correlation was observed in serum urea and urine GSH in automobile painters on natural orange juice at 4 weeks showing improvements on renal biomarkers that might induce renal injury. Moreover, taking synthetic vitamin c in automobile painters at 2 weeks showed a significant negative correlation between serum creatinine and increased excretion of urine lead. Furthermore, administration of synthetic vitamin c in automobile painters at 4 weeks showed improved antioxidant status and renal marker in significant positive correlation between urine GSH and urea, and significant negative correlation in urine GSH and urine lead. This finding was similar to Chiba *et al.* [54] which reported that the indices of Pb exposure in blood and urine of exposed workers to lead found significant correlation between the activities of superoxide dismutase, reduced glutathione, catalase and Pb.

V. CONCLUSION

It could be concluded that the therapeutic potential of orange juice (*C. sinensis*) are beneficial to human health which could be used as an alternative and affordable replacement for synthetic vitamin C in occupational painters exposed to lead toxicity. Orange juice confers protection in biochemical markers of lead toxicity in automobile painters at 4 weeks. It was observed that there was an improvement in reduction of serum renal biomarkers (urea and creatinine) compared to what were noticed during the administration of synthetic vitamin C. It was also observed that taking orange juice significantly reduced serum lead, urine ALA and urine GSH in orange juice. It will not be out of assumption that taking oral orange juice is very efficient in combating lead toxicity due to occupational exposure which might be as a result of phytochemical, proximate, and antioxidant components of natural orange juice where vitamin C is inclusive.

CONTRIBUTION TO KNOWLEDGE

Orange juice prevents lead toxicity earlier at 2 weeks. Synthetic vitamin c modulates the effect of lead toxicity at 4 weeks. A phenomenon of genetic polymorphism of Alad-1 seems to be a reasonable explanation for the differences in the degree of renal damage as demonstrated by results of serum urea and creatinine concentrations among the two categories of painters at baseline (0 week). Although further study [genetic] is required to confirm whether this is the case. This study established that lead toxicity impacts urinary GSH excretion.

- *Competing Interests*

Authors have declared that no competing interests exist.

- *Authors' Contributions*

AA Iyanda designed the study, AA Iyanda, OF Adesiyan, AA Adesiyan wrote and draft the manuscript, OF Adesiyan, AA Adesiyan, MO Akiibinu and S.A Kumuyi did the analysis. All authors prepared and approved the final manuscript.

- *Ethical Approval*

Ethical approval of Reference No: OSHREC/PRS/569T/158 was obtained from the Health Planning, Research and Statistics Department, Ministry of Health, Osun State Secretariat, Abere, Osogbo, Osun State. Informed consent was obtained from each participant.

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