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Effect of Vitamins C and E Intake on Blood Lipid Concentration, Lipid Peroxidation, Superoxide Dismutase and Catalase Activities in Rabbit Fed Petroleum Contaminated Diet

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Abstract: The effect of exposure to petroleum contaminated diet on the blood antioxidant defence system, lipid peroxidation and lipid profile as well as possible protective roles of vitamins E and C were studied in rabbits. Oxidative stress induction by crude oil was indicated by significantly ($P<0.05$) increased lipid peroxidation and a non-significant decrease in superoxide dismutase and catalase activities. A similar pattern was also detected in the lipid profile: total cholesterol and LDL-cholesterol insignificantly increased while HDL-cholesterol and triglyceride significantly decreased relative to rabbits fed normal diet. The reciprocal relationship between HDL-cholesterol and LDL-cholesterol in addition to compromised antioxidant enzymes could predispose exposed animals to coronary heart disease. However, pre-treatment of the diet with vitamins C and E exhibited a protective role on the toxic effect of crude oil on lipid profile, lipid peroxidation as well as antioxidant enzymes. The order of protection was vitamins E + C > vitamin E > vitamin C. These observations seemed to suggest that the protective role of vitamins C and E is synergistic. The protective role of the vitamins is probably time-dependent as significant ($P<0.05$) restoration of lipid profile as well as antioxidant enzymes activities to control values was effected after four weeks of exposure. It is therefore suggested that toxic effect of petroleum may be reduced by dietary supplementation of vitamins C and E.

Key words: Coronary heart disease, catalase, lipid profile petroleum, superoxide dismutase

Introduction

Nigeria is a major petroleum producing country. One drastic effect associated with its exploration and exploitation is the contamination of the immediate environment with petroleum hydrocarbons (Amadi *et al*., 1993). Most of the land in oil producing areas in Nigeria is under cultivation because the mainstay of people living in the region are farming and fishing (Egborge, 1991). This may result in contamination of agricultural produce. The petroleum hydrocarbon eventually gets into man and animals through ingestion of contaminated food or bioconcentration through the food chain (Jessup and Leightion, 1996).

The ingestion of petroleum hydrocarbon has been reported to induce oxidative stress (Val and Almeida-Val, 1999) through the generation of free radical (Achuba and Osakwe, 2003). It has been established that free radical generation with subsequent oxidative modification leads to lipid peroxidation (Halliwell, 1994) that damages critical cellular macromolecules such as DNA, lipids and proteins (Breimer, 1990; Romero *et al*., 1998; Souza *et al*., 1999); that results in inactivation of antioxidant enzymes (Pigeolet *et al*., 1990).

Vitamin E is a lipid soluble free radical scavenger that protects the membrane from lipid peroxyl radical (Buttner and Burns, 1996). Similarly, vitamin C is the water soluble antioxidant that reacts with peroxyl radicals formed in the cytoplasm before they reach the membrane (Khoja and Marzouki, 1994); and serves to regenerate the reduced Vitamin E (Tanaka *et al*., 1997). A good number of studies have established the effectiveness of antioxidant Vitamins against oxidative stress (Farris, 1991; Verma and Nair, 2001; Ognjanovic *et al*., 2003). The aim of this paper was to establish a possible protective role of Vitamins C and E against petroleum induced oxidative stress.

Materials and Methods

Twenty five male English rabbits (initial mean weight 1.45kg), aged about 16 weeks were purchased from a local animal dealer in Benin City, Nigeria. The animals were housed singly in clean metal hutches and acclimatised on growers mash (Product of Bendel Feed and Flour Mills (BFFM) Ltd, Ewu, Nigeria for three months prior to the commencement of the experiment. The animals were divided into five groups, each containing five rabbits. Members of each group were housed singly in clean metal hutches and the feeding was conducted at room temperature of 28°C, with lighting for 12hr each day. Rabbits in the control group were fed with growers mash only, while group A was fed with feed contaminated with crude oil at 2.5% (w/w) / kg of feed. Group B were fed with mash contaminated with petroleum at 2.5% (w/w) / kg of feed plus 500mg of vitamin E. Group C were fed with feed contaminated with petroleum at 2.5% (w/w) / kg of feed plus 500mg of

Table 1: Effect of Vitamin C, E and C+E intake on level of lipid peroxidation, in rabbit fed contaminated diet

	Weeks of Exposure	Normal diet	Diet + crude petroleum			
			Control	Vit C	Vit E	Vits. C+E
Lipid peroxidation MDA/ml	0	11.2±1.33	11.3±1.8	11.±1.6	11.2±1.1	11.3±1.41
	1	11.6±1.41	14.4±1.93	13.3±191	14.6±1.21	11.9±1.66
	2	12.1±1.21	16.3±1.08	15.1±2.23	15.5±3.26	13.6±3.01
	3	12.3±1.33	20.2±1.11*	15.8±2.39	16.3±3.26	13.8±2.72
	4	12.3±1.60	21.1±2.11*	16.6±2.43	15.8±2.31	14.4±2.61
	5	11.8±1.40	22.1±1.80*	16.9±2.51	15.3±2.26	14.3±3.33
	6	11.6±1.43	22.0±1.79*	17.9±2.46	15.4±2.28	14.2±2.54
	7	12.0±1.52	22.1±2.0*	15.8±3.11	16.1±2.31	13.4±3.10

Results are expressed as means ± SEM of determinations from five rabbits P<0.05 significant difference of rabbits fed contaminated diet relative to rabbit fed normal diet.

vitamin C. Group D was fed with feed contaminated with petroleum at 2.5% (w/w) / kg of feed plus 500mg each of vitamin E and C. The animals were exposed to the feed for seven weeks.

Preliminary investigation had established that this level of petroleum oil was tolerable to the rabbits on a prolonged basis without any drastic effect. The petroleum feed was prepared fresh everyday. Before administration of feed, the feeds were mixed with water so as to achieve a texture acceptable to the animals. Clean drinking water was liberally provided while stale feed remnants were regularly discarded.

At the end of each period, blood samples were collected from veni puncture of the ear into heparinized tubes and plasma separated by centrifugation at 3000g for 20 min at 4°C. Aliquots of centrifuged plasma were taken for the determination of total cholesterol, high density lipoprotein cholesterol, low-density lipoprotein-cholesterol and triglycerides using enzymatic methods (Siedel *et al.,* 1981, Wahlefeld, 1974; Burstein *et al.,* 1970). The concentration of lipid peroxide (LP) was determined as thiobarbituric acid reactive substance (TBARS) in the blood according to Gutteridge and Wilkins (1982).

Blood for the determination of antioxidant enzymes was centrifuged to separate plasma and erythrocytes. Isolated erythrocytes were washed three times with 3 volumes of ice-cold 155 mmol/NaCl and hemolysates containing about 50g haemoglobin per litre (McCord and Fridovich, 1969) was used for the determination of catalase activities according to Beutter (1982). For the determination of superoxide dismutase (SOD) activity by the epinephrine method of Misra and Fridovic (1972), lysates were diluted with distilled water (1:7v/v) and treated by chloroform-ethanol (0.6:1v/v) in order to remove heamoglobin according to Tsuchihashi (1923). Manganese dependent SOD was analyzed in the presence ImM NaCN to suppress Cu/ZnSOD activity and the cytosolic Cu/ZnSOD activity was determined as the difference between total and cyanide-sensitive enzyme activity (Crapo *et al.,* 1978). The enzyme activities were assayed with a SP 1800 UV/VIS spectrophotometer. All reagents used were of analytical grade.

The results were expressed as means ± SEM. All results were compared with respect to the control animals as well as to the animals fed with crude oil only. Comparisons between treated and control rabbits were made by using analysis of variance and the Duncan Multiple Range Test used to compare the means. P<0.05 were considered as significant.

Results

The results (Table 1) indicated a significant (P<0.05) increase in lipid peroxidation in rabbit fed crude petroleum contaminated diet relative to animals fed normal diet. But adding vitamins restored the levels of lipid peroxidation to control values.

Petroleum – induced changes in plasma total SOD, Cu/ZnSOD and MnSOD are depicted in Table 2. A non significant decrease in the activities of total SOD and Cu/ZnSOD relative to animals fed non contaminated diet was observed. surprisingly, a significant (P<0.05) increase in MnSOD was noted. However, pre-treatment of the diet with antioxidant vitamins tended to restore the activities of the enzyme to control values.

Table 3 shows the alterations in catalase activity by crude petroleum contaminated diet. The activity of the enzyme decreased in rabbits fed petroleum contaminated diet relative to rabbits fed normal diet. However, these changes tended to be restored to control values by pre-treatment of the diet with antioxidant vitamins. The alteration in the lipid profile of rabbits fed petroleum contaminated diet is depicted in Table 4. There is an insignificant increase in total cholesterol and LDL-Cholesterol. However a significant (P<0.05) decrease in HDL-cholesterol and triglycerides in animals fed petroleum contaminated diet relative to animals fed normal diet was observed.

Discussion

Consumption of crude oil contaminated diet induced the formation of lipid peroxidation products in the blood of

Table 2: Effect of Vitamin C, E and C + E intake on the level of superoxide dismutase enzyme activities of rabbit fed crude petroleum contaminated diet

		Diet + crude petroleum				
	Weeks of Exposure	Normal diet	Control	Vit C	Vit E	Vits. C+E
Total SOD Units/ml	0	8.98±3.11	8.93±2.60	8.97±3.00	8.92±0.00	8.98±3.01
	1	9.51±2.17	10.3±2.21	9.96±2.41	10.0±1.11	11.2±2.31
	2	9.66±1.88	8.76±1.92	8.97±1.99	8.89±2.3	±10.11±1.22
	3	9.81±2.16	8.02±2.11	7.66±1.71	8.45±2.11	9.00±3.11
	4	9.68±2.13	7.11±1.00	7.43±1.64	8.21±2.13	9.12±1.66
	5	9.65±2.00	7.02±1.12	7.21±1.87	8.10±1.66	9.03±1.81
	6	9.68±2.16	7.04±1.28	7.30±1.65	8.28±1.53	8.26±1.81
	7	9.88±2.11	6.92±2.3	8.11±1.77	8.08±1.60	9.01±1.94
Cu/Zn SOD Units/ml	0	8.13±1.42	8.14±1.10	8.13±1.40	8.11±1.50	8.15±1.60
	1	8.20±1.51	8.81±1.21	9.05±1.66	8.72±1.6	10.0±2.4
	2	8.23±1.80	7.64±1.99	8.87±1.72	8.11±1.8	9.21±1.61
	3	8.24±1.84	7.02±2.11	7.52±1.63	7.62±1.11	8.32±1.88
	4	8.23±1.79	6.34±1.66	6.61±1.54	6.09±2.0	7.55±1.92
	5	8.22±1.81	6.01±1.32	6.42±1.48	6.99±2.11	7.18±2.0
	6	8.22±1.83	5.90±1.29	6.04±1.33	7.18±1.40	7.14±1.58
	7	8.22±1.86	6.10±1.31	7.4±1.85	7.20±1.51	8.02±1.44
MnSOD Units/ml	0	0.52±0.24	0.55±0.21	0.56±0.22	0.52±0.11	0.56±0.12
	1	0.53±0.22	0.91±0.24	0.80±0.12	1.00±0.12	1.11±0.11
	2	0.49±0.41	0.99±0.31*	1.11±0.11	1.22±0.10	1..31±0.10
	3	0.55±0.21	1.01±0.32*	1.21±0.27	1.30±0.20	1.40±0.20
	4	0.53±0.11	1.05±0.22*	1.16±0.31	1.33±0.30	1.42±0.22
	5	0.51±0.24	1.07±0.31*	15.1±0.43	1.31±0.31	1.43±0.33
	6	0.50±0.33	1.08±0.23*	1.18±0.26	1.30±0.29	1.42±0.31
	7	0.51±0.24	1.06±0.33*	1.20±0.41	1.31±0.20	1.43±0.22

Results are expressed as means ± SEM of determinations from five rabbits P<0.05 significant difference of rabbits fed contaminated diet relative to rabbit fed normal diet.

Table 3: Effect of Vitamin C, E and C + E intake on the level of catalase enzyme activities of rabbit fed crude petroleum contaminated diet

		Diet + crude petroleum				
	Weeks of Exposure	Normal diet	Control	Vit C	Vit E	Vits. C+E
Catalase (Unit / ml)						
	0	7.11±1.71	7.12±1.62	7.11±1.81	7.13±1.55	7.31+1.66
	1	7.21±1.62	6.41±1.31	6.32±1.92	6.60±1.71	7.03+1.55
	2	7.00±2.11	6.11±1.21	6.06±1.73	6.20±1.82	6.60+1.22
	3	7.21±1.66	6.03±1.11	6.03±1.61	6.12±1.52	6.41+1.78
	4	7.12±1.72	5.63±1.30	5.58±1.62	5.60±1.66	6.56+1.66
	5	7.07±1.66	4.44±0.22*	5.56±1.44	5.11±1.16	6.55+1.51
	6	7.08±1.58	4.28±0.95*	5.52±1.23	5.08±1.13	6.54+1.45
	7	7.11±1.43	4.30±1.10*	5.50±1.33	5.3±1.16	6.71+1.36

Results are expressed as means ± SEM of determinations from five rabbits *P<0.05 significant difference of rabbits fed contaminated diet relative to rabbit fed normal diet.

rabbitl.

Various authors have earlier posited that petroleum induces the formation of free radicals, which react with some cellular components such as membrane lipids and produce lipid peroxidation products (Onwurah, 1999; Shertzer *et al.*, 1994; Bronk and Gores, 1991; Anozie and Onwurah, 2001; Khan *et al.*, 2001). This predisposes the animals to oxidative stress (Halliwell 1989; 1992 Liu and Mori, 1994). Besides acting as a mediator in oxidative stress, higher levels of lipid

Table 4: Effect of Vitamin C, E and C+E intake on plasma lipid profile of rabbit fed crude oil

Lipid Type	Weeks of Exposure	Normal diet	Contaminated diet			
			Control	Vit E	Vit C	Vits. E+C
Total Cholesterol (mg/dl)						
	0	62.3±0.5	61.3±0.4	62.8±0.5	62.3±0.8	63.3±0.6
	1	61.9±0.3	63.3±0.1	64.1±1.0	64.5±1.3	65.1±1.4
	2	62.1±0.8	64.8±0.4	63.6±0.7	65.6±0.8	64.3±1.2
	3	62.3±0.3	65.1±0.8	63.2±0.1	64.9±0.4	63.1±0.5
	4	62.5±1.1	65.3±1.3	61.5±1.2	64.7±1.3	64.3±0.6
	5	63.4±0.7	65.5±1.2	61.8±1.3	64.7±0.3	64.1±0.8
	6	62.2±0.5	67.4±0.7*	62.8±0.5	64.8±0.5	63.2±0.3
	7	63.5±0.6	67.1±0.5*	63.1±0.3	64.3±0.6	63.8±1.1
HDL-Cholesterol (mg/dl)	0	40.1±1.3	41.2±1.4	40.3±1.6	41.2±1.5	40.2±1.6
	1	41.9.±0.7	40.3±1.6	41.4±1.1	41.8±1.7	42.5±1.8
	2	42.0±0.8	36.8±1.7	41.8±0.6	36.7±1.0	40.3±1.4
	3	41.8±1.1	35.5±2.0	39.6±0.5	36.4±0.8	40.0±0.8
	4	41.7±1.4	34.4±1.8*	38.1±1.3	35.7±1.3	38.8±1.6
	5	41.6±1.3	33.2±0.5*	38.2±1.4	35.8±1.5	39.6±1.4
	6	41.8±0.8	35.2±1.1	37.0±0.5	36.0±0.3	38.0±0.6
	7	41.6±0.7	34.3±2.1	37.3±1.4	35.4±0.7	39.1±1.3
LDL-Cholesterol (mg/dl)	0	35.1±0.1	35.0±1.0	35.2±0.3	35.1±0.1	35.1±0.2
	1	34.6±2.2	35.61±0.7	35.5±1.6	36.3±0.3	34.3±1.5
	2	35.5±1.4	36.8±0.5	36.2±0.1	36.7±1.1	35.3±2.2
	3	36.3±1.2	38.8±1.5	37.1±0.2	37.3±1.3	36.0±1.8
	4	36.1±0.11	40.3±1.7*	37.3±0.5	38.1±1.2	36.3±1.9
	5	36.6±0.5	41.0±2.2*	37.4±0.6	38.4±1.4	36.4±0.8
	6	36.0±0.6	40.2±.08*	37.4±0.5	38.4±.05	36.8±0.8
	7	38.6±1.86	44.50±2.0	37.1±1.0	38.3±2.0	36.2±1.0
Triglycerides (mg/dl)	0	73.3±1.3	73.4±1.6	74.1±1.7	73.3±1.8	73.3±2.3
	1	74.1±1.7	62.0±3.0	63.3±2.2	60.0±3.0	71.5±4.1
	2	74.3±1.6	58.8±2.2	70.1±2.4	57.1±2.0	68.9±3.5
	3	75±0.21	58.1±1.5*	65.6±3.0	58.8±2.5	70.5±1.6
	4	73±0.11	54.6±1.3*	64.7±2.9	56.7±3.3	71.6±3.5
	5	71±0.24	51.1±3.1*	64.5±3.3	50.1±3.0	71.1±2.2
	6	70±0.33	50.0±1.4*	64.8±1.1	51.4±1.5	70.0±1.4
	7	71±0.24	48.5±2.3*	66.6±3.3	54.5±2.1	71.2±1.8

Results are expressed as means SEM of determinations from five rabbits *P<0.05 significant difference of rabbits fed contaminated diet relative to rabbit fed normal diet.

peroxidation affect cellular functions; culminating in disease processes (Munkittrick *et al*., 1998; 2000; Bailey *et al.,* 1992; 1996).

Moreover, the process of lipid peroxidation is under the control of an efficient cellular system involving among others SOD and catalase (Shika, 1996) that works in tandem to dismutate reactive oxygen species (Achuba and Osakwe, 2003). A decrease in the activities of these enzymes is accompanied by increased formation of reactive oxygen species (Trush and Kensler, 1991; Sies, 1991). This tallies with the non significant decrease in the activities of SOD (Table 2) and catalase (Table 3) obtained in this study and earlier observation of Pigeolet *et al*. (1990) who reported that oxygen radicals and peroxide are able to inactivate antioxidant enzymes.

The role of free radicals in the pathogenesis of altherosclerosis via oxidation of low-density lipoprotein which damages the arterial walls has been recognized (Harman, 1992). The ingestion of crude petroleum contaminated diet imposed a reciprocal relationship between HDL-cholesterol and LDL-cholesterol in the plasma of rabbit (Table 4). The decrease in HDL-cholesterol with a corresponding increase in LDL-cholesterol is a primary risk factor for coronary heart disease (Mckee and Mckee, 1999; Glew, 1997). Ben-David *et al*. (2001) submitted that the ingestion of petroleum caused reduction in blood glucose. This may shift the demand for metabolic substrate to lipid, thus explaining the significant decrease in the level of triglycerides in rabbits fed the petroleum contaminated diet relative to control animals (Table 4). Increase in the metabolism of lipids has been reported to induce generation of free radicals (Patockova *et al.,* 2003). However, a number of studies have established that antioxidant vitamins prevent lipid oxidation and oxidative damage, thus impede the progression of

atherosclerosis (Berry, 1992, Gaziano and Hennekens, 1992; Sato *et al.,* 1990).

The pretreatment of the feed with vitamins E and C during the exposure to the experimental rabbits decreases the toxic effect of crude oil. This is exhibited in the restoration of antioxidant enzymes and lipid profile to control values (Table 1, 2, 3 and 4). The protective role of these vitamins is greater when vitamins E and C were administered simultaneously. However, vitamin E was more effective then vitamin C, possibly because vitamin E protects the polyunsaturated fatty acid from peroxidation whereas vitamin C acts in the water-soluble compartment and has a sparing effect on vitamin E by regenerating the reduced form of vitamin E (Khoja and Marzouki, 1994; Tanaka *et al.,* 1997). Thus vitamins E and C were reported to synergististicaly inhibit oxidation of LDL-cholesterol (Sato *et al.,* 1990) and prevent cell destruction (Beyer, 1994; Chen and Tappel, 1995; Lass and Sohal, 2000). Moreover, there is also evidence that vitamin C increase HDL-cholesterol level and may also lower total cholesterol in the blood, thus reducing the risk of cardiovascular disease.

It is pertinent to conclude that ingestion of petroleum contaminated diet could predispose humans to cardiovascular diseases. However supplementation of diet with vitamins E and C could ameliorate petroleum-induce changes in lipid profile and antioxidant enzymes. Comparatively, vitamin E appeared more effective than vitamin C. On the whole, the data presented depict that vitamins E and C could act synergistically in preventing oxidative damage to lipids.

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