Original Article

Effect of Vitamins C and E on hepatic and renal function in albino rats treated with gasoline

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Submitted: Feb. 15, 2012; Accepted: Nov. 12, 2012; Published: Dec. 17, 2012.

ABSTRACT

The effects of Vitamins C and E were observed in rats treated with gasoline in this study. The rats were fed diets containing antioxidants vitamins E, C and E plus C while gasoline was injected intraperitoneally at various concentrations. The liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alkphos), urea and creatinine were used as indicators to assess the effect of these vitamins. It was observed that Vitamin E, C and combination of both increased the LD_{100} and LD_{50} of gasoline suggesting that the vitamins conferred protection on the rats while also reversing the induction of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alkphos), urea and creatinine caused by gasoline. The study showed that gasoline as a free radical caused enzymes induction while treatment with Vitamins E, C and combination of both vitamins lowered the induced enzymes, urea and creatinine.

Keywords: Gasoline, Oxidation, Vitamin C, Vitamin E

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INTRODUCTION

Gasoline (Petrol) is used as a fuel for engines in cars. It is produced from petroleum in the refining process. Gasoline is a pale brownish or pink liquid and it is very inflammable. Gasoline as a source of free radical is a generic term used to describe volatile petroleum fuels used primarily in internal combustion engines. It is a complex mixture of hydrocarbon compounds predominantly in the C_3-C_{12} range with a boiling point distribution between 120 to 400^{o}F (48.89 ^{o}C -204.44 ^{o}C) (77 to 340^{o}F (25 to171.11 ^{o}C) for aviation gasoline) and specific

gravity of about 0.74g/cm^3 .The general public is most commonly exposed to unleaded gasoline in form of vapours that evaporate during fuelling of vehicles at service stations (Page and Mehlman 1989).In previous studies, unleaded gasoline vapour was found to be liver tumour promoter and hepatocarcinogen in female mice but not in male mice (MacFarland *et al.,* 1984,Magaw *et al*., 1993) while Standeven *et al* (1995) reported gasoline as liver tumour promoter in both male and female mice. Exposure pathways therefore include breathing vapours at a service station when filling cars fuel tank, working at a service station, using equipment that runs on gasoline, such as a lawn mower, drinking contaminated water, and being close to a spot where gasoline has spilled or leaked into soil. There is therefore increase in the number of individuals who are constantly exposed to the light fraction of petroleum products (Dede, *et al.,* 2003). Inhaling or swallowing large amounts of gasoline can cause death and high concentration of gasoline is irritating to the lining of the stomach when swallowed.

Antioxidants have long been known to reduce the free radical mediated oxidative stress caused by elements and compounds in the environment (Dede and Nganwuchi, 2003, Dede, *et al.,* 2003). Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, and bone. It also plays an important role in the synthesis of the neurotransmitter, nor-epinephrine, synthesis of carnitine and metabolism of cholesterol to bile acids (Frei, 2003). Vitamin C is known to function as a highly effective antioxidant in living organisms (Carr and Frei, 1999). The antioxidant properties of vitamin c are thought to protect smokers, as well as people exposed to air pollution from the harmful effects of free radicals. Food sources such as Grape fruit juice, Orange, Straw berries, Tomato, Sweet red pepper, Broccoli, Orange juice and vegetables vary in their Vitamin C content. Vitamin E is synthesized by plants and is an antioxidant that protects all membranes and other fat- soluble parts of the body, such as low- density lipoprotein cholesterol, from damage. Some of the food sources of vitamin E include Alfalfa sproats, avocado, Bee pollen, Carrot, chickweed, Cumfrey Root, Dadelion Root, garlic, greens (leafy), lemon grass, marsh mallow and mushrooms. Others are seeds, sunflower seeds and sunlight. Vitamin E is absorbed from the intestine through lymph. It circulates through the body plasma in associations with Beta-lipoprotein. Vitamin E has been used in connection with the following conditions like anemia, burns, epilepsy, immune function for elderly people, intermittent claudication, rheumatoid arthritis, tardire dyskinesia, Alzheimer's disease, Angina, atherosclerosis, Bronchitis, cold sores, Down's syndrome, Dysmenorrhea, heart attack, leukoplakia, osteoarthritis, Parkinsons disease, preclampsia, stroke, skin ulcers, infertility, age related cognitive decline etc (Traber, 1999).

The aim of this study is to determine the effect of vitamin C and E on hepatotoxic and renal damage caused by gasoline in albino rats using aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alkphos), urea and creatinine as indicators.

MATERIALS AND METHODS

Test Animals

One hundred and fifty (150) male albino rats of 0.175kg average weight were obtained from the department of pharmacology and Toxicology, university of Port Harcourt animal house. The rats were fed adlibitum with rat pellets and water and acclimatize for two weeks prior to commencement of study in four separate groups. The petrol sample (gasoline) used in this study was obtained directly from AP filling station, University of Port Harcourt, near Port Harcourt. The vitamin supplements (E and C) used for the study were obtained from a pharmacy store, Ebus Pharmacy, Port Harcourt. Commercially prepared Alanine aminotransferase, aspartate aminotransferase, urea and creatinine reagents were obtained from Randox Diagnostics, London while alkaline phosphatase reagents was obtained from Quimica Clinica Aplicada (QCA) Spain

Animal Studies

This group consisted of 20 rats, which were divided into 5 cages, each cage containing 4 rats.

Preliminary study was carried out to determine LD_{100} and LD_{50} of gasoline by intraperitoneal administration of gasoline at 80, 160, 200 and 240 g/kg while the last group was given normal saline to serve as control and the number of death monitored in all the groups and recorded. The LD_{50} was done by Arithmetic method of Karber (Dede, and Igbigbi, 1997).

Twenty five (25) albino rats divided into 5 groups of 4 albino rats each were administered with gasoline intraperitoneally at concentrations of 20, 40, 80, and 160g/kg while the control rats in group 1 were administered with 0.9% normal saline. Signs and symptoms of toxicity due to gasoline were observed in the rats. The rats were considered dead when they no longer responded to agitation.

One hundred and five albino rats divided into 3 treatments of 7 groups each for vitamin E, vitamin C and vitamin C and vitamin E treated rats were fed with diet containing vitamin E, vitamin C and combination of vitamins C and E in their feed for 2 weeks, after which the rats were administered with gasoline intraperitoneally at concentrations of 20, 40, 80, 120, 160, 200 and 240g/kg. The control rats in cage 1 were administered with 0.9% normal saline. Signs and symptoms of toxicity were observed. The dose difference, number of dead rats and the means death at each dose level were recorded. The computation of LD_{50} was carried out using Arithmetic method of Karber (Dede and Igbigbi, 1997).

Biochemical Studies

Urine samples were collected from the rats in groups 1,3, and 4 by suprapubic aspiration and tested for Vitamin C. Vitamin C levels in the urine examined were found to be positive in groups 3 and 4 rats, and tested negative in group 1 rats. The dipstick detection method was used. This method is based on the discoloration of Tillman reagent (2,6, dichlorophenol indophenols). In the presence of vitamin C, a colour change takes place from blue to red (Strove and Makarova, 1989).

Blood samples were collected from rats in groups 1,2 and 4, using the tail of the rats. Colour reaction for tocopherol (vitamin E) was tested for in these groups. Groups 2 and 4 rats tested positive, while group one rats tested negative. The method of

saturation was based on the property of tocopherol to give under the action of strong oxidants (for example concentrated nitric acid), compounds of quinoid structure colored red (Strove and Makarova, 1989).

Determination of ALT and AST was done by monitoring the concentrations of pyruvate hydrazone formed with 2, 4 dinitrophenylhydrazine. Five hundred microlitre (0.5ml) of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT respectively. One hundred microlitre (0.1ml) of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37ºC for 30minutes. Five hundred microlitre (0.5ml) of 2, 4 dinitrophenylhydrazine was dispensed into all test tubes. One hundred microlitre (0.1ml) of sample and control was dispensed into their respective blank test tube. The contents of each test tube was mixed and allowed to stand for 20minutes at 25ºC. 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared (Reitman, and Frankel, 1957).

Alkaline Phosphatase activity was done by Phenolphthalein Monophosphate method .The test tubes were respectively labeled sample, standard and control. One millilitre (1.0ml) of distilled water was pipetted into each tube followed by a drop of the substrate into each test tube. All the test tubes were incubated at 37ºC for 5minutes. Ten microlitre (0.1ml) of sample, standard and control were dispensed into their respective test tubes. The test tubes were incubated at 37ºC for 20minutes. Five milliliter (5ml) of colour developer was added to each test tube, mixed, and read at 550nm using water as blank. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard (Babson *et al* 1966).

Urea estimation was done by Urease - Berthelot colorimetric method. Ten (10) microlitre of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank respectively. Hundred (100) microlitre of urea reagent 1 was added to all the tubes and incubated at 37^{O}C for 10 minutes. Two thousand five hundred (2500) microlitres of urea solutions 2 and 3 was added to all the tubes, mixed and incubated at 37^{O}C for 15 minutes. The absorbance of the sample, control and standard were read at 546nm against the content of the blank tube. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard (Weatherburn 1967).

Creatinine estimation was done by Jaffe's colorimetric method. Five hundred (500) microlitres of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank respectively containing five hundred (500) microlitres of trichloroacetic acid (TCA).The contents were mixed and spun at 2500rpm for 10minutes. One (1ml) millilitre of supernatant from each tube was added into respectively labeled test tube containing one millilitre (1ml) of reagent mixture of Picric acid and sodium hydroxide (500 microlitres each).The contents were mixed and stand at 25^{O}C for 20 minutes. The absorbance of the sample, control and standard were read

at 546nm against the content of the blank tube. The concentration of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard (Henry 1974).

Statistical Analysis

The biochemical data were subjected to some statistical analysis. Values were reported as Mean $\pm$ SEM while student's t-test was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16.A value of $P<0.05$ was accepted as significant.

RESULT

The result showed an increase in LD_{100} in Vitamin E (240g/Kg) vitamin C (280g/Kg) treated albino rats compared with the gasoline treated (160g/Kg) while there was synergism effect of vitamins E and C as it further increase the LD_{100} to 320g/Kg. Also gasoline had LD_{50} of 95 ± 0.175g/kg while treatment with Vitamin E increased the LD_{50} to 140 ± 0.175g/kg, Vitamin C to 165 ± 0.175g/kg and vitamins E and C to 200 ± 0.175g/kg as shown below in table 1. The LD_{100} of 160g/Kg in gasoline was significantly different from 240 g/kg, 280g/kg and 320g/kg obtained in vitamins E, C and combination of C and E treated rats. Also the LD_{50} of 95 ± 0.175g/kg in gasoline treated was significantly different from 140 ± 0.175g/kg, 165 ± 0.175g/kg and 200 ± 0.175g/kg obtained in Vitamins E, C and combination of C and E treated rats.

TABLE 1. Lethal dose in rats treated with gasoline and vitamins E and C

	GASOLINE TREATED		VITAMIN E TREATED		VITAMIN C TREATED		VITAMIN C and E TREATED	
CAGE	Dose level g/kg	No dead g/kg	Dose level g/kg	NoDead g/kg	Dose level g/kg	NoDead g/kg	Dose level g/kg	NoDead g/kg
1	0.00	0	0.00	0	0.00	0	0.00	0
2	20.00	1	40.00	0	40.00	0	40.00	0
3	40.00	2	80.00	1	80.00	1	80.00	0
4	80.00	3	120.00	2	160.00	2	120.00	1
5	160.00	5	160.00	2	200.00	2	160.00	2
6			200.00	3	240.00	3	240.00	2
7			240.00	5	280.00	5	280.00	3
							320.00	5
LD_{50}i	95 ± 0.175g/kg		140 ± 0.175g/kg		165 ± 0.175g/kg		200 ± 0.175g/kg	

There was also dose dependent increase in aspartate amino transferase (AST) activity of gasoline treated rats compared with vitamin E treated rats. The activities (U/L) of 12± 1.15,141± 9.00, 156± 18.33, 167± 9.29 and 176± 15.88 in gasoline was significantly different from 16± 0.58, 70± 11.54,120± 11.54 and 140± 20.00 obtained in vitamin E treated at concentrations of 40,80, 120 and 160g/Kg respectively while 150± 11.54 and 160± 7.64 were activities at concentrations of 200 and 240g/Kg. Vitamin C treated rats also had significant dose dependent reduction in AST compared with gasoline treated. At 0.00 Vitamin C treated had value of 14± 1.16 while the other activities include 80± 10.00,100± 15.28,120± 15.28,125± 25.66,140± 30.55 and 141± 6.66 at concentrations of 40,80, 120, 160,200 and 240g/Kg respectively. The combined treatment of vitamins C and E showed a significant improvement compared with gasoline treated. The 0.00 dose had AST activity of 12± 1.16 while the other activities include 60± 5.00, 69± 6.66, 100± 11.55, 130± 5.77, 164± 8.72, 170± 20.82 and 182± 9.87 at concentrations of 40,80, 120, 160,200,240 and 320g/Kg respectively as shown in table 1 below.

TABLE 2. Effects of vitamins C and E on aspartate amino transferase in rats treated with gasoline

ASPARTATE AMINO TRANSFERASE (U/L)							
CONCENTRATION (g/Kg)	VITAMIN E	P VALUE	GASOLINE	VITAMIN C	P VALUE	VITAMINS C AND E	P VALUE
0.00	16± 0.58	0.020	12± 1.15	14± 1.16	0.423	12± 1.16	0.05
40.00	16± 1.53	0.005	141± 9.00	80± 10.00	0.024	60± 5.00	0.009
80.00	70± 11.54	0.100	156± 18.33	100± 15.28	0.039	69± 6.66	0.019
120.00	120± 11.54	0.127	167± 9.29	120± 15.28	0.112	100± 11.55	0.028
160.00	140± 20.00	0.259	176± 15.88	125± 25.66	0.133	130± 5.77	0.092
240.00	150± 11.54			140± 30.55		164± 8.72	
280.00	160± 7.64			141± 6.66		170± 20.82	
320.00						182± 9.87	

There was also dose dependent increase in aspartate amino transferase (ALT) activity of gasoline treated rats compared with vitamin E treated rats. The activities

(U/L) of 86± 8.33,171± 36.93, 177± 8.88 and 196± 12.22 in gasoline was significantly different from 7± 0.58, 22± 3.47, 23± 3.00 and 33± 6.25 obtained in vitamin E treated rats at concentrations of 40,80, 120 and 160g/Kg respectively while 34± 3.06 and 45± 7.64 were activities at concentrations of 200 and 240g/Kg. Vitamin C treated rats also had significant dose dependent reduction in ALT compared with gasoline treated. At 0.00 Vitamin C treated had value of 8± 1.16 while the other activities include 17± 3.06,18± 4.16,34± 3.06,39± 4.93,40± 8.66 and 50± 5.00 at concentrations of 40,80, 120, 160,200 and 240g/Kg respectively. The combined treatment of vitamins C and E showed a significant improvement compared with gasoline treated. The 0.00 dose had ALT activity of 8± 1.00 while the other activities include 17± 3.06, 17± 3.00, 34± 2.09, 34± 1.00, 39± 1.00,80± 11.55 and 90± 15.28 at concentrations of 40,80, 120, 160,200,240 and 320g/Kg respectively as shown below in table 3.

TABLE 3. Effects of vitamins C and E on alanine amino transferase in rats treated with gasoline

ALANINE AMINO TRANSFERASE (U/L)							
CONCENTRATION (g/Kg)	VITAMIN E	P VALUE	GASOLINE	VITAMIN C	P VALUE	VITAMINS C AND E	P VALUE
0.00	8± 1.15	0.102	13± 1.52	8± 1.16	0.038	8± 1.0	0.185
40.00	7± 0.58	0.010	86± 8.33	17± 3.06	0.007	17± 3.06	0.017
80.00	22± 3.47	0.051	171± 36.93	18± 4.16	0.050	17± 3.00	0.046
120.00	23± 3.00	0.002	177± 8.88	34± 3.06	0.007	34± 2.09	0.002
160.00	33± 6.25	0.009	196± 12.22	39± 4.93	0.012	34± 1.00	0.005
240.00	34± 3.06			40± 8.66		39± 1.00	
280.00	45± 7.64			50± 5.00		80± 11.55	
320.00						90± 15.28	

There was also significant difference in alkaline Phosphatase activity (U/L) of 76

±8.33, 322± 24.03, 420± 91.65, 574± 14.00 in gasoline treated compared with 40± 5.77, 80± 11.55, 89± 12.42, 90± 5.77 at concentrations of 40,80, 120 and 160g/Kg respectively while 98± 6.11 and 110± 5.77 were obtained at concentrations of 200 and 240g/Kg respectively. Vitamin C treated rats also had significant dose dependent reduction in alkaline phosphatase compared with gasoline treated. At 0.00 Vitamin C treated had value of 34± 3.46 while the other activities include 45± 2.89, 50± 5.77, 52± 5.29, 59± 5.20, 59± 0.58 and 80± 5.00 at concentrations of 40,80, 120, 160,200 and 240g/Kg respectively. The combined treatment of vitamins C and E showed a significant improvement compared with gasoline treated. The 0.00 dose had alkaline phosphatase activity of 40± 5.77 while the other activities include 40± 7.64, 49± 5.20, 59± 0.58, 69± 10.69, 70± 10.00, 98± 6.11 and 130± 2.89 at concentrations of 40,80, 120, 160,200,240,280 and 320g/Kg respectively as shown in table 4 below.

TABLE 4. Effects of vitamins C and E on alkaline phosphatase in rats treated with gasoline

	ALKALINE PHOSPHATASE (U/L)						
CONCENTRATION (g/Kg)	VITAMIN E	P VALUE	GASOLINE	VITAMIN C	P VALUE	VITAMINS C AND E	P VALUE
0.00	29± 0.58	0.206	36± 3.46	34± 4.80	0.296	40± 5.77	0.670
40.00	40± 5.77	0.087	76± 8.33	45± 2.89	0.043	40± 7.64	0.082
80.00	80± 11.55	0.020	322±24.03	50± 5.77	0.005	49± 5.20	0.005
120.00	89± 12.42	0.058	420±91.65	52± 5.29	0.057	59± 0.58	0.059
160.00	90± 5.77	0.002	574±14.00	59± 5.20	0.001	69± 10.69	0.000
240.00	98± 6.11			59± 0.58		70± 10.00	
280.00	110± 5.77			80± 5.00		98± 6.11	
320.00						130± 2.89	

The result of urea concentration (mmol/l) in gasoline treated showed 8.2±1.04,10.5±0.29, 12.8±2.56 and 15.7±2.93 compared with 5.5±0.50, 5.3±0.35, 6.6±1.70 and 6.9±0.49 at concentrations of 40,80, 120 and 160g/Kg respectively while 7.2±0.92 and 10.6±0.31 were concentrations at 200 and 240g/Kg respectively. Vitamin C treated rats also showed significant dose dependent reduction in urea compared with gasoline with value of 6± 0.5 at 0.00g/Kg while the other activities include 6.4± 0.23, 6.8± 1.01, 7± 1.16,7.8± 0.12,8± 1.00 and 8.1± 1.01 at concentrations of 40,80, 120, 160,200 and 240g/Kg respectively. The combined treatment with vitamins C and E showed a significant improvement compared with 0.00g/Kg at 7± 0.58 while the other concentrations include 4.3± 0.35, 4.9± 0.06, 6.2± 0.7, 6.8± 1.01, 8.6± 0.81,10.6± 0.81 and 11.5± 0.58 at concentrations of 40,80, 120, 160,200,240,280 and 320g/Kg respectively as shown below in table 5. Creatinine concentration (umol/l) in gasoline treated albino rats was 166±16.00, 190±5.77, 210±60.00 and 380±29.06 while in vitamin E treated the concentrations were 50±5.77, 69±4.94,

69±1.00 and 69±1.00 at gasoline concentrations of 40, 80, 120 and 160g/Kg respectively while 70±10.00 and 160±5.77 were creatinine concentration obtained at 200 and 240g/Kg. Vitamin C treated rats also showed significant dose dependent reduction in creatinine with concentrations of 60± 5.78 at 0.00g/Kg while the other activities include 58± 4.62, 66± 8.51,70± 10.00,71± 5.20,80± 5.77 and 180± 36.06 at concentrations of 40,80, 120, 160,240 and 280g/Kg respectively. The combined treatment with vitamins C and E showed significant improvement with 68± 6.93 at 0.00g/Kg while the other concentrations include 50± 5.77, 66± 6.50, 70± 11.55, 75± 6.50, 80± 5.77,80± 2.89 and 160± 17.32 at concentrations of 40,80, 120, 160,200,240,280 and 320g/Kg respectively as shown below in table 6 .

TABLE 5. Effect of vitamins c and e on urea in rats treated with gasoline

UREA (MMOL/L)							
CONCENTRATION (g/Kg)	VITAMIN E	P VALUE	GASOLINE	VITAMIN C	P VALUE	VITAMINS C AND E	P VALUE
0.00	5.0±0.58	0.050	6.4±0.61	6.0± 0.5	0.512	7.0± 0.58	0.824
40.00	5.5±0.50	0.054	8.2±1.04	6.4± 0.23	0.197	4.3± 0.35	0.037
80.00	5.3±0.35	0.014	10.5±0.29	6.8± 1.01	0.075	4.9± 0.06	0.004
120.00	6.6±1.70	0.149	12.8±2.56	7.0± 1.16	0.101	6.2± 0.7	0.192
160.00	6.9±0.49	0.114	15.7±2.93	7.8± 0.12	0.119	6.8± 1.10	0.116
240.00	7.2±0.92			8.0± 1.00		8.6± 0.81	
280.00	10.6±0.31			8.1± 1.01		10.6± 0.81	
320.00						11.5± 0.58	

TABLE 6. Effects of vitamins c and e on creatinine in rats treated with gasoline

CREATININE (UMOL/L)							
CONCENTRATION (g/Kg)	VITAMIN E	P VALUE	GASOLINE	VITAMIN C	P VALUE	VITAMINS C AND E	P VALUE
0.00	60±5.78	0.423	70±5.78	60±5.78	0.423	68± 6.93	0.785
40.00	50±5.77	0.032	166±16.00	58.0± 4.62	0.033	50± 5.77	0.009
80.00	69±4.94	0.000	190±5.77	66.0± 8.51	0.013	66± 6.50	0.009
120.00	69±1.00	0.144	210±60.00	70.0± 10.00	0.164	70± 11.55	0.187
160.00	69±1.00	0.011	380±29.06	71.0± 5.20	0.013	75± 6.50	0.016
240.00	70±10.00			80.0± 5.77		80± 5.77	
280.00	160±5.77			180.0± 36.06		80± 2.89	
320.00						160± 17.32	

AST (U/L) activity of 160.00 ± 7.60 in gasoline treated rats was significantly higher than 117.00 ± 10.00 obtained in Vitamin C treated rats.ALT (U/L) activity of 162.50 ±25.80 was also higher than 33.00 ± 5.00 in vitamin C treated rats. The Vitamin C treatment reduced alkaline phosphatase activity (U/L) from 348.00 ± 104.00 in gasoline treated to 57.50 ± 12.30 in Vitamin C treated. Creatinine concentration (umol/l) of 87.50 ± 7.30 in Vitamin C treated was significantly lower than 236.50 ± 48.67 in gasoline treated while urea concentration (Mmol/L) of 7.40 ± 0.30 in Vitamin C was significantly lower than 11.80 ± 1.60 in gasoline treated. Also AST (U/L), ALT (U/L),alkaline phosphatase (U/L) activities, urea (Mmol/L) and creatinine concentrations (umol/l) obtained in Vitamin E treated 109.00 ± 23.00, 21.00 ± 5.0 , 84.50 ± 24.00,7.00 ± 0.80 and 82.00 ± 8.00 respectively were significantly lower than the gasoline treated as shown in table 7 below. AST (U/L) activity of 160.00 ± 7.60 in gasoline treated rats was significantly higher than 126.00 ± 20.00 obtained in Vitamin C and E treated rats. ALT (U/L) activity of 162.50 ±25.80 was also higher than 44.00 ± 11.20 in vitamin C and E treated rats. The Vitamin C and E treatment reduced alkaline phosphatase activity (U/L) from 348.00 ± 104.00 in gasoline treated to 73.80 ± 30.00.Creatinine concentration (umol/l) of 83.00 ± 10.00 in Vitamin C and E treated was significantly lower than 236.50 ± 48.67 in gasoline treated while urea concentration (Mmol/L) of 7.56 ± 0.40 in Vitamin C and E was significantly lower than 11.80 ± 1.60 in gasoline treated as shown in table 7 below.

Table 7. Overall effects of vitamins C, E and combinations of vitamins C and E on hepatic and renal indicators in rats treated with gasoline

PARAMETER	VITAMIN C	P VALUE	GASOLINE	VITAMIN E	P VALUE	VITAMIN C AND E TREATED	P VALUE
AST (U/L)	117.00 ± 10.00	0.000	160.00 ± 7.60	109.00 ± 23.00	0.005	126.00 ± 20.00	0.005
ALT (U/L)	33.00 ± 5.00	0.08	162.50 ±25.80	21.00 ± 5.0	0.0001	44.00 ± 11.20	0.009
ALP (U/L)	57.50 ± 12.30	0.062	348.00 ± 104.00	84.50 ± 24.00	0.001	73.80 ± 30.00	0.058
Creatinine(umol/L)	87.50 ± 7.30	0.025	236.50 ± 48.67	82.00 ± 8.00	0.001	83.00 ± 10.00	0.021
Urea (mmol/L)	7.40 ± 0.30	0.036	11.80 ± 1.60	7.00 ± 0.80	0.035	7.56 ± 0.40	0.010

DISCUSSION

The increase in LD_{100} and LD_{50} in Vitamins E,C and combination of C and E treated rats suggested that the treatment with these vitamins conferred some protection against gasoline toxicity by increasing the concentration of gasoline that will cause LD_{100} viz - a- viz LD_{50}. Changes in behavioral pattern with respect to onset of symptoms (weakness, reduced movement, and ataxia, loss of consciousness, respiratory distress, and coma) and death was faster in the untreated group than the treated groups. Deaths in the untreated rats started occurring at 20g/kg whereas in the treated groups, death was recorded from 80g/kg. The onset of symptoms in the treated rats was 10mins, 13 minutes, and

20 minutes for the vitamin E only, vitamin C only and vitamin C plus E treated groups respectively, while onset of symptoms in the gasoline treated groups was 5 minutes. These symptoms occurred mostly at 160g/kg, 200g/kg, 280g/kg and 320g/kg dose levels. The quick onset of symptoms of toxicity and death observed in the untreated group could be due to the high lipid solubility of gasoline as reported by O' Conner and Huggen, (1988). The small size of gasoline also accounts for the faster and higher degree of penetration into biological system, which was responsible for the rapid depression of the central nervous system leading to respiratory depression and death. The treatment with vitamin C reversed this Phenomenon since it has the ability of anti oxidation whereas vitamin E acts mainly as a free radical chain breaking antioxidant in liposomes and cellular membrane (Lui, 1995). Vitamin E has been reported to confer protection on albino rats treated with gasoline (George and Adegoke 2011).

Gasoline caused induction of enzymes such as AST, ALT and ALK Phos in this study while supplementation of diets with vitamins C, E and combination of C and E reduced the induction of the enzymes such as AST, ALT and ALK Phos in this study. This is suggestive of protection against gasoline toxicity. Liver is the central organ of metabolism and act as an organ of storage. Many potentially toxic substances are metabolized by cells especially by the hepatic parenchyma cells. Metabolic action by the hepatic parenchyma cells has been regarded as an important defense system against toxicants and the transformations involved have been referred to as detoxification (Zimmerman, 1974). Elevated serum activity of the two aminotransferases (AST and ALT) is the most frequently measured indicator of liver disease (Reichling and Kaplan, 1988).The treatment with gasoline increased the liver AST which treatment with Vitamins E, C and combination of both E and C reduced as seen it table 2.This may be as result of antioxidant nature of these vitamins. AST is also diffusely represented in the heart, skeletal muscle, kidneys, brain and red blood cells while ALT has low concentrations in skeletal muscle and kidney (Wroblewski 1958); an increase in ALT serum levels is therefore more specific for liver damage. The level of serum ALT activity has been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity (Beckett *et al*., 1989). ALT might have leaked from damaged cells, due to increased permeability of the hepatocellular membrane, or due to necrosis, indicating organ dysfunction (Mclntyre and Rosalki 1992). Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain of reactions (Joseph *et al*., 2009), that damage living cells, spoil foods, degrade materials such as rubber, gasoline, lubricating oil.

The role of liver in metabolic conversion is due to its susceptibility to chemical injury (Zimmerman, 1974). Liver enzymes such as lactate dehydrogenase (LDH), glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic acid transaminase (GPT), alkaline phosphatase and gamma glutamyl transpeptidase (GGT) are considered to be biochemical markers for assessing liver function. Elevation of alanine aminotransferase (ALT or GPT) activity appears to reflect hepatic disease and it is more specific for hepatic disease than aspartate aminotransferase (AST or GOT)

because of the biological location of the enzymes. Gasoline treatment caused dose dependent increase in ALT. The great susceptibility of liver to damage by chemical agent is presumably a consequence of its primary role in metabolism of foreign substances (Zimmerman, 1974). However the elevation of AST and ALT along with the alkaline phosphates (ALKPHOS) activity may reflect some inflammatory disease or injury to the liver (Dede *et al*., 2001) which were reversed by treatment with the Vitamins E, C and E plus C. This may be due to the fact that vitamin C helps to recycle vitamin E exerting long term antioxidant effect. Studies by Burton, *et al*., (1982), Igarashi, *et al.,* (1999), and Sealey, *et al.,* (2002) supported this report. Braide *et al* (2011a) reported that sugar diet reversed the haematoxicity caused by crude petroleum contaminated diet by increasing the Hb and PCV concentrations and reducing the white blood cell count caused by petroleum contaminated diet. Gari diet has also been shown to reduce enzymes induced by petroleum hydrocarbon (Braide*, et al* 2011b).Treatment of these rats with vitamins E, C and vitamins E and C caused reduction in the inducible enzymes occasioned by the gasoline. The delayed onset of symptoms of toxicity in the treated groups suggested that vitamin C and vitamin E conferred some degree of protection to the albino rats.

The study revealed that gasoline caused increase in urea and creatinine concentrations of albino rats. Urea and Creatinine are markers of nephrotic damage. The Kidneys play a special role in concentrating toxic substances within its tubules and excreting them. These functions render it susceptible to damage by certain chemical substances. The kidney is the major organ of excretion of metabolites of gasoline components (Rickert et al 1979) causing dose dependent increase in creatinine concentration in gasoline treated rat. The treatment with Vitamins E, C and E plus C reversed the elevated urea and creatinine concentrations seen in gasoline treated rats. This finding is in agreement with Burton, *et al.,* (1982), *Frei*, *et al.,* (1989) and Tanaka*, et al.,* (1997), that vitamin C and vitamin E as antioxidant, protects tissues against oxidative stress within the body.

The study revealed overall increases in ALT, AST and alkaline Phosphatase, urea and Creatinine in gasoline treated rats compared with vitamins E,C and E plus C treated rats. This observation is similar to study by Dede *et al.,* (2004) using gasoline while studies by Wachukwu *et al.,* (2004) and Ayalogu *et al.,* (2001) reported elevated enzymes levels in rats treated with gasoline. Though the activity of either enzyme particularly AST may be elevated also in extra hepatic disease. However the elevation of AST and AST along with the elevation of ALP activity may reflect some inflammatory disease or injury to the liver. Elevation of alanine amino transferase (ALT) activity appears to reflect hepatic disease and it is more specific for hepatic disease than aspartate amino transferase (AST) because of the biological location of the enzymes (Dede *et al*., 2001). In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity) (Rej 1989). Treatment with Vitamins E, C and E plus C reversed the elevated enymes and renal biomarkers. Gasoline as a free radical has been reported to cause decrease haemoglobin concentration with increase white cell

count while treatment with Vitamins E, C and combination of both vitamins increased the haemoglobin concentration and decreasing the white cell count (George *et al.,* 2011). Antioxidants are molecules, which interact with free radicals and terminate the chain reaction before vital molecules are damaged. They donate an electron to stabilize a free radical. Experiments showed that the antioxidant activity of ascorbic acid involves a hydrogen transfer rather than an election transfer. It has the ability of anti oxidation whereas vitamin E acts mainly as a free radical chain breaking antioxidant in liposomes and cellular membrane (Lui, 1995).

CONCLUSION

This study has shown that gasoline causes hepatic dysfunction and renal damage while feeding on anti oxidants Vitamins C and E or a combination of the two vitamins help to reversed the hepatic and renal damage by repressing the induced enzymes and elevated renal indicators caused by gasoline due to antioxidant nature of Vitamins C and E.

ACKNOWLEDGEMENTS

The authors are grateful to Management of Labmedica Services, Aggrey road and SIGMA laboratories, Port Harcourt for the biochemical analysis and Mr Jide Ogunnowo of Pharmacology department, University of Port Harcourt for his help in the animal study.

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