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Effect of Vitamins C and E on Hematological Parameters in Albino Rats Treated with Gasoline

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Abstract: The effects of Vitamins C and E on haematoxicity caused by gasoline were observed in rats treated with gasoline in this study. The rats were fed diet containing antioxidants as vitamins E, C and E plus C, while gasoline was injected intraperitoneally at various concentrations. The haemoglobin and white cell count were monitored as indicators to assess the effect of these vitamins on haematoxicity. 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 decreased haemoglobin and increased white cell count caused by gasoline. The study showed that gasoline as a free radical decreased haemoglobin concentration and increased white cell count while treatment with Vitamins E, C and combination of both vitamins increased the haemoglobin concentration and decreased white cell count. Antioxidant activity of vitamin C (ascorbic acid) involves a hydrogen transfer rather than an electron transfer that confer the ability of anti oxidation whereas vitamin E acts mainly as a free radical chain breaking antioxidant in liposome and cellular membrane. Therefore the Vitamin C is involved in transfer of electron to reduce the effect of free radical while Vitamin E breaks the free radical chain event caused by gasoline. Therefore consumption of diet containing vitamins E and C could help to ameliorate the oxidation effect caused by gasoline by reversing haematoxicity.

Key words: Haematoxicity · Ascorbic Acid · Antioxidant · Gasoline

INTRODUCTION

Adequate intake of vitamin C helps in the treatment of cardiovascular diseases (vasodilation and hypertension), cancer, diabetes mellitus and common cold [1]. Food sources such as Grape fruit juice, Orange, Strawberries, Tomato, Sweet red pepper, Broccoli, Orange juice and vegetables vary in their Vitamin C content. Vitamin E as alpha or gamma tocopherol has been suggested as a potent antioxidant. Clinical studies carried out by researchers, provided information on some of the functions of vitamin E. It is useful in the treatment of Alzheimer's disease [2]. Vitamin E also helps stop arteriosclerosis [3].

Reports had shown that Vitamin C and Vitamin E when administered together, results in a synergistic interaction. Vitamin C was observed to be useful in regenerating Vitamin E [3]. Useful antioxidants especially Vitamin C and Vitamin E have been reported to ameliorate the cellular damage caused by free radicals formations. The administration of vitamin C to rats conferred some level of protection to the rats against gasoline toxicity and damage to tissues [4]. Some of the food are sources of vitamin E, which include Alfalfa sproats, avocado, Bee pollen, Carrot, chickweed, Cumfrey Root, Dadelion Root, garlic, greens (leafy), lemon grass, marsh mallow and mushrooms. 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 [4].

Gasoline is used as fuel for motor vehicles and power plants. It is also used as pesticides and cleaning agent. Gasoline contains over 500 hydrocarbons that may have between 3 to 12 carbons per molecule and gasoline used to have a boiling range from 30°C to 220°C at atmospheric pressure. There are several known toxins in gasoline, some of which are human carcinogen [5]. Unleaded gasoline vapour was found to be liver tumour promoter and hepatocarcinogen in female mice but not in male mice [5, 6] while Standeven *et al*. [7] showed that unleaded gasoline acted as a liver tumour promoter in both male and female mice. Gasoline, a crude petroleum

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product caused dose dependent decrease in haemoglobin concentration and white blood cell counts [8, 9]. Many of the harmful effects seen after exposure to gasoline are due to the individual chemicals in the gasoline mixture, such as benzene and lead. 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. Target tissues are the bones, brain, heart, kidneys, liver, nervous system and pancreas [10].

The aim of this paper was to establish a possible protective role of vitamins C and E against gasoline induced changes on haematological indices using haemoglobin and white cell counts (total and differential) as indicators.

MATERIALS AND METHODS

Experimental Animals: One hundred and fifteen (115) male albino rats of 175g 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 and C) used for the study were obtained from a pharmacy store, Ebus Pharmacy, Port Harcourt.

Animal Studies

Toxicity Test: This group consisted of 25 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 body weight, 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 [11].

Treated Groups: A: 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 160 g/kg body weight, 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.

B-Seventy albino rats divided into 2 treatments of 7 groups each for vitamin E and vitamin C treated rats were fed with diet containing vitamin E and vitamin C, respectively in their feed for 2 weeks, after which the rats were injected 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 computation of LD_{50} was carried out using Arithmetic method of Karber [11].

Haematological Studies: The Haemoglobin (Hb) estimation was done by the cyanomethaemoglobin method using Drabkin's solution [12]. Total white blood cell (TWBC): White blood cell diluting fluid (Turkes solution) contains a weak acid (Glacial acetic acid) to lyse the red cell and a stain to stain the nucleus of the white cells [13]. White cell differential count was done using thin blood smear film [13].

Statistical Analysis: The biochemical data were subjected to some statistical analysis. Values were reported as Mean±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

There was also dose dependent decrease in haemoglobin concentration (g/dl) of gasoline treated rats compared with vitamin E treated rats. The concentration (g/dL) of 17.6±1.50, 13.2±1.62, 11.9±0.06, 11.0±0.58 and 10.3±0.17 in gasoline was significantly different from 20.0±3.33, 20.1±3.38, 19.2±0.46, 18.2±0.46and 16.1±0.64, obtained in vitamin E treated at concentrations of 0.00, 40,80, 120 and 160g/Kg, respectively while 15.4±0.35 and 13.1±0.58 were concentrations at doses of 200 and 240g/Kg. Vitamin C treated rats also had significant dose dependent reduction in haemoglobin compared with gasoline treated. At 0.00 Vitamin C treated had value of 18.2±0.46while the other concentrations include 14.8±2.43, 14.6±1.39, 14.3±0.17, 14.0±0.58, 13.9±0.06 and 10.6±0.98 at doses 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 concentration of 19.0±1.73 while the other concentrations include 20.8±1.27, 19.1±0.52, 16.2±1.27, 15.4±0.81, 15.4±0.64, 14.8±2.43 and 14.1±0.64 at doses of 40,80, 120, 160,200,240 and 320g/Kg respectively as shown in Table 1 below.

Table 1: Effects of vitamins C and E on haemoglobin in rats treated with gasoline

	Haemoglobin (g/dL)								
Concent Ration (g/Kg)	Gasoline	Vitamin E	P Value	Gasoline	Vitamin C	P Value	Gasoline	Vitamins C AND E	P Value
0.00	17.6±1.50	20.0±3.33	0.131	17.6±1.50	18.2±0.46	0.213	17.6±1.50	19.0±1.73	0.707
40.00	13.2±1.62	20.1±3.38	0.107	13.2±1.62	14.8±2.43	0.186	13.2±1.62	20.8±1.27	0.094
80.00	11.9±0.06	19.2±0.46	0.005	11.9±0.06	14.6±1.39	0.179	11.9±0.06	19.1±0.52	0.006
120.00	11.0±0.58	18.2±0.46	0.000	11.0±0.58	14.3±0.17	0.048	11.0±0.58	16.2±1.27	0.086
160.00	10.3±0.17	16.1±0.64	0.006	10.3±0.17	14.0±0.58	0.032	10.3±0.17	15.4±0.81	0.015
240.00		15.4±0.35			13.9±0.06			15.4±0.64	
280.00		13.1±0.58			10.6±0.98			14.8±2.43	
320.00								14.1±0.64	

Table 2: Effects of vitamins C and E on white cell count in rats treated with gasoline

	White cell count (g/dL)								
Concent Ration (g/Kg)	Gasoline	Vitamin E	P Value	Gasoline	Vitamin C	P Value	Gasoline	Vitamins C and E	P Value
0.00	7.4±0.81	7.6±0.35	0.05	7.4±0.81	7.6±0.35	0.05	7.4±0.81	9.9±0.21	0.160
40.00	10.6±1.33	6.9±0.95	0.142	10.6±1.33	7.0±1.16	0.284	10.6±1.33	7.9±0.06	0.173
80.00	8.2±0.46	6.3±0.40	0.127	8.2±0.46	6.9±0.67	0.368	8.2±0.46	6.9±0.67	0.309
120.00	5.3±0.49	5.6±0.18	0.483	5.3±0.49	6.0±0.29	0.267	5.3±0.49	6.6±0.65	0.923
160.00	4.3±0.71	4.2±0.06	0.678	4.3±0.71	5.8±0.46	0.119	4.3±0.71	6.6±0.35	0.008
240.00		4.2±0.13			5.6±0.23			6.4±0.17	
280.00		4.0±0.17			4.6±0.23			5.4±0.17	
320.00								4.6±0.06	

Table 3: Effects of vitamins C and E on neutrophils percent in rats treated with gasoline

	Neutrophils (%)								
Concent Ration (g/Kg)	Gasoline	Vitamin E	P Value	Gasoline	Vitamin C	P Value	Gasoline	Vitamins C and E	P Value
0.00	31±5.00	32±0.1	0.05	31±5.00	50±5.00	0.05	31±5.00	54±1.16	0.580
40.00	47±1.73	45±1.67	0.902	47±1.73	45±1.67	0.914	47±1.73	60±2.89	0.035
80.00	29±0.58	48±6.11	0.104	29±0.58	51±4.93	0.057	29±0.58	50±5.78	0.081
120.00	42±0.58	49±0.58	0.05	42±0.58	50±5.00	0.284	42±0.58	49±0.58	0.050
160.00	40±2.89	51±4.93	0.138	40±2.89	60±2.89	0.020	40±2.89	49±5.20	0.184
240.00		60±5.00			52±6.11			54±1.53	
280.00		50±5.00			42±4.62			43±1.73	
320.00								46±2.30	

There was also dose dependent decrease in white cell count ($X10^9$/l) of gasoline treated rats compared with vitamin E treated rats. At 0.00 gasoline had count of 7.4±0.81 $X10^9$/l. The count ($X10^9$/L) of 10.6±1.33, 8.2±0.46, 5.3±0.1 and 4.3±0.71 in gasoline was significantly different from 6.9±0.95, 6.3±0.40, 5.6±0.18 and 4.2±0.06 obtained in vitamin E treated at concentrations of 40,80, 120 and 160g/Kg, respectively while 4.2±0.13 and 4.0±0.17 were counts at concentrations of 200 and 240g/Kg. Vitamin C treated rats also had significant dose dependent reduction in white cell count ($X10^9$/l) compared with gasoline treated. At 0.00 Vitamin C treated had count of 7.6±0.35while the others include 7.0±0.16, 6.9±0.67, 6.0±0.29, 5.8±0.46, 5.6±0.23 and 4.6±0.71 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 white cell count of 7.9±0.21 while the other activities include 7.9±0.06, 6.9±0.67, 6.6±0.65, 6.6±0.35, 6.4±0.17, 5.4±0.17 and 4.6±0.06 at concentrations of 40, 80, 120, 160,200,240 and 320g/Kg respectively as shown in Table 2 below.

The neutrophil counts (%) were 31±5.00, 47±1.73, 29±0.58, 42±0.58 and 40±2.89 at dose of 0.00, 40, 80, 120 and 160g/Kg, respectively while vitamin E treated rats had counts of 32±0.1, 45±1.67, 48±6.11, 49±0.58, 51±4.93, 60±5.00 and 50±5.00 at doses 0.00,40,80, 120, 160,200 and 240g/Kg respectively. Vitamin C treated rats had 50±5.00, 45±1.67, 51±4.93, 50±5.00, 60±2.89, 52±6.11 and 42±4.62 at doses 0.00,40,80, 120, 160,200 and 240g/Kg respectively. The combined treatment of vitamins C and E had 54±1.16, 60±2.89, 50±5.78, 49±0.58, 49±5.20,54±1.53, 43±1.73 and 46±2.30 at doses of 40,80, 120, 160,200,240 and 320g/Kg, respectively as shown in Table 3 below.

Table 4: Effects of vitamins C and E on lymphocytes in rats treated with gasoline

	Lymphocytes (%)								
Concent Ration (g/Kg)	Gasoline	Vitamin E	P Value	Gasoline	Vitamin C	P Value	Gasoline	Vitamins C and E	P Value
0.00	60±0.07	60±0.07	0.05	60±0.07	50±5.77	0.173	60±0.07	40±5.78	0.040
40.00	53±0.73	50±5.00	0.770	53±0.73	55±5.77	0.368	53±0.73	36±2.36	0.196
80.00	63±4.04	46±2.30	0.093	63±4.04	49±5.20	0.007	63±4.04	48±8.08	0.266
120.00	52±6.11	49±5.20	0.804	52±6.11	48±3.52	0.503	52±6.11	51±5.86	0.928
160.00	54±1.53	47±4.04	0.317	54±1.53	40±1.67	0.067	54±1.53	49±0.58	0.130
240.00		40±1.67			46±5.03			46±5.20	
280.00		50±5.77			58±9.17			49±1.16	
320.00								54±11.37	

Table 5: Effects of vitamins C and E on monocytes percent in rats treated with gasoline

	Monocytes (%)								
Concent Ration (g/Kg)	Gasoline	Vitamin E	P Value	Gasoline	Vitamin C	P Value	Gasoline	Vitamins C and E	P Value
0.00	9±0.58	8±2.08	0.122	9±0.58	0±0.00	0.004	9±0.58	6±1.00	0.035
40.00	0±0.0	5±0.58	0.013	0±0.0	0±0.00	0.05	0±0.0	4±2.30	0.225
80.00	8±0.5	6±0.58	0.195	8±0.5	0±0.00	0.004	8±0.5	2±0.58	0.002
120.00	6±0.5	2±0.58	0.005	6±0.5	2±0.58	0.005	6±0.5	0±0.00	0.007
160.00	6±0.5	2±1.16	0.035	6±0.5	0±0.00	0.016	6±0.5	2±1.16	0.035
240.00		0±0.0			2±0.58			0±0.00	
280.00		0±0.0			0±0.00			8±1.00	
320.00								0±0.00	

The lymphocyte counts (%) were 60±0.07, 53±0.73, 63±4.04, 52±6.11 and 54±1.531 at dose of 0.00, 40,80, 120 and 160g/Kg respectively while vitamin E treated rats had counts of 60±0.07, 50±5.00, 46±2.30, 49±5.20, 47±4.04, 40±1.67 and 50±5.77 at doses 0.00,40,80, 120, 160,200 and 240g/Kg respectively. Vitamin C treated rats had 50±5.77, 55±5.77, 49±5.20, 48±3.52, 40±1.67, 46±5.03 and 58±9.17 at doses 0.00,40,80, 120, 160,200 and 240g/Kg respectively. The combined treatment of vitamins C and E had lymphocyte counts of 40±5.78, 36±2.36, 48±8.08, 51±5.86, 49±0.58, 46±5.20, 49±1.16 and 54±11.37 at doses of 0.00, 40, 80, 120, 160,200,240 and 320g/Kg respectively as shown in Table 4 below.

The monocyte counts (%) were 9±0.58, 0±0.0, 8±0.5, 6±0.5 and 6±0.5 at dose of 0.00, 40, 80, 120 and 160g/Kg respectively while vitamin E treated rats had counts of 8±2.08, 5±0.58, 6±0.58, 2±0.58 and 2±1.16 at doses 0.00,40,80, 120, 160 while doses 200 and 240g/Kg both had 0±0.0 count. Vitamin C treated rats had 0±0.00 at doses 0.00,40,80, 160,200 while 120 and 240g/Kg had 2±0.58 each. The combined treatment of vitamins C and E had lymphocyte counts of 6±1.00, 4±2.30, 2±0.58, 2±1.16and 8±1.00 at doses of 0.00, 40, 80, 160,200 and 280 while 120, 240 and 320g/Kg had 0±0.00 count as shown in Table 5 below.

Haemoglobin concentration (g/dl) of 11.60±0.60 in gasoline treated rats was significantly lower than 17.00±1.00 obtained in Vitamin C treated rats. Haemoglobin concentration of 11.60±0.60 in gasoline treated rats was significantly lower than 13.70±0.60 obtained in Vitamin E treated rats. Haemoglobin concentration of 11.60±0.60 in gasoline treated rats was significantly lower than 16.50±0.90 obtained in Vitamin C plus E treated rats. White blood cell count of 7.10±1.30 was also higher than 5.20±0.50, 5.90±0.30 and 6.30±0.60 in vitamin C, Vitamin E and vitamin C and E treated rats respectively. Neutrophil count of 39.50±3.70 was also lower than 50.50±2.10, 50.00±2.30and 50.10±2.10 in vitamin C, Vitamin E and vitamin C and E treated rats respectively. Lymphocyte count of 55.50±2.50 was higher than 47.00±1.60, 49.30±2.70 and 47.50±2.10 in vitamin C, Vitamin E and vitamin C and E treated rats respectively. Monocyte count of 6.70±0.05 was also higher than 2.50±0.80, 0.70±0.30 and 4.00±1.00 in vitamin C, Vitamin E and vitamin C and E treated rats respectively as shown below in Table 6.

Table 6: Overall effects of vitamins C and E on haematological parameters in rats treated with gasoline

Parameter	Gasoline	Vitamin C	P Value	Gasoline	Vitamin E	P Value	Gasoline	Vitamin C	P Value
Haemoglobin (HB) (g/dl)	11.60±0.60	17.00±1.00	0.000	11.60±0.60	13.70±0.60	0.016	11.60±0.60	16.50±0.90	0.002
White Blood Count (WBC) (x$10^{9/L}$)	7.10±1.30	5.20±0.50	0.543	7.10±1.30	5.90±0.30	0.238	7.10±1.30	6.30±0.60	0.881
Neutrophil (N) (%)	39.50±3.70	50.50±2.10	0.009	39.50±3.70	50.00±2.30	0.107	39.50±3.70	50.10±2.10	0.027
Lymphocyte (L)(%).	55.50±2.50	47.00±1.60	0.158	55.50±2.50	49.30±2.70	0.108	55.00±2.50	47.50±2.10	0.090
Monocyte (M) (%)	6.70±0.05	2.50±0.80	0. 078	6.70±0.50	0.70±0.30	0.600	6.70±0.50	4.00±1.00	0.297

DISCUSSION

There was a significant decrease of haemoglobin in all the groups, but more significant with the gasoline treated group compared with the vitamin E, C and E plus C treated rats. The result of low haemoglobin in gasoline treated rats is similar to reports by Ovuru and Ekweozor [14] in rabbits, Leighton *et al*. [15] in young herring gulls and Atlantic puffins, Sunmonu and Oloyede [16] on African catfish (*Clarias gariepinus*) and rats [17]. The result of this study showed that gasoline caused dose dependent decrease in haemoglobin concentrations in albino rats while feeding with vitamins C, E and E plus C caused increase haemoglobin concentration. 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 [18].

Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain of reactions [19], that damage living cells, spoil foods, degrade materials such as rubber, gasoline, lubricating oil. Antioxidants terminate these chain reactions through the removal of free radical intermediates and inhibition of other oxidation reactions [20]. This is why plants and animals maintain complex systems of multiple antioxidants, such as glutathione, vitamin C and vitamin E along with some enzymes like catalase, superoxide dismutase and various peroxidases. Braide *et al.* [21] 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 [22]. Proliferation of white blood cells was higher in the gasoline treated rats and showed a dose dependent decrease in gasoline treated suggesting that gasoline caused immune response while the white cell counts were reduced in vitamins E, C and E plus C treated rats.

Differential leucocytes count showed a decrease in neutrophils and increase in lymphocytes in the gasoline treated group, this result is in agreement with the findings of Hogstedt *et al*. [23], who reported increase frequency and size of lymphocytes in occupational exposure to benzene in petrol. However, in the vitamin treated group, an increase of neutrophils count was recorded when compared to lymphocyte count. There is no clear understanding of the variation in the neutrophil /lymphocyte ratio, but the toxicity of petrol to haematopoetic cells in the bone marrow could be due to several of its metabolites that are formed in relatively highly concentrations and act in an additive or synergetic manner to disrupt a range of mechanisms that regulate blood cells formation. Vitamin E has been reported to confer protection on albino rats treated with gasoline [24].

CONCLUSION

This study has shown that gasoline causes haematoxicity while feeding on anti oxidants Vitamins C and E or combination of the two vitamins help to reversed the haematoxic damage by increasing the haemoglobin and reducing the white cell count caused by gasoline due to antioxidant nature of Vitamins C and E.

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REFERENCES

1. Frei, B., 2003. To C or not to C. That is the question! Am Coll. Cardiol., 42: 253-255.

2. Sano, M., C. Ernestoe, R.G. Thomas, M.R. Klauber, K. Schafer, M. Grundman, P. Woodbury, J. Growdon, C.V. Cotman, E. Pfeiffer, L. Schneider and L.J. Thal, 1997. A controlled trial of selejihini alpha-tocopherol, or both as treatment for Alzheimers disease. N. Engl. J. Med., 336: 1216-1222.
3. Saloneu, R.M., 2003. Six year effect of combined vitamin C and E supplementation on artheleroscleroticprogression. Circulation, l: 947-953.
4. Dede, E.B. and C. Ngawuchi, 2003. The Effect of Vitamin C on gasoline poisoned rats.Proceedings of Nigeria Environmental Society Conference. 13th Annual General Meeting Bayelsa State pp: 16.
5. Macfarland, H.N., C.E. Ulrich, C.E. Holdworth, D.N. Kitchenv, W.H. Halliwell and S.C. Blum, 1984. A chronic inhalation study with unleaded gasoline vapour. J. American College of Toxicol., 3: 231-248.
6. Magaw, R.J., W.R. Richter and J.A. Mac Gregor, 1993. A reexamination of liver tumours in mice exposed to wholly vapourized unleaded gasoline. J. American College of Toxicol., 12: 195-199.
7. Standeven, A.M., D.C. Wolf and T.L. Goldwothy, 1995. Altered hepatic foci cell proliferation liver gasoline tumour promotion. Environmental Health Perspective, 105: 696-700.
8. Wachukwu, C.K., E.B. Dede, C.C. Ozoemena and S.E. Amala, 2004. Effect of gasoline on blood and liver functions of albino rats (*Ratus ratus*). J. Medical Laboratory Sci., 13: 1-5.
9. Dede, E.B., C.P.R. Chike and O.A. Adegoke, 2004. The effect of selenium (antioxidant) on gasoline toxicity in male albino rats (*Ratus ratus*). African J. Applied Zoology and Environmental Biol., 6: 128-134.
10. Pouls, M., 2000. Oral Chelation and nutritional replacement. Health Education Alliance for Life and Longevity, (HEALL), 5: 53.
11. Dede, E.B. and P.S. Igbigbi, 1997. Determination of LD_{50} of Metakelfin in rats. J. Science, Metascience, 111: 1-7.
12. Cheesbrough, M., 2000. Measurement of Hemoglobin: in District Laboratory Practice in Tropical Countries. Part II: 299-302.
13. Sood, R.D., 1990. White blood cell count in: Medical Laboratory Technology (Methods and Interpretation) Published by Jaypee Brothers Medical Publishers, New Delhi, India. pp: 152-160.
14. Ovuru, S.S. and I.K.E. Ekweozor, 2004. Haematological changes associated with crude oil ingestion in experimental rabbits. Afr. J. Biotech., 3: 346-348.
15. Leighton, F.A., Y.Z. Lee, A.D. Rahimtula, P.J. O'Brien and D.B. Peakall, 1985. Biochemical and functional disturbance effect in red blood cells of herring gulls ingesting Prudhoe Bay Crude oil. Toxicology and Applied Pharmacol., 81: 25-31.
16. Sunmonu T.O. and O.B. Oloyede, 2008. Haematological response of African Catfish (*Clarias gariepinus*) and rat to Crude oil exposure. The Internet J. Haematol., 4(1): 20-26.
17. Akaninwor, J.O., E.A. Okeke and E.O. Ayalogu, 2006. Effect of diets contaminated with crude petroleum products (Bonny light and Forcados) on the haematological parameters of Wistar albino rats. J. Nigerian Environmental Society, 3: 160-166.
18. Liu, Z.L., 1995. Antioxidant activity of Vitamin E and C derivatives in membrane mimetic systems In: Bioradicals detected by ESR *spectroscopy*. Ohyanishiguch, H. and Packer, L., Eds. Birkhauser, V.B., Switzerland, pp: 259-275.
19. Joseph, N.M., M. Sabharwal, A. Shashi, A. Mahor and S. Rawal, 2009. Intl. J. Pharmaceutical Sciences and Research, 1: 1.
20. Sies, H., 1997. Oxidative stress: oxidants and antioxidants Exp Physiol., 82: 291.
21. Braide, A.S., O.A. Adegoke, E.O. Bamigbowu and M.B.O. Ayodele, 2011. Effect of sugar on some heamatological parameters in albino rats fed with petroleum contaminated diet. International J. Applied Biological Res., 3: 90-99.
22. Braide, A.S., O.A. Adegoke and E.O. Bamigbowu, 2011. Effect of Cassava based diet on hepatic proteins in albino rats fed with crude oil contaminated diet. J. Appl. Science and Environmental Management, 15: 223-229.
23. Hogstedt, B., A. Holmen, A. Karlson, G. Rarhle, K. Nillius and K. Vestlund, 1991. Petrol Pump Mechanics had increased frequencies and sizes of Micronuclei in Lymphocytes stimulated by Pokeweed Mitogen. Mutation Res., 263: 51-55.
24. George I.M. and O.A. Adegoke, 2011. Effect of vitamin E on biochemical parameters in albino rats treated with gasoline. J. Scientific Res., 3: 641-649.