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Effect of Vitamin E on Biochemical Parameters in Albino Rats Treated with Gasoline

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Abstract

The study was carried out to assess the effect of antioxidant vitamin E on the toxicity caused by gasoline in albino rats. The study was carried out by monitoring the aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alkphos), urea and creatinine in albino rats fed diet containing vitamin E to saturation and injecting gasoline intraperitoneally. It was observed that the LD_{100} and LD_{50} increased significantly in vitamin E fed rats compared to the gasoline fed rats. Also the enzymes monitored were reduced in vitamin fed rats compared with the gasoline fed rats while the kidney function improved with the vitamin E rats by the lowering of the urea and creatinine elevated by gasoline. This suggests that the vitamin E conferred some protection on the rats by its antioxidant nature. Therefore this study showed that gasoline as a free radical caused hepatotoxicity and renal damage while vitamin E can confer protection against toxicity caused by gasoline by donating an electron to stabilize the free radical and terminating the chain reaction before vital molecules are damaged. Hence feeding on diet containing vitamin E should be encouraged.

Keywords: Hepatotoxicity; Gasoline; Antioxidant; Enzymes.

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1. Introduction

Gasoline is used as fuel for motor vehicles and power plants. It is also used as pesticides and cleaning agent. Gasoline is a product of the fractional distillation of crude petroleum. It contains over 500 saturated and unsaturated hydrocarbons which have aliphatic, aromatic or branched chains. The hydrocarbons may have between 3 to 12 carbons and a boiling point range from 30^{O}C to 220^{O}C at atmospheric pressure [1] and specific gravity of 0.74g/cm. The toxicity of gasoline is widely acknowledged. There is risk involved even with only minute or short- term exposure to gasoline. Signs and symptoms of gasoline

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toxicity are associated with abdominal pain, anemia, anorexia, anxiety, bone pain, brain damage, confusion, constipation, convulsions, dizziness, drowsiness, fatigue, headaches, hypertension, inability to concentrate, indigestion, irritability, loss of appetite, loss of muscle co-ordination, memory difficulties, miscarriage, muscle pain, pallor, tremors, vomiting and weakness. These effects are highly contributed by the presence of lead and benzene in gasoline. Target tissues are the bones, brain, heart, kidneys, liver, nervous system and pancreas [2]. Gasoline, a crude petroleum product caused dose dependent decrease in haemoglobin concentration and white blood cell counts [3,4].

Antioxidants are molecules, which can safely interact with free radicals and terminate the chain reaction, before vital molecules are damaged. Although there are several enzymes systems within the body that scavenge free radicals, the principal micronutrient (vitamin) antioxidants are vitamins E, beta-carotene, and vitamin C. 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 [5]. The aim of this study is to determine the effect of vitamin E on hepatotoxic and renal effect caused by gasoline in albino rats using aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alkphos), urea and creatinine as indicators.

2. Materials and Methods

Test animals

Eighty (80) male albino rats of 0.175 kg average weight were obtained from the department of pharmacology and Toxicology, university of Port Harcourt animal house. The rats were fed ad libitum 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) used for the study was obtained from a pharmacy store, Ebus Pharmacy, Port Harcourt.

Animal studies

This group consisted of 20 rats, which were divided into 5 cages, each cage containing 5 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 [6].

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 [6].

Twenty five (25) albino rats divided into 5 groups of 5 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.

Thirty five albino rats divided into 7 groups for vitamin E were fed with diet containing vitamin E in their feed for 2 weeks, after which the rats were administered with gasoline intraperitoneally at concentrations of 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 $LD_{50}$was carried out using Arithmetic method of Karber [6].

Biochemical studies

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 [7].

Determination of ALT and AST was done by monitoring the concentrations of pyruvate hydrazone formed with 2, 4 dinitrophenylhydrazine. 0.5ml of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT respectively. 0.1ml of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37ºC for 30minutes. 0.5ml of 2, 4 dinitrophenylhydrazine was dispensed into all test tubes. 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 [8].

Alkaline phosphatase activity was done by Phenolphthalein Monophosphate method .The test tubes were respectively labeled sample, standard and control. 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. 0.1ml of sample, standard and

control were dispensed into their respective test tubes. The test tubes were incubated at 37ºC for 20minutes. 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 [9].

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) µl of urea reagent 1 was added to all the tubes and incubated at 37^{o}C for 10 minutes. 250 µl 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 [10].

Creatinine estimation was done by Jaffe's colorimetric method. Five hundred (500) ml of sample, standard, control and distilled water was pipette into test tube labeled sample, standard control and blank, respectively containing five hundred (500) ml of trichloroacetic acid (TCA).The contents were mixed and spun at 2500rpm for 10minutes. 1000 ml of supernatant from each tube was added into respectively labeled test tube containing 1000 ml of reagent mixture of picric acid and sodium hydroxide (500 ml 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 [11].

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.

3. Results

The result presented in Table 1 showed an increase in LD_{100} in vitamin E treated albino rats (240$\pm$0.175g/kg) compared with the gasoline treated (160$\pm$0.175g/kg). Also the LD_{50} was increased by vitamin E(140$\pm$0.175g/kg) treated rats compared with gasoline treated. (95$\pm$0.175g/kg).

There was also significant difference in alkaline phosphatase activity (U/L) of 76 $\pm$8.33, 322$\pm$24.03, 420$\pm$91.65, 574$\pm$14.00 in gasoline treated compared with 40$\pm$5.77, 80$\pm$11.55, 89$\pm$12.42, 90$\pm$5.77 at concentrations of 40,80, 120 and 160g/kg, respectively while 98$\pm$6.11 and 110$\pm$5.77 were obtained at concentrations of 200 and 240g/kg respectively.

Table 1. Median lethal dose in rats treated with gasoline.

	Gasoline treated without vitamin E		Gasoline treated with vitamin E	
Cage	Dose level (g/kg)	No. dead	Dose level (g/kg)	No. dead
1	0.00	0	0.00	0
2	20.00	1	40.00	0
3	40.00	2	80.00	1
4	80.00	3	120.00	2
5	160.00	4	160.00	2
6			200.00	3
7			240.00	4
LD_{50}	LD_{50}ip = 95 ± 0.175g/kg		LD_{50}ip = 140 ± 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 (Table 2). 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 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.

Table 2. Effect of vitamin E on hepatic enzymes in rats treated with gasoline.

	Alkaline phosphatase			Aspartate aminotransferase			Alanine aminotransferase		
Conc. (g/kg)	GT without vitamin E	GT with vitamin E	*P* value	GT without vitamin E	GT with vitamin E	*P* value	GT without vitamin E	GT with vitamin E	*P* value
0	36±3.46	29±0.58	0.206	12±1.15	16±0.58	0.020	13±1.52	8±1.15	0.102
40	76±8.33	40±5.77	0.087	141±9.00	16±1.53	0.005	86±8.33	7±0.58	0.010
80	322±24.03	80±11.55	0.020	156±18.33	70±11.54	0.100	171±36.93	22±3.47	0.051
120	420±91.65	89±12.42	0.058	167±9.29	120±11.54	0.127	177±8.88	23±3.00	0.002
160	574±14.00	90±5.77	0.002	176±15.88	140±20.00	0.259	196±12.22	33±6.25	0.009
200		98±6.11			150±11.54			34±3.06	
240		110± 5.77			160±7.64			45±7.64	

GT = Gasoline treated.

Also dose dependent increase in alanine amino transferase (AST) activity was observed in 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.

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 (Table 3). Creatinine concentration (µmol/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.

Table 3. Effect of vitamin E on urea and creatinine in rats treated with gasoline.

	Urea			Creatinine		
Conc. (g/kg)	GT without vitamin E	GT with vitamin E	*P* value	GT without vitamin E	GT with vitamin E	*P* value
0.00	6.4±0.61	5.0±0.58	0.050	70±5.78	60±5.78	0.423
40.00	8.2±1.04	5.5±0.50	0.054	166±16.00	50±5.77	0.032
80.00	10.5±0.29	5.3±0.35	0.014	190±5.77	69±4.94	0.000
120.00	12.8±2.56	6.6±1.70	0.149	210±60.00	69±1.00	0.144
160.00	15.7±2.93	6.9±0.49	0.114	380±29.06	69±1.00	0.011
200.00		7.2±0.92			70±10.00	
240.00		10.6±0.31			160±5.77	

GT = Gasoline treated.

Table 4. Overall effect of vitamin E on biochemical parameters in rats treated with gasoline.

Parameter	Gasoline treated without vitamin E	Gasoline treated with vitamin E	*P* value
AST (U/L)	160.00 ± 7.60	109.00 ± 23.00	0.05
ALT (U/L)	162.50 ±25.80	21.00 ± 5.0	0.0001
ALP (U/L)	348.00 ± 104.00	84.50 ± 24.00	0.001
Creatinine (µmol/L)	236.50 ± 48.67	82.00 ± 8.00	0.001
Urea (mmol/L)	11.80 ± 1.60	7.00 ± 0.80	0.05

There was significant decrease in AST (U/L) activity of vitamin E treated 109±23 compared with 160±7.6 in gasoline treated. Also there was significant decrease in ALT activity (U/L) of 21±5 in vitamin E compared with 162.5±25.8 in gasoline treated rats. Alkaline phosphatase activity was reduced by vitamin E diet to 84.5± 24.0 from 348±104 obtained in gasoline treated rats. The concentrations of 82±8 and 7.0± 0.8 obtained for creatinine (μmol/L) and urea (mmol/L) in vitamin E treated rats were significantly lower than 236.50±48.67 and 11.8±1.6 obtained in gasoline treated rats as shown in Table 4 below.

4. Discussion

The increase in LD_{100} and LD_{50} in vitamins 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} vis-a-vis 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 gasoline rats started occurring at 20g/kg whereas in the vitamin E treated groups, death was recorded from 80g/kg.

The biochemical parameters monitored during the acute toxicity study were aspartate aminotransferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP). Other parameters monitored include creatinine (CR) and urea (UR) levels. Enzymes were also used as markers because they are found in body tissues; they frequently appear in the serum following cellular injury or sometimes in smaller amounts, from degraded cells or storage area. Hence serum enzymes levels are often useful in the diagnosis of particular diseases or physiologic abnormalities [12].

In this study, the four groups examined produced a dose dependent increase in the levels of the three enzymes. Biochemical studies by Obianime *et al.* [13] revealed that gasoline given intraperitoneally induced a time dependent increase in liver enzyme activity. Wachukwu *et al.* [3] reported that there was a corresponding increase in the activity of the liver enzymes, AST, ALT and ALP with increase in the concentration of petrol while Uboh *et al.* [14] reported that exposure of male and female rats to 17.8 $cm^3h^{-1}m^{-3}$ of Premium Motor Spirit (PMS) blend unleaded gasoline (UG) vapors for 6 hr/day, 5 days/week for 20 weeks caused hepatotoxicity. Also previous studies observed that gasoline vapors induced proatherogenic changes in serum lipid profile and signs of hepatic oxidative stress [15-17], haematotoxicity [18, 19], reproductive toxicity [20] and nephrotoxicity [21] in male and female rats.

From this study, the increase in activity was highest with ALT, which is more specific for hepatic diseases than AST and ALP in the untreated group. The result further showed that in vitamin E treated rats the enzymes were reduced compared with the gasoline treated rats. This is similar to reports by other authors using vitamins A and E [14]. This may be attributed to the anti oxidant nature of vitamin E by removing the free radical. Biological locations can also influence or affect the release of these enzymes. The increase in these three enzymes may reflect some inflammatory disease or injury to the liver (hepatocellular disease). Krishan and Veena [22] also observed increase in levels of

AST, ALT and ALP in serum of fish exposed to 2,3,4 – triaminoazo benzene resulting to hepatocellular damage. Dheer, *et al.* [23], Mohssen [24] and Sharpe, *et al.* [25] are studies that indicated increase in the activity of liver enzyme following liver damage in fish and albino mouse exposed to toxic substances.

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 [26]. Also vitamin E help to ameliorate the kidney function by reducing the urea and creatinine values. Vitamin E might have aided the kidney to excrete the gasoline thus protecting the kidney as well as the rats. Vitamin E acts mainly as a free radical chain breaking antioxidant in liposomes and cellular membrane [27]. The study revealed overall increases in ALT, AST and alkaline Phosphatase, urea and Creatinine in gasoline treated rats compared with vitamins E, treated rats. This observation is similar to study by Dede *et al.* [4].

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. Antioxidants have long been known to reduce the free radical mediated oxidative stress caused by elements and compounds in the environment [28, 29].The function of vitamin E in the study was to donate an electron to stabilize a gasoline which is a free radical.

5. Conclusion

This study has shown that gasoline as a free radical causes hepatotoxicity and renal damage while feeding on antioxidant vitamin E reversed the hepatotoxicity and the renal damage.

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