AMELIORATION OF ARSENIC-INDUCED TOXICITY BY ETHANOL LEAF EXTRACT OF *PHYLLANTUS AMARUS LINN* AND VITAMIN C IN MALE ALBINO RATS

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ABSTRACT

The ameliorative effect of ethanol leaf extract of *Phyllantus amarus* (EEPA) and vitamin C in arsenic-induced toxicity was studied. Thirty-six (36) male albino rats divided into six groups of six (6) rats each were used for the study. Arsenic toxicity was induced in three of the groups by daily intake of 100 ppm of arsenic as Dimethylarsenate (DMA) in their drinking water. Two of the arsenic-exposed groups were treated with 200 and 500 mg/kg bwt of EEPA and vitamin C respectively. The third group was not treated during arsenic exposure. The fourth and fifth groups were positive control for *P. amarus* and Vitamin C respectively, while another group served as the normal control. All treatments were done orally for six weeks. The effects of treatments on lipid profile, lipid peroxidation and liver function were thereafter studied. Increased levels of total cholesterol, LDL-cholesterol and malondialdehyde (MDA) were observed in plasma and lymphocytes of untreated arsenic-exposed rats compared to the control group. Arsenic increased total cholesterol and LDL-cholesterol concentrations, while triacylglycerol concentration was reduced significantly. Treatments with EEPA and Vitamin C however ameliorated the dyslipidemia observed in arsenic-exposed groups. Exposure to DMA increased plasma activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of the animals, while plasma activity of ALT in rats treated with Vitamin C was not different compared to the control. Both treatments however, had no significant effect ($p > 0.05$) on the activity of plasma AST. *P. amarus* may therefore play a role in ameliorating arsenic-induced dyslipidemia in male albino rats.

Keywords: Dimethylarsenate, *Phyllantus amarus,* vitamin C, lipid profile and liver function

INTRODUCTION

Arsenic is a naturally occurring chemical element found in abundance in the Earth's crust and in small quantities in rock, soil, water and air. It is a metalloid which exists in two forms: the organic which is less toxic and the inorganic form which is highly toxic (Vahidnia *et al.*, 2007; WHO, 2003). It is present in the forms of oxides or sulfides or as a salt of iron, sodium, calcium, copper etc. About one third of the arsenic in the atmosphere comes from natural sources, such as volcanoes, and the rest comes from anthropogenic sources (Arpan and Biswajit, 2016; Garelick *et al.*, 2008; WHO, 2003). Contamination of ground water with arsenic is a global problem and millions of people are at a risk of arsenicosis (Huang *et al.*, 2015; Baig *et al.*, 2015: Maharjan *et al.*, 2005).

Human exposure to arsenic is majorly

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through food (Mandal, 2017; Kotz, 2011), water (Huang *et al.*, 2015; WHO, 2003) and can sometimes be inhaled in the air through man-made activities like mining, burning of charcoal and by industrial processes (Singh *et al.,* 2011). Exposure to high level of arsenic is reported to be associated with heart diseases (Monrad *et al.*, 2017; Rasheed *et al.*, 2016; Chen and Karagas, 2013; Tseng *et al.*, 2003), cancer (Monrad *et al., 2017;* Smith *et al.*, 1992), stroke (Rasheed *et al.*, 2016; Chou *et al.,* 2007), chronic respiratory diseases (Hendryx, 2009) and diabetes (Grau-Perez *et al*, 2017; Kile and Christiani, 2008).

Vitamin C is a water-soluble and non-enzymic antioxidant (Madhavi *et al.*, 2017; Sirmali *et al.*, 2014; Combs and Gerald, 2012). When present in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids, carbohydrates and nucleic acids from damage by free radicals and reactive oxygen species (ROS) that are generated during normal metabolism, by active immune cells, and through exposure to toxins and pollutants (Roy *et al.*, 2014; Nikolic *et al.*, 2006). Vitamin C also participates in redox recycling of other important antioxidants. For example, it is known to regenerate vitamin E from its oxidized form (Traber and Stevens, 2011; Nikolic *et al.*, 2006).

Phyllantus amarus belongs to the *Euphorbiaceae.* It is an ethno botanical plant that is distributed in almost all tropical countries (Verma *et al.,* 2014; Obianime and Uche, 2009, Oluwafemi and Debiri, 2008). It primarily contains lignans (Verma *et al.,* 2014; Sharma *et al.*, 1993; Somanabandhu *et al.,* 1993), flavonaoids (Ajibua *et al.,* 2017; Umoh *et al.*, 2013; Sharma *et al.*, 1993) and tannins (Ajibua *et al.*, 2017). *P. amarus* also contains other compounds like hydrolysable

tannins namely phyllanthusiin D (Verma *et al.,* 2014; Umoh *et al.*, 2013; Houghton *et al.,* 1996), amariin (Foo, 1993) and amarulone (Rao and Bramley, 1971). Other components include amarinic acid and alkaloids like ent-norsecurinine, sobubbialine, epibubbialine; diarylbutane, nyrphyllin and a neolignan, phyllnirurin (Ajibua *et al.,* 2017; Somanabandhu *et al.*, 1993). *Phyllanthus* has been used in Ayurvedic medicine for many years and has been shown to have a wide number of therapeutic uses. These include employing the whole plant for jaundice, gonorrhea, frequent menstruation (Verma *et al.,* 2014; Oluwafemi and Debiri, 2008), antimicrobial (Shah *et al.*, 2017; Saranraj and Sivasakthivelan, 2012), anti-inflamatory (Adeolu and Sunday, 2013) and diabetes (Shetti *et al.*, 2012; Povi *et al.*, 2011; Cailxto *et al.*, 1978). Although, *Phyllanthus amarus* has been very useful traditionally, there is no documented study on its use during arsenicosis hence this study.

MATERIALS AND METHODS
Dimethylarsenate (DMA) (trade name- Cacodylic acid) used was obtained from Sigma Chemical Company, St. Louis, MO USA. Vitamin C used was a product of Kunimed Pharmachem Ltd Lagos, Nigeria. Other reagents used were of analytical grade and were prepared using distilled water.

Plant material and extract preparation
Fresh leaves of *Phyllantus amarus* were harvested from Alabata village in Odeda L.G.A Ogun State Nigeria and authenticated by a Taxonomist in Federal University of Agriculture, Abeokuta Ogun state Nigeria. The extract of the plant was prepared according to the method of Adewole *et al.* (2011). Fresh leaves of the plant were air-dried at room temperature and pulverized using an electric blender. 150 g of the ground leaves was

weighed and percolated in 750 ml of absolute ethanol for 48 hours. The solution was filtered and concentrated at room temperature (25 ± 2°C) to give a yield of 4.47% (w/w) which was later reconstituted with distilled water to obtain the required stock used for this experiment.

Animals

Adult male albino rats with average weight of 200 g obtained from the animal house of the Physiology Department of the University of Ibadan, Ibadan, Nigeria were used for the study. The rats were housed in cages maintained at 25 ± 2 °C and 12 hours light and dark cycle. The animals had free access to rat pellets and water *ad-libitum.*

Experimental Grouping

Adult rats were randomly divided into six groups of six rats each. Rats in group I served as the control; groups II, III and IV were exposed 100 ppm of arsenic as Dimethylarsenate (DMA) in drinking water. Group III and IV were treated with 200 mg/kg body weight of the ethanolic leaf extract of *P. amarus* (EEPA) and 500 mg/kg body weight vitamin C respectively (treatment was done concurrently with arsenic exposure). Group V and VI were control groups for the Vitamin C and EEPA treatments they were given 500 mg/kg body weight vitamin C and 200 mg/kg body weight EEPA respectively. All the substances were administered orally to the animals for 6 weeks.

Sample collection

At the end of the treatment, animals were fasted overnight and sacrificed under light ether anaesthesia. Blood samples were collected from abdominal artery into heparinized tubes while kidney and liver were harvested into physiological saline and then blotted dry.

The blood samples were thereafter centrifuged at 4000 rpm for 5 minutes to obtain the plasma used for analysis. Homogenate (10 %) of the organs were obtained using 0.25M sucrose for enzyme assays while chloroform-methanol mixture (2:1 v/v) was used to obtain the homogenate used for lipid analysis.

Lymphocytes isolation

The lymphocytes were isolated using the method described by Skoog and Beck (1956). Briefly, the heparinized blood was mixed with Ficoll reagent in the ratio 3:2 by layering the blood carefully on the Ficoll solution. The mixture was centrifuged for 20 minutes at 1500 rpm at room temperature. The solution above the Ficoll layer contained the lymphocytes. Without touching the Ficoll layer, a sterile pipette tip was used to remove the lymphocytes into Eppendorf tubes and then stored at -20°C for further analysis.

Lipid extraction

Lipid extraction from animal tissues was done using the method described by Folch *et al.* (1957). To obtain samples used for lipid analysis, 0.2 mL of the isolated lymphocytes was mixed with 1.8 mL chloroform-methanol (2:1) mixture while 0.2 g of liver and kidney was homogenized in 1.8 mL of chloroform-methanol (2:1) mixture. The homogenate was shaken every 5 minutes for 30 minutes, centrifuged at 4000 rpm for 10 minutes and the supernatant collected. 0.2 mL of 0.05 M of KCl was then added to remove other non-polar components of the supernatant from the mixture. This was then mixed at room temperature for 5 minutes and centrifuged. The lower layer was removed into dry Eppendorf tubes and was

used for lipid analysis.

Extraction and assay of phospholipid was carried out by a method described by Stewart (1980), a method based on complex formation between ammonium ferrothiocyanate and phospholipids.

Lipid extract (0.1 mL) was evaporated to dryness at 60°C, cooled and 2 mL chloroform was added followed by 2 mL ammonium ferrothiocynate. The contents were mixed and the phases allowed to separate. The chloroform layer was then removed

and the absorbance read at 488 nm against a blank.

HDL-cholesterol was isolated according to the method of Gidez *et al.* (1982). An aliquot of heparin-manganese chloride solution (0.025 mL) was added to 0.25 mL of plasma in an Eppendorf tube. The resultant mixture was thoroughly shaken and left to stand at room temperature for 10 minutes. The mixture was then centrifuged at 4000 rpm for 10 minutes. The clear supernatant obtained is the high density lipoproteins (HDL) fraction.

Low density lipoprotein-cholesterol (LDL-cholesterol) was estimated using Friedewald

formula: $$LDL\ (mg/dL) = TC(mg/dL) - HDL(mg/dL) - TG/5\ (mg/dL)$$

while the quotient TG/5 is VLDL value; TC= total cholesterol (Friedwald *et al.*, 1972)

Biochemical Analyses

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by colorimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine as described by Reitman and Frankel, (1957). Total cholesterol and the triacylglycerol concentration were determined based on the methods of Allain (1974) and Bucolo and David, (1973) respectively while the extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS) using malondialdehyde (MDA) as standard by the method of Buege and Aust (1978). These parameters were determined in the plasma, lymphocytes and in tissue homogenates. Total protein was determined by Biuret method as described by Tietz (1995). Cupric ions in an alkaline medium interact with protein peptides bonds. After incubation for 30 minutes at room temperature, absorbance of the coloured mixture is read against a reagent blank at 546 nm using a

spectrophotometer.

Statistical Analysis

Values are expressed as mean ± standard error mean (SEM). The data were statistically analyzed using one way Analysis of Variance (ANOVA). The level of homogeneity among the groups was tested using Duncan's Multiple Range Test (DMRT). P values < 0.05 were considered to be significant.

RESULTS

The levels of the total cholesterol and LDL-cholesterol in the plasma were observed to be highest in the group of rats administered DMA only (group II) having values of 101.66±0.96 and 45.17±0.50 mg/dL respectively when compared with the control (group I) (78.18±1.26 mg/dL and 7.48±2.66 mg/dL) respectively (Table 1), while triacylglycerol level was significantly (p < 0.05) reduced in this group (Table 1). Rats administered DMA and later given EEPA or vitamin C (groups III and IV) significantly had their

total cholesterol reduced by 36 % and 31 % while LDL cholesterol was reduced by 70 % and 88 % compared with DMA only group (group II) (Table 1). There was no significant difference ($p > 0.05$) in phospholipid levels in all the exposed groups when compared with the control (I), although HDL-TG and VLDL levels were significantly ($p < 0.05$) lowered in the DMA only group (II).

The values of the lipid indices observed in the lymphocytes were highest in the group administered DMA only (Table 2). However, EEPA and Vitamin C treatments significantly ($p < 0.05$) reduced the arsenic-induced hyperlipidemia (Table 2).

In DMA only (II) group, MDA concentrations increased significantly ($p < 0.05$) in all the compartments studied (Table 3). However, treatment with EEPA significantly reduced MDA in the plasma and lymphocytes. Although, a reduction in MDA concentration was observed in the treated group, these values were not significant ($p > 0.05$) in the liver and kidney homogenates. EEPA was observed to have a better ameliorative effect on the extent of lipid peroxidation as indicated by the concentrations of MDA in the plasma and lymphocytes (Table 3).

Table 4 shows that the animals exposed to 100 ppm DMA only, had the highest ALT activity in the plasma, kidney as well as in the liver homogenate (7.08 ± 0.78 U/L, 10.13 ± 0.92 U/mg protein, 17.34 ± 0.64 U/mg protein) respectively. However, the group exposed to DMA and then treated with EEPA (group III) had significantly reduced ALT only in the kidney homogenate (about 45 % reduction) while the group treated with vitamin C (group IV) had ALT values reduced significantly only in the plasma (Table 4).

Table 5 shows the results of the activity of AST in the different groups. All groups exposed to DMA had increased AST activities in the plasma, ranging from 6.13 ± 0.49 to 6.67 ± 0.48 U/l. Treatments did not have any effect on increased kidney AST activity observed in the DMA group. Likewise in the liver, an AST activity of 9.92 ± 0.79 U/mg protein was observed in the exposed but treated with EEPA (group III).

Table 1: Plasma lipid profile of the animals

Groups n=6	Total Cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	TG (mg/dL)	HDL-TG (mg/dL)	Phospholipids (mg/dL)	VLDL (mg/dL)
Control (I)	78.18 ± 1.26[a]	58.10 ± 3.46[a]	7.48 ± 2.66[ab]	63.06 ± 0.32[a]	52.04 ± 8.01[a]	103.68 ± 22.43[a]	12.61 ± 1.09[a]
Arsenic only (II)	101.66 ± 0.96[b]	31.61 ± 2.00[b]	45.17 ± 5.02[c]	39.38 ± 6.01[c]	25.50 ± 2.45[c]	94.08 ± 19.37[a]	7.88 ± 0.67[b]
Arsenic + EEPA (III)	64.70 ± 0.95[a]	49.20 ± 3.95[a]	13.50 ± 3.45[b]	61.66 ± 7.94[a]	35.00 ± 4.01[b]	100.85 ± 14.76[a]	10.05 ± 0.95[a]
Arsenic + vit C (IV)	70.53 ± 1.23[a]	54.97 ± 3.82[a]	5.51 ± 1.24[a]	50.26 ± 5.95[b]	41.88 ± 6.36[b]	115.43 ± 7.15[a]	10.05 ± 0.95[a]
Vit C only (V)	68.01 ± 0.59[a]	53.64 ± 3.21[a]	3.28 ± 1.07[a]	55.41 ± 6.46[b]	31.50 ± 1.07[b]	110.97 ± 10.59[a]	11.08 ± 1.01[a]
EEPA only (VI)	86.63 ± 0.47[a]	60.18 ± 2.13[a]	16.45 ± 4.73[b]	53.47 ± 6.04[b]	32.60 ± 3.20[b]	120.59 ± 16.51[a]	10.06 ± 0.95[a]

Values are mean ± S.E.M. Values within a column having different superscripts are significantly different at p < 0.05

Table 2: Lymphocytes lipid profile of the animals (µg/mg protein)

Group n=6	Total Cholesterol	Triacylglycerol	Phospholipid
Control (I)	13.62 ± 1.12[a]	10.86 ± 1.81[a]	25.23 ± 1.21[a]
Arsenic only (II)	63.46 ± 3.61[c]	49.13 ± 2.59[c]	171.67 ± 5.72[c]
Arsenic +EEPA (III)	24.47 ± 2.31[b]	25.44 ± 1.01[b]	66.39 ± 3.25[b]
Arsenic +vit C (IV)	25.31 ± 1.52[b]	29.75 ± 2.99[b]	73.56 ± 3.17[b]
Vit C only (V)	30.01 ± 2.41[b]	17.05 ± 1.21[a]	48.71 ± 4.01[a]
EEPA only (VI)	27.39 ± 2.49[b]	16.23 ± 1.61[a]	44.80 ± 1.94[a]

Values are mean ± S.E.M. Values within a column having different superscripts are significantly different at p < 0.05

Table 3: Lipid peroxidation as indicated by malondialdehyde (MDA) Concentration in the animals

Control (I)	26 ± 9.6^a	18 ± 1.4^a	24 ± 2.4^{ab}	22 ± 4.7^a
Arsenic only (II)	45 ± 7.5^b	35 ± 1.2^b	38 ± 3.6^b	39 ± 4.4^b
Arsenic +EEPA (III)	21 ± 3.8^a	19 ± 0.8^a	30 ± 1.4^b	36 ± 3.4^b
Arsenic +Vit C (IV)	31 ± 6.4^a	24 ± 1.0^b	36 ± 1.4^b	35 ± 3.1^b
Vit C only (V)	30 ± 1.3^a	21 ± 1.3^a	16 ± 2.5^a	21 ± 5.7^a
EEPA only (VI)	24 ± 2.0^a	19 ± 1.5^a	12 ± 2.7^a	22 ± 2.3^a

Values are mean $\pm$ S.E.M. Values within a column having different superscripts are significantly different at $p < 0.05$

Table 4: Plasma, kidney, and liver alanine aminotransferase (ALT) activities in the animals

Groups n=6	Plasma (U/L)	Liver (U/ mg protein)	Kidney (U/ mg protein)
Control (I)	5.08 ± 0.50^b	6.58 ± 1.05^a	6.92 ± 1.16^{ab}
Arsenic only(II)	7.08 ± 0.78^c	17.34 ± 0.64^c	10.13 ± 0.92^b
Arsenic + EEPA (III)	6.56 ± 0.46^c	15.83 ± 0.41^{bc}	5.55 ± 0.91^a
Arsenic + vit C (IV)	5.15 ± 0.59^b	12.30 ± 0.46^b	7.58 ± 0.74^{ab}
Vit C only (V)	3.79 ± 0.54^a	7.29 ± 1.40^a	5.93 ± 0.46^{ab}
EEPA only (VI)	3.97 ± 0.47^a	6.42 ± 0.67^a	5.83 ± 0.67^a

Values are mean $\pm$ S.E.M. Values within a column having different superscripts are significantly different at $p < 0.05$

Table 5: Plasma, kidney, and liver aspartate aminotransferase (AST) activities in the animals

Groups n=6	Plasma (U/L)	Liver (U/ mg protein)	Kidney (U/mg protein)
Control (I)	4.25 ± 0.49^a	7.17 ± 0.31^a	5.50 ± 0.59^a
Arsenic only(II)	6.67 ± 0.48^b	5.00 ± 0.83^a	8.25 ± 0.55^b
Arsenic + EEPA (III)	6.42 ± 0.47^b	9.92 ± 0.79^b	7.25 ± 4.33^b
Arsenic +Vit C (IV)	6.13 ± 0.49^b	6.42 ± 0.79^a	7.88 ± 0.96^b
Vit C only (V)	5.51 ± 0.40^{ab}	7.88 ± 1.66^{ab}	6.22 ± 0.83^{ab}
EEPA only (VI)	4.43 ± 0.54^a	6.60 ± 0.23^a	5.60 ± 1.59^a

Values are mean $\pm$ S.E.M. Values within a column having different superscripts are significantly different at $p < 0.05$

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DISCUSSION

Arsenic (As) and its compounds are ubiquitous in nature and exhibit both metallic and non-metallic properties (WHO, 2003; Ishinishi *et al.*, 1986). Reports have shown chronic arsenic exposure to cause oxidative damage in lymphocytes and a down regulation of antioxidant mechanisms (Roy *et al.*, 2014; Ghosh *et al.*, 2008). Although, arsenic has been reported to cause oxidative stress, which results in cell damage (Monrad *et al.*, 2017; Grau-Perez *et al.*, 2017; Roy *et al.*, 2014; Vizcaya-Ruiz *et al.*, 2009), to the best of our knowledge, there is no report on the effects of arsenic on the lipids of the lymphocytes. Our results showed that there was hypercholesterolemia, hypertriglyceridemia and hyperphospholipidemia with values (63.46 ± 3.61µg/mg protein, 49.13 ± 2.59 µg/mg protein and 171.67 ± 5.72 µg/mg protein) respectively in the lymphocytes of the male wistar rats as a result of exposure to 100 ppm DMA. However, treatments with vitamin C and EEPA significantly ameliorated arsenic-induced hyperlipidemia in this compartment (Table 2). From our results, the extent of lipid peroxidation as indicated by the concentration of MDA in the animals was increased in the plasma and lymphocytes. Although treatment with vitamin C and EEPA reduced the concentration of MDA in the plasma, in the lymphocytes only vitamin C reduced MDA concentration (Table 3). This could be as a result of the ability of vitamin C to scavenge reactive oxygen species thereby reducing this oxidative stress marker. Oxidative stress is caused by an imbalance between production of reactive oxygen species and biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (Bhattacharyya *et al.*, 2014; Reuter *et al*, 2010; Sies, 1985). There have also been reports that disturbances in the nor-

mal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA (Oluwole, 2011; James, 1994). Results of our study showed that exposure to arsenic as DMA caused oxidative stress as indicated by the significant increase in MDA concentration in all exposed groups.

Some enzymes found primarily in organs can also be found in low quantities in extracellular fluids. However, during tissue damage through leakage arising from altered permeability, serum levels of such enzymes can be increased (Muthumani, 2013; Das *et al.*, 2012; Patel and Kalia, 2010; Wills, 1985). Aminotranferases (ALT and AST) have been reported to be markers of liver and kidney damage (Sangappa *et al.*, 2017; Muthumani, 2013; Wills, 1985). AST has been shown to be similar to ALT since both enzymes are associated with liver parenchymal cells (Wills, 1985). The difference is that ALT is found majorly in the liver, with clinically insignificant quantities found in kidneys, heart, and skeletal muscle, while AST ubiquitous in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells (Nalpas *et al.*, 1986). ALT is therefore a more specific indicator of liver inflammation than AST, because diseases affecting these other organs such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma (Adil *et al.*, 2016; Ghouri *et al.*, 2010) may cause the elevation of AST levels. It has been observed that biological indicators of the hepatic damage such as AST and ALT showed increased activity as a result of arsenic exposure in drinking water (Milan *et al.*, 2017; Das *et al.*, 2012; Santra *et al.*, 1999). These reports support our findings as shown in Tables 4 and 5. The group ad-

ministered DMA only for six weeks (group II) had increased plasma activities of ALT (39 %) and AST (57 %). One would have expected a corresponding decrease in the renal and hepatic activities of these enzymes. Surprisingly, renal and hepatic ALT increased by 46 and 164 % respectively while renal AST increased by 50 % in animals exposed to DMA only (group II). Arsenic has been reported to inhibit both pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (α-KGDH) complexes- enzymes found in the tricarboxylic acid cycle (a pathway involved in energy generation (Erik *et al.*, 2009; Navas-Acien *et al.*, 2006). Inhibition of PDH and α-KGDH results in the accumulation of pyruvate and α-ketoglutarate (Schiller *et al.*, 1977). The homeostatic response to this accumulation would be increased synthesis of AST and ALT to metabolize these substrates to alanine and glutamate.

In EEPA and Vitamin C treated groups (groups III and IV), activity of ALT in the plasma was not significantly different from the control (group I). This could be due to the antioxidant properties of both treatments thus help alleviate effects of the toxicant on the tissues. The importance of vitamin C as a radical scavenger in extracellular fluids, trapping radicals and protecting biomolecules from peroxidative damages have been reported by Madhavi *et al.* (2017) and Sirmali *et al.* (2014).

Phytochemical screening of *P. amarus* revealed the presence of flavanoids, alkaloids, lignans and tannins thereby suggesting that the plant has good medicinal properties. The presence of flavonoids makes the plant a source of antioxidants which have been reported by Ajibua *et al.* (2017); Nguyen *et al.* (2017) and Lim and Murtijaya (2007).

The presence of alkaloids and lignans also indicated the anti-inflammatory properties of the plant (Sangeeta *et al.*, 2017). Studies have suggested that *P. amarus* has hepatoprotective, nephroprotective and cardio protective properties as demostated by the findings of Sangeeta *et al.* (2017), Oluwole, (2011) Lim and Murtijaya, (2007) and Harish and Shivanandappa (2006).

Lipids have been reported to be one of the most susceptible targets of free radicals (Pratt *et al.*, 2011; Rajani and Purnima, 2009). From our study, we observed that exposure to arsenic caused an oxidative change in the lymphocytes and plasma lipids thereby resulting in dyslipidemia shown in the exposed groups. Epidemiological studies have associated arsenic exposure with elevated risks of hypertension (Wu *et al.*, 2014; States *et al.*, 2009; Rahman *et al.*, 1999; Chen *et al.*, 1995) carotid atheroslecrosis (Wu *et al.*, 2014; Wang *et al.*, 2002), and ischemic heart disease (Hsueh, *et al.*, 1998; Tseng *et al.*, 2003). Based on the findings of this study, *P. amarus* and vitamin C may therefore play a role in the amelioration of arsenic-induced pathologies.

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