

PROTECTIVE EFFECTS OF VITAMINS C AND E AGAINST GASOLINE VAPOURS INDUCED HAEMATOLOGICAL, BIOCHEMICAL AND LIPID PROFILE CHANGES IN MALE RATS

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ABSTRACT

The present work was designed to investigate the changes in some hematological, biochemical parameters and lipid profile as well as possible protective role of vitamin C and E against gasoline vapours induced male rats.

The rats exposed to gasoline vapours were divided into 4 groups: group-1 treated with gasoline vapours only, group-2 treated orally with 200 mg/kg body weight of vitamin C and group-3 treated with 150 mg/ kg body weight of vitamin E and group-4 treated with vitamins C and E. The duration of the treatment was 6 weeks. Fasting blood samples were collected serum were prepared for estimation of some haematologic parameters (Hb content, PCV, RBCs count and lipid profile (total cholesterol, triglyceride (TGs), (HDL-C), (LDL-C) and (VLDL-C). Liver function was assessed by measuring the activities of liver marker enzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)). Total serum protein (TSP) and albumin were measured. The present results showed that gasoline vapours caused significant ($p \leq 0.05$) decrease in TSP and albumin concentration and significant ($p \leq 0.05$) increase in serum ALP, ALT and AST activities in comparison with the control group.

Also, exposure to gasoline vapours induced significant ($p \leq 0.05$) decrease in Hb, PCV, RBC and significant ($p \leq 0.05$) increase in TC, TGS, LDL-C and VLDL-C and decrease in HDL-C compared to control. However concomitant treatment with gasoline vapours and administration of vitamins C and E exhibited a protective role on the observed toxic effect of gasoline vapours in male rats.

The results of the present study indicated that toxic effects of gasoline vapours may be reduced by dietary supplementation of vitamins C and E.

Keywords: Gasoline Vapours, vitamin C, Vitamin E, lipid profile, Haematology, Liver enzymes, Serum total protein, Albumin.

INTRODUCTION

Gasoline, a fractionated product of crude oil, is a mixture of over 500 hydrocarbons that may have between 3-12 carbon atoms. It is used as fuel for automobiles and some electricity-generating machines (Kinawy, 2009 and Uboh *et al.*, 2012). Human population is directly or indirectly exposed to this pollutant in the course of their day-to-day activities (Rabble and Wong, 1996). Gasoline enters the body orally (accidental), Transdermally, and by inhalation but inhalation route is more important because more persons may be affected (Ugwoke *et al.*, 2005). Source of exposure to gasoline involves refueling stations, motor mechanical workshops, and traffic-congested areas and occupationally (Rabble and Wong, 1996). It is well known that exposure to gasoline vapours cause serious health hazards to humanity (Uboh *et al.*, 2009).

It has been reported that gasoline vapours induced changes in serum lipid profile and haematotoxicity (Uboh *et al.*, 2007a, 2007b). Also, several studies have reported that petroleum and its products have the ability to increase the level of liver markers enzymes (Iheoliaha, 2009; Udem *et al.*, 2009 and Mattie *et al.*, 1995). Vitamin A, vitamin C, vitamin E and selenium are among nutrients with antioxidant activity. Humans obtain vitamin C from vegetable, fruit and other plant food because they are unable to synthesize it. Vitamin C can suppress activities of free radicals such as reactive oxygen species (ROS), the high levels of which are thought to initiate cancer through oxidative damage to DNA. Vitamin E is the major lipid-soluble antioxidant in serum and has the ability to inhibit the generation of singlet oxygen, free radicals, lipid radicals and lipid hydroperoxides (Datta *et al.*, 2012).

Oxidative stress is one among the molecular mechanisms responsible for the toxicity associated with exposure to gasoline vapours, which disturbs the antioxidant defense system and produced alteration in lipid peroxidation (Uboh *et al.*, 2007a, 2008b).

From the above mentioned data, it was thought that it is important to investigate the possible protective effect of vitamins C and E against haematological, biochemical and lipid profile changes induced by exposure to gasoline vapours in male rats.

MATERIALS AND METHODS

Experimental animals:

This study was carried out on twenty male rats (Wistar albino). The rats weighed 180-200 g and were of more or less the same age (3-3.5 month old). The experimental animals were obtained from the animal house of faculty of medicine (Alexandria University). They were allowed to acclimatize, one week before the commencement of the experiment, under normal laboratory conditions and were allowed free access of a standard balanced laboratory diet (wheat, milk and carrot) and tap water. Animals were randomly divided into eight groups of five animals each and were housed in steel cage.

Treatment of rats with vitamins E and C:

Vitamins E and C were solubilized in olive oil and distilled water solvent, respectively. Prophylactic doses of vitamin E (150mg/kg body weight) and vitamin C (200 mg/kg body weight) were used in the present study. Administration was done by oral gavaging using intragastric syringe. The used vitamin solutions were kept at room temperature and protected from direct contact with air and light to avoid degradation.

Exposure to gasoline vapours:

The experimental animals were exposed to gasoline vapours for one hour daily for 6 weeks. Exposure was achieved by using the method of Ugwoke *et al.* (2005): soaking 5 ml of commercially procured gasoline 80 in 20 g cotton wool, which was plastered on one end of the animal cages. The vapour was allowed to mix with the ambient air of the cages and the larger environment and about 1-2 ml of the gasoline was vapourized in the ambient air of each animal cage for one hour. The exposure modality stimulated a general occupational oil depot environment in which gasoline saturated the ambient air to which unprotected workers in the fuel station are exposed daily for hours. The piece of soaked cotton was daily changed.

Gasoline was obtained from fuel station as 1 liter clean bottle. It was used concentrated with octane number 80 (red color).

Experimental design:

The 4 rat groups were arranged as follows:

G1: Control (n = 5) allowed to respire fresh air with no exposure to gasoline vapours. G2: Negative control (n = 5) daily exposed to vapours of gasoline solution of octane number 80 only for 1 hour for 6 weeks. G3: (n = 5) daily exposed to vapours of gasoline solution of octane number 80 only for 1 hour and concomitantly treated with vitamin (C) and vitamin E for 6 weeks. G4: (n = 5) administered with vitamins C and E for 6 weeks.

Preparation of blood

At the end of the experiment, blood sample was collected from each animal from retro-orbital sinus of the eye (after a mild anesthesia by inhalation) using heparinized capillary tube into clean bottle containing ethylene diaminetetracetic acid (EDTA) as anticoagulant for haematological analysis.

Determination of haematological parameters

Faulkner and King Method (1970) was used for determination of red blood cell count (RBCs) and packed cell volume (PCV) or haematocrit value. Reagent kits for determination of for haemoglobin content (Hb) were purchased from Biodiagnostics (Egypt).

Preparation of serum

Part of the collected blood, using capillary tube into clean bottle, was allowed to clot for a minimum of 30 min before centrifuged to obtain serum (1500 x 15 min) and stored at -20°C until the analysis.

Determination of serum lipid concentration.

Aliquots of serum were taken for the determination of total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C) using kits purchased from BIOMED Diagnostics (Germany), SPINREACT (Spain), and BioSystems (Spain). Very low density lipoprotein cholesterol (VLDL-C) and Low density lipoprotein cholesterol (LDL-C) were calculated by the following equations:

$$\text{VLDL-C} = \text{TGs}/5$$

$$\text{LDL-C} = \text{TC} - (\text{TGs}/5 + \text{HDL}).$$

The biochemical analysis

Activities of serum aminotransferases (ALT and AST) and alkaline phosphatase were measured using BioMed Diagnostics kit, Germany, according to the instructions of the supplier. Serum total protein level was carried out according to the manual of BioMed Diagnostics kit, Germany. Serum albumin level was determined as outlined in SPECTRUM Diagnostics kit.

Statistical analysis

Results were presented as mean \pm standard error of the mean (SE) obtained from five animals. SPSS program was used for the statistical analysis of data with (one -way ANOVA) to compare the groups. In all the cases, a difference was considered significant $p \leq 0.05$.

RESULTS

The mean and standard error (SE) of the haematological parameters of all experimental groups are presented in Table 1.

The results of this study showed that inhalation of gasoline significantly ($p \leq 0.05$) lower the values of Hb content, PCV and RBC count at a mean level of $10 \pm 0.20\%$, $34 \pm 0.58\%$ and 3.81 ± 0.11 million cells/ μL respectively relative to a mean level of $12.44 \pm 0.12\%$, $45 \pm 1.45\%$ and 4.52 ± 0.08 million cells/ μL obtained for the control group. Inhalation of gasoline 80 with concomitant treatment with vitamin C and vitamin E significantly ($p \leq 0.05$) increase the values of Hb content, PCV and the RBC count at a mean level of $11.90 \pm 0.07\%$, $39.2 \pm 1.02\%$ and 4.12 ± 0.08 million cells/ μL when compared to gasoline 80 inhaled group. No significant ($p \leq 0.05$) changes in the studies parameters were observed among group 4 when compared to control.

From Table 2, it is obvious that inhalation of gasoline 80 significantly ($p \leq 0.05$) increased the values of total cholesterol (Tc), triglycerides (TGs), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) at a mean level of 204.75 ± 1.49 mg/dl, 206.5 ± 2.53 mg/dL, 160.20 ± 1.04 mg /dL and 32 ± 0.41 mg /dL respectively relative to a mean level of 112.40 ± 2.14 mg /dL, 125.4 ± 1.08 mg /dL, 68.16 ± 0.57 mg /dL and 22.4 ± 0.81 mg /dL for control group. Also, the results showed that high density lipoprotein-cholesterol (HDL-C) level was significantly ($p \leq 0.05$) decreased at mean level of 32.75 ± 0.85 mg /dL relative to that obtained for control rats (44 ± 0.63 mg /dL).

Inhalation of gasoline 80 with concomitant treatment with vitamin C and vitamin E significantly ($p \leq 0.05$) decreased the values of TC, TGs, LDL-C and VLDL-C at a mean level of 183.75 ± 1.89 mg /dL, 138.50 ± 1.04 mg /dL, 93.04 ± 0.93 mg /dL and 26 ± 0.59 mg /dL when compared to gasoline 80 inhaled group.

The results also showed that inhalation of gasoline 80 with concomitant treatment with vitamin C and vitamin E significantly ($p \leq 0.05$) increased the HDL- C value at a mean level of 36.80 ± 1.02 when compared to gasoline 80 inhaled group.

No significant ($p \leq 0.05$) changes in the studies parameters were observed among group 4 when compared to control. Here, it was observed that vitamin c and vitamin E showed ameliorative effect against gasoline 80 induced haematological and lipid profile changes.

As shown in Table 3, the results of this study revealed that the activities of ALT, ALP and AST obtained for male rats exposed to gasoline vapours only were significantly ($p \leq 0.05$) increased at a mean of 65.0 ± 1.78 , 126.0 ± 1.91 and 97.75 ± 1.25 (U/L), respectively relative to a mean level of 32.80 ± 2.50 , 45.40 ± 0.93 and 32.60 ± 0.68 (U/L) compared with activities obtained for rats in control group. The activities of ALT, ALP and AST in rat exposed to gasoline vapours and concomitantly treated with vitamin C and vitamin E were significantly ($p \leq 0.05$) lower at a mean of 51.50 ± 2.25 , 76.50 ± 2.10 and 64.25 ± 1.80 (U/L) compared to the activities obtained for male rats exposed to gasoline vapours only. Also, the results of this study (Table 1) showed that exposure to gasoline vapours significantly ($p \leq 0.05$) decreased the concentrations of serum total protein and albumin at a mean of 6.23 ± 0.06 (g/dL) and 3.49 ± 0.9 (g/dL), respectively relative to a mean level of 8.73 ± 0.09 (g/dL) and 5.65 ± 0.9 (g/dL), respectively compared with activities obtained for rats in control group. It was observed that there was a significant ($p \leq 0.05$) increase in serum total protein and albumin in rat exposed to gasoline vapours and concomitantly treated vitamin C and vitamin E. No significant ($p \leq 0.05$) changes in the studied parameters were observed among group IV when compared to control.

Table 1. The effect of gasoline vapours on Hb, PCV and RBCs in male rats.

	N	Hb (%)	PCV (%)	RBCs (million cells/ μ L)
Group I	5	12.44 ^a \pm 0.12	45.0 ^a \pm 1.45	4.52 ^a \pm 0.08
Group II	5	10.0 ^b \pm 0.20	34.0 ^b \pm 0.58	3.81 ^b \pm 0.11
Group III	5	11.90 ^c \pm 0.07	39.20 ^c \pm 1.02	4.12 ^c \pm 0.08
Group IV	5	12.24 ^a \pm 0.09	44.40 ^a \pm 0.93	4.37 ^a \pm 0.01
F (p)		19.103* (<0.001*)	10.136* (<0.001*)	12.633* (<0.001*)

Normally distributed data was expressed in mean \pm SE and was compared using F test (ANOVA) and was using Post Hoc Test (LSD) for comparison between groups

The different superscripts are significant

*: Statistically significant at $p \leq 0.05$

Table 2. Effect of vitamin C, E and C+ E intake on serum lipid profile of male rats exposed to gasoline 80 vapours.

	N	TC (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Group I	5	112.40 ^a \pm 2.14	125.40 ^a \pm 1.08	44.0 ^a \pm 0.63	68.16 ^a \pm 0.57	22.40 ^a \pm 0.81
Group II	5	204.75 ^b \pm 1.49	206.50 ^b \pm 2.53	32.75 ^b \pm 0.85	160.20 ^b \pm 1.04	32.0 ^b \pm 0.41
Group III	5	183.75 ^c \pm 1.89	138.50 ^c \pm 1.04	36.80 ^c \pm 1.02	93.04 ^c \pm 0.93	26.0 ^c \pm 0.59
Group IV	5	118.60 ^a \pm 1.72	127.40 ^a \pm 1.44	42.20 ^a \pm 0.37	69.60 ^a \pm 0.79	22.58 ^a \pm 0.31
F (p)		244.561* (<0.001*)	273.796* (<0.001*)	14.415* (<0.001*)	831.619* (<0.001*)	12.784* (<0.001*)

Normally distributed data was expressed in mean \pm SE and was compared using F test (ANOVA) and was using Post Hoc Test (LSD) for comparison between groups

The different superscripts are significant

*: Statistically significant at $p \leq 0.05$

Table 3. Effect of vitamin C and vitamin E on some biochemical parameters in male rats exposed to gasoline 80 vapours.

	N	ALT (U/L)	ALP (U/L)	AST (U/L)	Albumin (g/dL)	Total protein (g/dL)
Group I	5	32.80 ^a \pm 2.50	45.40 ^a \pm 0.93	32.60 ^a \pm 0.68	5.65 ^a \pm 0.06	8.73 ^a \pm 0.09
Group II	4	65.0 ^b \pm 1.78	126.0 ^b \pm 1.91	97.75 ^b \pm 1.25	3.49 ^b \pm 0.9	6.23 ^b \pm 0.06
Group III	4	51.50 ^c \pm 2.25	76.50 ^c \pm 2.10	64.25 ^c \pm 1.80	5.55 ^a \pm 0.16	7.58 ^c \pm 0.13
Group VI	5	35.80 ^a \pm 2.20	48.20 ^a \pm 1.83	33.20 ^a \pm 1.28	5.60 ^a \pm 0.13	8.30 ^a \pm 0.03
F (p)		23.110* (<0.001*)	282.180* (<0.001*)	262.811* (<0.001*)	28.260* (<0.001*)	96.648* (<0.001*)

The data was expressed in mean \pm SE and was compared using F test (ANOVA) and was using Post Hoc Test (LSD) for comparison between groups

The different superscripts are significant

*: Statistically significant at $p \leq 0.05$

DISCUSSION

Moszczynski and Lisiewicz (1983) and Synder and Hedli (1996) reported that some of gasoline vapours components are haematotoxic in humans (workers subjected to benzene, toluene and xylene) while Abubakar *et al.* (2013) reported the same observations in experimental animals (rats exposed to gasoline vapours). In the present study, gasoline 80 inhalation significantly reduced the PCV in treated male rats. This observation is in agreement with study of Igwebuiki *et al.* (2007) who reported that Nigerian Qua Iboe Brent crude oil reduced PCV in treated rats.

The present study showed that exposure to vapours of gasoline 80 decreased the RBC count, Hb content and PCV in treated rats. Similar results were obtained by Gangar *et al.* (2010) in mice treated with benzo (a) pyrene, Uboh *et al.* (2008) in rats treated with gasoline vapours, Orisakwe *et al.* (2005) in rats treated with Nigerian Bonny light crude oil, Youssef *et al.* (2003) in rats treated with cypermethrin and Al-Harbi *et al.* (1992) in rats treated with Doxorubicin.

Leighton *et al.* (1983) and Igwebuik *et al.* (2007) reported that treating rats with crude oil was associated with anemia, of the haemolytic type. Also, Gasoline 80 inhalation may cause anemia where there was a decrease in Hb content in treated rats. Since a number of crude oil components are lipophilic in nature, biological membranes may be the target sites where adverse effects of gasoline 80 occur. Lowering of RBC count coupled with low Hb content here may be due to destructive action of gasoline on erythrocytes and as a result of which the viability of the cells may be affected as reported by Karuppasamy (2000). Also, it was observed that gasoline 80 inhalation caused a significant decrease in RBC count while treatment with vitamin C and vitamin E ameliorated the observed alternations in haematological parameters induced by gasoline 80 vapours. Ameliorative effects of vitamins (A and E) on haematotoxicity induced by gasoline vapours were reported in some studies (Uboh *et al.*, 2008; Uboh *et al.*, 2010a).

The present study revealed that the values of TC, TGs, LDL-C, and VLDL-C increased while the value of HDL-C decreased in serum of gasoline 80 inhaled male rats compared with control. These results are similar to the work of Aberare *et al.* (2011) who revealed an increase in means of plasma TC and LDL-C levels in rats exposed to gasoline fumes, Uboh *et al.* (2008) who demonstrated that exposure to gasoline vapours increased TC, TGs, LDL-C, VLDL-C and decreased HDL-C in rats exposed to gasoline vapours, Uboh *et al.* (2005) discovered an increase in TGS and TC in rats exposed to petrol and kerosene fumes and Achuba (2005) revealed alternation in blood lipid concentration in rabbit fed petroleum contaminated diet. Similar results were observed in orchietomized rats who showed modulated plasma lipid profile (Deyhim, 2007) and patients on regular hemodialysis (Khajehdehi, 2000). Halim *et al.* (1997) and Owu *et al.* (1998) reported that triglyceride and cholesterol are used as indices of the liver cells damage. The increase in lipid profile, demonstrated in this study, may be an indication that inhalation of gasoline vapours may affect lipid metabolism as a result of liver damage. Oxidative stress and lipid peroxidation are among the main hypotheses of mechanism of hepatocyte injury (Public Health Service, 1995). It is well known that the oxidative stress produces reactive oxygen species (ROS) such as OH^\cdot , O_2^\cdot hydrogen peroxide, peroxy hydroxyl and superoxide radicals attack protein, lipid and nucleic acids which are important cell constituents provoking severe cellular alterations resulting in cell damage or death, due to their high reactivity. Peroxy radicals are formed by lipid peroxidation, which are central species in the peroxidation chain, and cause damage of cellular membranes and key organelles such as mitochondria (Robertson *et al.*, 2001 and Linden *et al.*, 2008). Increased lipid peroxidation and oxidative stress have been reported to be associated with exposure to gasoline vapours (Uboh *et al.* 2007). Oxidative stress is one of the mechanisms by which exposure to gasoline is involved in hyperglycemia and hypercholesterolemia (Uboh *et al.*, 2010). In this study, it was also observed that inhalation of gasoline 80 with concomitant treatment with vitamin C and vitamin E brought improvement in the alteration in serum lipid concentration induced by gasoline 80 vapours in male rats. Similar results were obtained by Khajehdeli (2000); Achuba (2005); Achuba and Otuya (2006) and Gaur and Dixit (2011). This effect of vitamins E and C may be due to their free radical scavenging activity, as antioxidants, and ability to inhibit lipid peroxidation. It has been proved that vitamins have possible protective role against oxidative stress induced by crude oil in blood of rabbit (Achuba and Otuya, 2006).

Atherosclerosis is a cause of deaths and is characterized by low level of high HLD-C, high levels of TC (hypercholesterolaemia), LDL-C and TGs in the plasma (Gambhir *et al.*, 2001; Nasiruddin and Ahmad, 2006). Exposure to gasoline vapours is a crucial factor responsible for the increase in atherosclerotic risk (Uboh *et al.*, 2008a). Ability of vitamins C and E to increase HDL-C level may be beneficial in reducing the risk of atherosclerosis caused by gasoline 80 inhalation.

Uboh *et al.* (2005) revealed that liver is one of the organs affected by gasoline vapours toxicity. The results of this study indicate that exposure of rats to gasoline vapour caused significant alterations in the biochemical parameters of liver function.

LAP, ALT and AST are among liver enzymes known as marker enzymes used for the assessment of the functional integrity of the liver cells (Jaeger and Hedegaard, 2002; Adaramoye *et al.*, 2008) and are usually raised in acute hepatotoxicity or mild hepatocellular injury (Jaeger and Hedegaard, 2002). In this study, there was a significant increase in the activities of serum ALP, ALT and AST following the exposure to gasoline vapour. Previous workers have also noted an increase in rat liver enzymes activities after chronic exposure to gasoline vapours (Harman *et al.*, 1981, Brad *et al.*, 1990, Wackukwu *et al.*, 2004 and Uboh *et al.*, 2012). Also, studies by Karpaki *et al.* (1998) indicated that the activities of ALT and AST were elevated in gas station workers. According

to Jacobs (1996) and Lin *et al.*, (2000), these marker enzymes are released into the circulation after cellular damage causing rises of the levels of these enzymes. The amounts of AST and ALT in blood are directly connected to the extent of the tissue damage. According to Reddy and Bhagyalakshmi (1994) and Kumar *et al.* (2005), the increase in serum ALP is perhaps due to increased synthesis of ALP while increase in ALP activity suggested enhanced breakdown of phosphates to release energy in view of impaired ATPase system. The AST and ALT are enzymes of amino acid metabolism and the increased energy demand, as a result of stress, may result in mobilization of potential energy sources including amino acid resulting in increase in ALT and AST (Reddy and Bhagyalakshmi, 1994). In this work, there was also a significant decrease in the level of protein and albumin concentration compared to control. These results agree with Iheoliola *et al.* (2009) study on female albino rats given water contaminated with 5% engine oil that shows a significant decrease in serum total protein and Uboh *et al.* (2012) who demonstrated that gasoline vapours induced decrease in serum total protein and albumin in male rats. The function of the liver is estimated by evaluating serum total protein and albumin. It is well known that albumin is among plasma protein synthesized by the liver. The significant decrease in serum total protein and albumin in gasoline 80 vapours exposed rats indicated that gasoline exposure in this study had an effect on the synthetic function of the liver. Jana and Bandyopadhyaya (1987) have stated that any toxic substance inhibits the protein synthesis activity. In case of lead, inhibition of protein synthesis may be due to the ability of lead to damage the endoplasmic reticulum which leads to reduction of protein synthesis (Sharma *et al.*, 2010). Similar effect may be done by lead present in gasoline. Sathyanarayana (2005) reported the metabolic status of proteins indicates the physiological status of animal. Also, Swamy *et al.* (1992) reported that the decrease in total proteins is due to their metabolic utilization. In addition, in this study, it was observed that administration of vitamins C and /or E to rats exposed to gasoline vapours produced an appreciable improvement in the hepatotoxic effect associated with exposure. The protective effect of vitamins against hepatotoxicity was documented by some authors. The protective effect of vitamin (A), (E) and vitamins C and E against gasoline – induced liver injury in rats was demonstrated by Uboh *et al.*, (2009), George and Adegoke (2011) and Uboh *et al.* (2012). Also, it has been shown that vitamins possess significant hepatoprotective activity against different hepatotoxic agents like Halothane, rifampacin, sodium nitrate and alcohol (Karakilick *et al.*, 2005, Tayal *et al.*, 2007, Krishnamoorthy and Sangeetha, 2008 and Daata *et al.*, 2012). Most of the liver damages are induced by lipid peroxidation (Muriel, 1987).

The protective effect of vitamin C and E, observed in this study, may be due to the action of these antioxidants against oxidative stress induced by gasoline 80 vapours. The exposure to gasoline 80 vapours may cause hematological, biochemical and lipid profile changes. Vitamins C and E appeared to be promising agents for amelioration of these changes. According to Datta *et al.* (2012), vitamin C acts in synergism with vitamin E where, vitamin E works in fats and oils which make it complementary to vitamin C which works in water.

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