Turk J Biol
36 (2012) 217-223
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doi:10.3906/biy-1004-111

Hepatoprotective effect of vitamins C and E against gasoline vapor-induced liver injury in male rats

Friday Effiong UBOH[1], Patrick Ekong EBONG[1], Henry Dan AKPAN[2], Itoro Friday USOH[2]

[1]Biochemistry Department, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, PMB 1115, Calabar - NIGERIA

[2]Biochemistry Department, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, PMB 1017, Uyo - NIGERIA

Received: 06.05.2010

Abstract: The protective effect of vitamins C and E against gasoline vapor-induced liver injury was investigated in rats. Liver injury was assessed from the activities of liver function diagnostic indices including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (γ-GT), alkaline phosphatase (ALP) activities, total serum protein (TSP), albumin concentrations, and the histological architectures of the liver tissues of the experimental animals. The results showed that gasoline vapors caused a significant ($P \leq 0.05$) decrease in TSP and albumin; an increase in serum ALT, AST, γ-GT, and ALP activities; and degenerative changes in the structural architecture of the liver tissues, i.e. an indication of hepatic injury, in comparison with the control group. The indicators of hepatic injury associated with exposure to gasoline vapor were reverted with either vitamin C or vitamin E administration, showing a protective effect of the vitamins against gasoline vapor-induced liver injury in rats. The hepatic injury reversion effect of vitamin E was observed to be insignificantly ($P \geq 0.05$) higher than that of vitamin C. The results of our study suggest a protective effect of vitamin C and vitamin E against gasoline vapor-induced liver injury, with vitamin E as a better option.

Key words: Gasoline vapor, vitamin C, vitamin E, liver enzymes, serum proteins, histopathology

Introduction

The liver is the major organ responsible for metabolism, detoxification, and secretory functions in the body. Hence, it regulates various important metabolic functions in mammalian systems. Hepatic damage is associated with the distortion of these metabolic functions. The liver tissue is reported to be one of the tissues with a high regenerative capacity (1). According to Rabelo et al. (2), hepatocytes exhibit a very good regenerative response to several stimuli, including massive destruction of hepatic tissue by toxins, viral agents, or surgical extraction. Regeneration of the liver tissues is a result of an organized and controlled response of the liver toward tissue damage induced by toxic agents, trauma, infections, or postsurgery resection. Different chemical agents, including gasoline vapor constituents, are known to be hepatotoxic (3).

There has been a sharp increase in the use of gasoline and other petroleum products in recent times. Gasoline in particular is widely used as fuel for automobiles and some electricity-generating machines. It is a very volatile liquid; its direct evaporation releases gasoline vapor, with several

organic and inorganic constituents, into the immediate environment. These organic and inorganic constituents of gasoline vapor are ubiquitous in the environment and constitute various components of the petroleum pollutants in the air. The effects of these pollutants are of great concern as they impact both the environment and human health. A large percentage of the human populace is directly or indirectly exposed to these pollutants in the course of their day-to-day activities. It is generally reported that those who are occupationally exposed constitute the population at greatest risk of frequent exposure (4,5). The potential health hazards associated with chronic or subchronic exposure to these ubiquitous pollutants in the environment has attracted the attention of the general public and the scientific community.

In animals, exposure to gasoline vapor has been reported to produce various toxicity effects in many tissues. In our previous studies, we observed that gasoline vapor induced proatherogenic changes in the serum lipid profile and signs of hepatic oxidative stress (3), hematotoxicity (6), reproductive toxicity (7), and nephrotoxicity (8) in male and female rats. The basic molecular mechanisms through which gasoline vapor constituents and other chemical agents express their toxicity effects may vary. For instance, it has been reported that the molecular mechanism that may be responsible for the toxicity of alcohol and cadmium involves oxidative stress, which disturbs the antioxidant defense system and produces reactive oxygen species (ROS), including hydrogen peroxide, superoxide, and hydroxyl radicals (9,10). In experimental rat models, exposure to gasoline vapor has also been reported to cause oxidative stress, which disturbs the antioxidant defense system and produces an alteration in lipid peroxidation (3).

The major concern of environmental and biochemical toxicologists in recent times has been devising measures that can abate the adverse effects associated with exposure to ubiquitous environmental pollutants. Since previous studies indicate that the toxicity effects associated with exposure to gasoline vapor constituents and other toxicants are an indication of tissue or tissue components, such as reactive metabolite species interactions in the body, the presence of antioxidants may provide protective measures against their toxicity effects. Some antioxidants are naturally present in the body, while others, such as antioxidant vitamins, are provided as micronutrients in the diet. Some vitamins (such as vitamins A, E, and C) are known to play an important role in ameliorating the toxicity effects of reactive species generated by chemical agents in biological systems. Vitamins C and E are known to be potent antioxidants (11-14). In our previous studies, it was observed that vitamin E expressed a higher hepatoprotective effect in rats exposed to gasoline vapor than vitamin A (15,16). These reports indicated that the vitamins may augment the function of endogenous free radical scavengers and, consequently, decrease the deleterious effects of gasoline vapor constituents on body cells. In view of the varying reports on the intrinsic antioxidant activity of these vitamins, the present study considers the comparative ameliorative effect of vitamins C and E on the changes in the histology and serum liver function diagnostic biochemical indices associated with exposure to gasoline vapor in male rats.

Materials and methods

Experimental animals

For this study, 24 mature male Wistar albino rats weighing 200.2 ± 30.1 g were obtained from the animal house of the College of Medical Sciences of the University of Calabar, Calabar, Nigeria. The rats were divided into 4 groups with 6 rats each, as follows:

1. Group I: Normal control group, no exposure to gasoline vapor.
2. Group II: Experimental control group, exposed to gasoline vapor only.
3. Group III: Experimental test group 1, exposed to gasoline vapor and concomitantly treated with vitamin E daily.
4. Group IV: Experimental test group 2, exposed to gasoline vapor and concomitantly treated with vitamin C daily.

The rats were acclimatized in the experimental animal house for 1 week before the commencement of the experiment; they were housed in stainless steel cages and fed with normal rat pellets. All rats in both test and control groups were allowed free access to food and water throughout the experimental period.

Exposure to gasoline vapor

The animals in the test groups were exposed to gasoline vapor in exposure chambers. A modified whole body inhalation exposure method, previously described (3,17,18), was used to expose the animals in test groups to ungraded concentrations of the vapor generated from direct evaporation of liquid gasoline. The Premium Motor Spirit blend of liquid unleaded gasoline used in this study was obtained from the Mobil refueling station on Marian Road in Calabar, Nigeria. The test animals were allowed to inhale the evaporating vapor in the chambers during the exposure period. An exposure period of 6 h (from 0900 to 1500 hours) daily, 5 days per week, was adopted for 10 weeks. The animals in Groups III and IV were administered vitamins E and C once daily, respectively, concomitant with exposure to gasoline vapor.

Treatment of the rats with vitamins E and C

Tablets of vitamin C (100 mg) obtained from Emzor Pharmaceutical Industries, Lagos, Nigeria, and capsules of vitamin E (Efishal 200™) from Shalina Laboratories, Pvt., Mumbai, India, were used in this study. Vitamins E and C were solubilized in vegetable oil and distilled water solvents, respectively. The vitamin C tablets were ground into powder to prepare a suspension containing 200 mg of vitamin C in 0.25 mL. Similarly, vitamin E capsules were cut open and carefully emptied into a clean container to prepare a suspension containing 400 IU of vitamin E in the same volume as vitamin C. The suspensions were kept at room temperature and protected from direct contact with air and sunlight to avoid degradation. The rats in Groups III and IV were administered 400 IU/kg of vitamin E (α-tocopherol) and 200 mg/kg of vitamin C once daily, respectively, concomitantly with exposure to gasoline vapor. Administration of the vitamins was done by oral gavaging using an intragastric syringe.

Collection and handling of blood and liver tissues for analysis

The animals were sedated with chloroform vapor and dissected for collection of blood and liver tissue specimens 24 h after the last day of the experimental exposures and treatments. Whole blood from each animal was collected by cardiac puncture into well-labeled plain sample tubes and allowed to clot for 3 h in ice water. The serum was separated from the clots by centrifuging at 10,000 rpm for 5 min, placed into well-labeled plain sample bottles, and used for enzyme, total protein, and albumin assays. The liver tissues were surgically removed and washed immediately with ice-cold saline. A sliced section of the liver tissue was fixed in a suitably treated formalin reagent for histological examination. All analyses were carried out within 48 h of tissue collection.

Biochemical and histopathological assays

Biochemical analyses carried out included measurement of the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GT), and alkaline phosphatase (ALP); serum total protein and albumin concentrations were also measured. The measurements of the concentrations of these biochemical parameters were done by spectrophotometric determination of their absorbances using analytical grade laboratory reagent kits. Laboratory reagent kits from Biosystems Laboratories (S.A. Costa Brava, Barcelona, Spain) were used to assess the activities of ALT, AST, and ALP in the serum; reagent kits from Randox Laboratories (Crumlin, United Kingdom) were used to assess the activities of γ-GT in the serum.

AST catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate, forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase and NADH. The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic concentration of AST present in the sample. ALT also catalyzes the reversible transfer of an amino group from alanine to α-ketoglutarate, forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase and NADH. The rate of decrease in the concentration of NADH, measured photometrically, is proportional to the catalytic concentration of ALT present in the sample.

Total serum protein and albumin concentrations were determined by spectrophotometric techniques using commercial kits (Spinreact de México, Naucalpan, Mexico). All analytical procedures were carried out following the respective manufacturers'

instructions. All absorbance readings were taken with a DREL3000 HACH model spectrophotometer.

The histopathological examination of the liver tissues was carried out in the Histopathology Department of the University of Calabar Teaching Hospital, Calabar, Nigeria. After rinsing the dissected liver in normal saline, tissue sections were taken from the organ. The tissue was fixed in 10% formosaline, dehydrated with 100% ethanol solution, and embedded in paraffin. It was then processed into sections 4-5 μm thick stained with hematoxylin and eosin and observed under a light microscope for any morphological changes.

Statistical analysis

Results were presented as mean ± standard error of the mean (SEM). SPSS for Windows was used for the statistical analysis of the data with one-way ANOVA. $P \leq 0.05$ was considered statistically significant.

Results and discussion

The results of our study of the hepatoprotective effect of vitamins C and E against gasoline vapor-induced liver injury in male rats are presented in the Table and Figures 1a-1d. The Table shows that exposure of male rats to gasoline vapor significantly ($P \leq 0.05$) increased the activities of serum ALT, AST, γ-GT, and ALP; it also decreased the concentrations of serum total protein and albumin. The results of this study also show that concomitant treatment of the rats exposed to gasoline vapor with vitamins C and E, respectively, resulted in a significant ($P \leq 0.05$) decrease in the activities of serum ALT, AST, *γ*-GT, and ALP, as well as an increase in the concentrations of serum total protein and albumin. The reported decrease in the activities of serum ALT, AST, *γ*-GT, and ALP, and increase in the concentrations of serum total protein and albumin were insignificantly ($P \geq 0.05$) higher in the rats treated with vitamin E than in the rats treated with vitamin C (Table). Figures 1a-1d show the histological sections of the liver tissue architecture of the different experimental rats. Figure 1a represents a typical liver section from a rat in the normal control group, showing normal cellular architecture. This section indicates distinct sinusoidal spaces and hepatocytes with cytoplasm and prominent nuclei. Figure 1b presents the photomicrograph of a typical section from the liver cell of an experimental test animal exposed to gasoline vapor. In this section, severe histopathological changes, such as centrilobular hepatic necrosis, tissue fatty change, Kupffer cells, ballooning degeneration, and infiltrating lymphocytes, were observed. The observations made from this section indicated liver injury, as compared to the section from the control group. This suggests that the cellular integrity of the liver tissues was altered by the constituents of gasoline vapor, and hence the derangement of their cellular functions. The results of the histopathological examinations of typical liver sections from experimental test rats treated with vitamins C and E are presented in Figures 1c and 1d, respectively. These sections depict normal hepatocytes similar to those of the control group. These sections suggest that the administration of vitamins C and E was able to restore possible histological damage associated with exposure to gasoline vapor.

Table. Effects of vitamins C and E on some liver function diagnostic indices in rats exposed to gasoline vapour.

Group	Treatment	ALT (U/L)	AST (U/L)	*γ*-GT (IU/L)	ALP (IU/L)	TSP (g/dL)	Albumin (g/dL)
I	Control	10.76 ± 2.56	12.46 ± 3.44	24.38 ± 5.64	266.56 ± 24.76	6.02 ± 2.33	3.96 ± 1.56
II	Gasoline vapor only	52.20 ± 18.25*	68.56 ± 20.61*	49.42 ± 9.22*	358.82 ± 65.21*	3.20 ± 0.56*	2.01 ± 0.60*
III	Gasoline vapor + vitamin C	11.82 ± 3.01^{a,c}	13.74 ± 3.11^{a,c}	22.93 ± 4.68^{a,c}	270.46 ± 56.23^{a,c}	5.68 ± 1.82^{a,c}	3.68 ± 1.02^{a,c}
IV	Gasoline vapor + vitamin E	11.16 ± 2.67^{a,b,c}	13.26 ± 2.86^{a,b,c}	23.68 ± 3.80^{a,b,c}	260.78 ± 54.30^{a,b,c}	5.88 ± 1.36^{a,b,c}	3.78 ± 1.22^{a,b,c}

Values are presented as mean ± SEM; n = 6: *$P \leq 0.05$ compared to Group I; $^{a}P \geq 0.05$ compared to Group I; $^{b}P \geq 0.05$ compared to Group III; $^{c}P \leq 0.05$ compared to Group II.

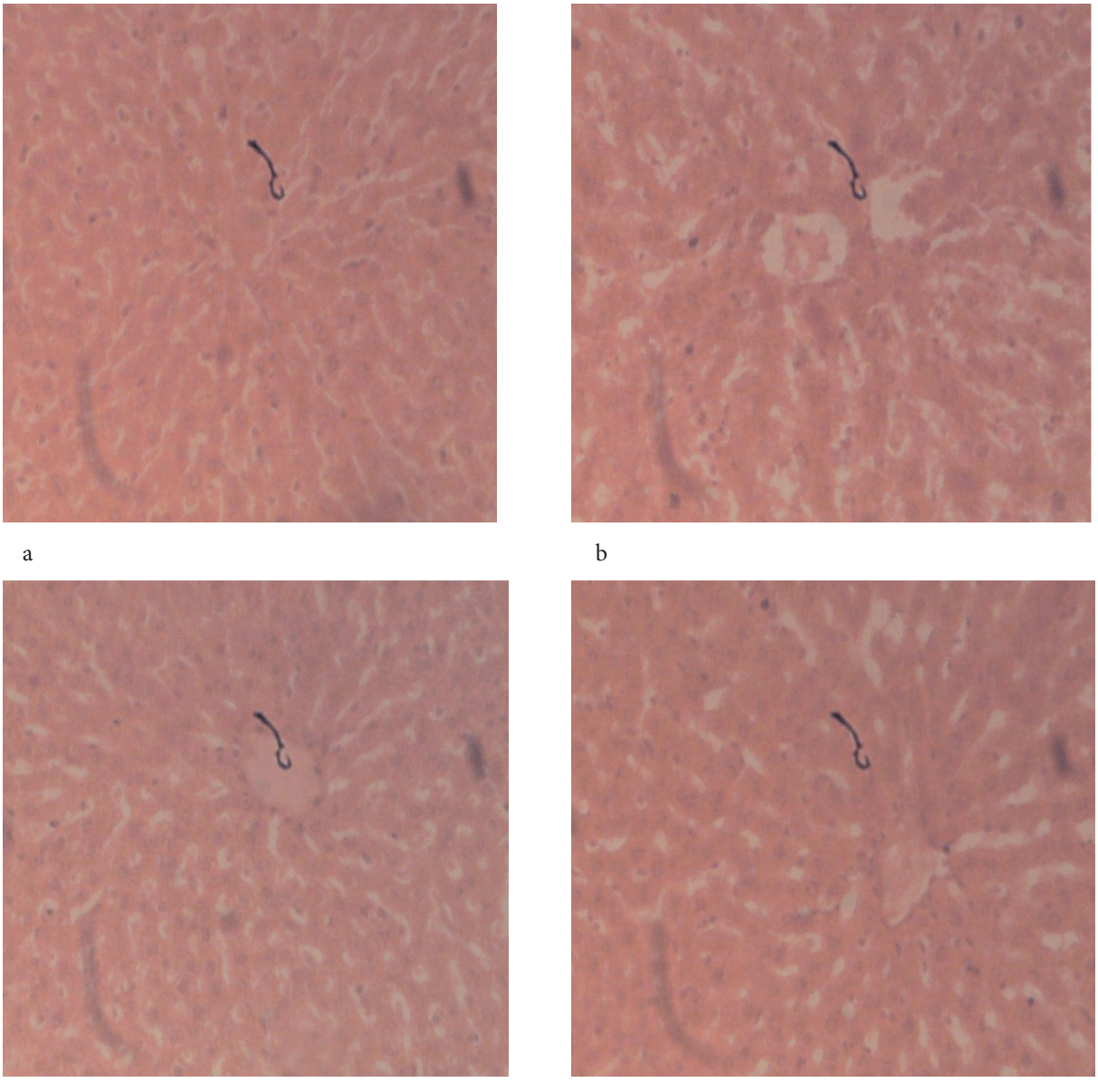


Figure 1. Photomicrographs (×100) of liver sections taken from experimental rats: a) section of liver tissue from normal rat, showing normal cellular architecture with distinct hepatic cells and sinusoidal spaces; b) section of liver tissue from rat exposed to gasoline vapor, exhibiting severe histopathological changes such as centrilobular hepatic necrosis, fatty change, Kupffer cells, ballooning degeneration, and infiltrating lymphocytes; c) section of liver tissue from rat exposed to gasoline vapor and treated with vitamin C, showing normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein; d) section of liver tissue from rat exposed to gasoline vapor and treated with vitamin E, showing normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein.

The results of this study indicate that exposure of rats to gasoline vapor caused significant alterations in the biochemical parameters of liver function. Liver enzymes such as ALT, AST, and ALP are known marker enzymes for the assessment of the functional integrity of the liver cells (19,20). These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication due to damage to the liver (19). In this study, the activities of serum ALT,

AST, ALP, and γ-GT were significantly increased, while the values of total protein and total albumin were statistically decreased following exposure to gasoline vapor. The recorded changes in these biochemical parameters were substantiated by the histopathological changes, characterized by diffuse ballooning degeneration and pyknotic nuclei of hepatocytes with lymphocytic infiltration of the hepatic parenchyma (indicative and reflective of acute hepatocellular injury). The present available data indicate that the constituents of gasoline vapor exert possible hepatotoxic effects, as the increase in the activities of serum ALT and ALP suggest liver damage. The results reported in this study are in agreement with our previous studies, which indicated that exposure to gasoline vapor induced severe adverse physiological and biochemical disturbances that affect the functional and structural integrity of the liver and kidney tissues in experimental animals (3,8,17,21).

In the present study, it was also observed that vitamin E and C administration to rats exposed to gasoline vapor produced an appreciable improvement in the hepatotoxic effect associated with exposure. Thus, it appears that the vitamins counteracted the hepatotoxic effect associated with gasoline vapor-generated free radicals and enhanced the antioxidant capacity of the several endogenous antioxidant factors. The results of this study correlate with the results of our earlier study on the hepatoprotective effects of vitamin A against gasoline vapor toxicity in rats (16). The observations from the present study agree with those of Ayo et al. (13), Chen et al. (22), Frei (23), and Ambali et al. (24), who reported that vitamin C is an effective antioxidant in various biological systems. According to Odigie et al. (25) and Idogun and Ajala (26), both animal and human studies have shown that ascorbic acid is a potent antioxidant that mediates its antioxidant effect by scavenging ROS. The foregoing indicates that, as an antioxidant agent, vitamin C may have inhibited the chain reactions of chemical agent-generated free radicals or scavenged the reactive free radicals before they reached their hepatic targets.

Vitamin E has been reported to express 2 important functions in the membranes: preventing ROS damage in polyunsaturated fatty acids as a liposoluble antioxidant and acting against damage caused to phospholipids as a membrane-stabilizing agent (27). In addition, vitamin E is known to act by breaking the antioxidant chain that prevents ROS-produced cell membrane damage (28). Factor et al. (29) demonstrated that vitamin E can directly reduce ROS production by interfering in the union between the membrane and the NADPH oxidase complex. In a correlating study, Ramírez-Farías et al. (30) reported that short-term antioxidant supplementation attenuates lipid peroxidation and protects against liver injury and dysfunction in an ethanol intoxication model during partial hepatectomy-induced liver regeneration.

The results of the present study suggest the existence of a hepatoprotective effect of vitamins C and E against gasoline vapor-induced hepatotoxicity in rats. The protective effect of these vitamins over the adverse effects of free radicals generated by the vapor's constituents and their efficiency in the regeneration of the cellular and physiological status of the liver tissues coincides with the observations of Ramírez-Farías et al. (30), Morales-González et al. (31,32), and Parra-Vizuet et al. (33) on glycine and vitamin E in ethanol-induced hepatic injury. Thus, the ameliorating effects of vitamins C and E on gasoline vapor-induced hepatotoxicity are likely to be mediated via the inhibition of free radical generation and free radical scavenging activity. In addition, vitamin E may be a better option than vitamin C in ameliorating gasoline vapor-induced hepatotoxicity.

Corresponding author:

Friday Effiong UBOH

Biochemistry Department,

Faculty of Basic Medical Sciences,

College of Medical Sciences,

University of Calabar, PMB 1115,

Calabar - NIGERIA

Email: fridayuboh@yahoo.com

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