



# Ascorbic Acid Supplementation Protects Against Gasoline Fume-Induced Oxidative Stress and Thyroid Dysfunction in Rats

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## Abstract

The protective effect of ascorbic acid against gasoline fume -induced oxidative stress and thyroid gland dysfunction was investigated in rats. Thirty-five (18 male and 17 female) rats weighing 200 gm to 250 gm were randomly segregated into five groups (n=7 per group). Rats in group 1 served as unexposed control, while rats in group 2 were exposed to GV alone. Rats in groups 3, 4 and 5 were exposed to Gasoline Vapor (GV) and orally administered ascorbic acid 100 mg/kg and 200 mg/kg and 280 mg/kg respectively for 14 days. Thereafter, all animals were sacrificed and blood obtained was used for the determination of serum levels of Triiodothyronine (T<sub>3</sub>), Tetraiodothyronine (T<sub>4</sub>), Thyroid Stimulating Hormone (TSH), Catalase (CAT) and Malondialdehyde (MDA) using standard methods.

Exposure to GV alone significantly (P<0.05) decreased serum levels of T<sub>3</sub> and CAT and increased MDA in both male and female rats. Serum levels of T<sub>4</sub>, TSH significantly increased in male, but decreased in female rats compared to the corresponding values in the control group. The GV-exposed thyroid section revealed follicular hypertrophy and hyperplasia, compacted follicles, congested blood vessels and almost extinct colloids. Co-administration of ascorbic acid caused a remarkable improvement in these parameters. Exposure to GV could be associated with oxidative stress and thyroid dysfunction which can be mitigated by the administration of ascorbic acid, a synthetic antioxidant.

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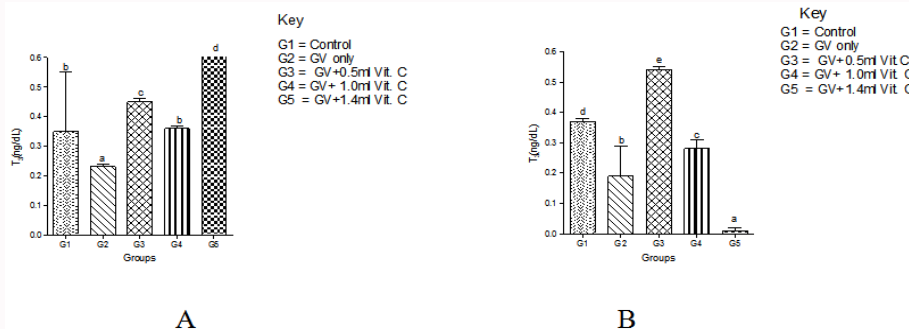
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**Keywords:** Petroleum vapor; Thyroid toxicity; Reactive oxygen species; Ascorbic acid

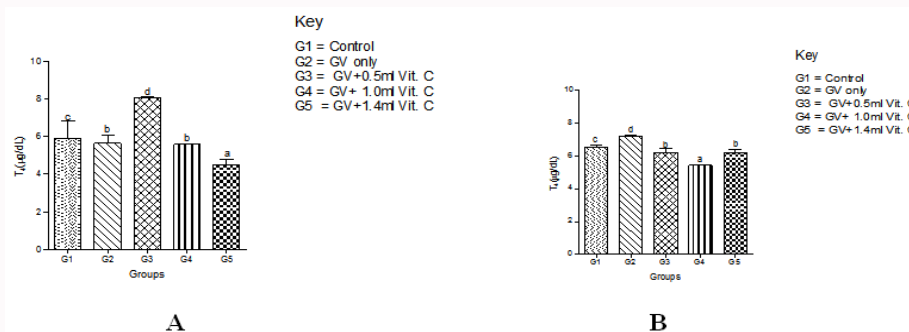
## Introduction

Gasoline is a colorless to pale brown or pink liquid produced by distillation, cracking, and reforming of crude oil. It is widely used domestically and industrially as a fuel in spark ignition and internal combustion engines in automobiles, trucks and aircrafts. It is a volatile liquid that evaporates easily into the atmosphere, constituting a significant environmental pollutant and hazard to the general population and in particular, to those who are occupationally exposed [1]. Exposure to gasoline can be either intentionally or accidentally through inhalation, ingestion or dermal routes. The most at risk population are the petrol pump attendants and workers in the petrochemical industries, who are exposed to the synergistic and/or additive adverse effects of the gasoline constituents [2]. When inhaled, the hydrocarbons are readily absorbed and may cause a wide range of adverse health effects which may vary depending on several factors including differences in exposure intensity and duration, as well as inter-individual differences due to variation in individuals' pharmacodynamics and pharmacokinetics related to the different gasoline components [3]. Evidence has shown that exposure to gasoline vapors (especially the light-chain volatile compounds (BTEX)) causes some levels of toxicity to the whole body and may induce organ toxicity [4,5]. These compounds are gradually released into the air, and they exist in both the vapor phase and the water soluble fraction, due to their high vapor pressure and water solubility. Benzene being an important component of BTEX compounds is particularly toxic to several endocrine glands including thyroid gland [4].

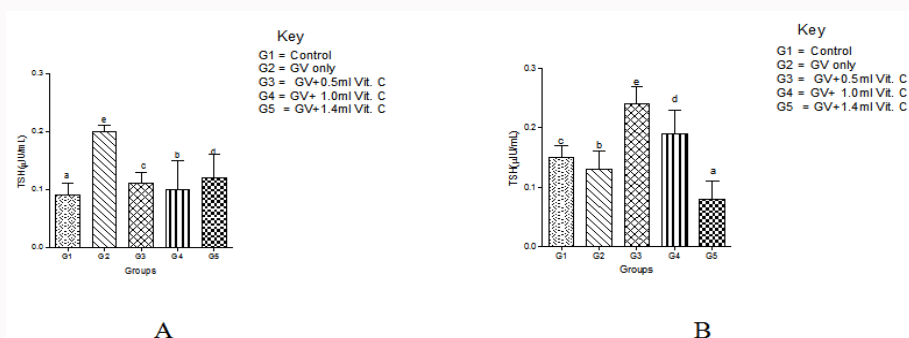
The thyroid gland plays a major role in the metabolism, growth and development of the human body. It helps to regulate many body functions by constantly releasing a steady amount of hormones into the bloodstream. Hormones produced by the thyroid gland include T<sub>3</sub> and T<sub>4</sub> or and calcitonin. The peripheral tissues are provided a constant supply of thyroid hormone through the activities of a sensitive and tightly regulated feedback control system, thyroid gland autoregulation,



**Figure 1:** Effect of Ascorbic acid on GV-induced changes in serum T<sub>3</sub> levels in Male (A) and Female (B) rats. Values are expressed as Mean ± SEM at P<0.05 relative to control. Different letters indicate significance while similar letters represent non-significance.



**Figures 2:** Effect of Ascorbic acid on GV-induced changes in serum T<sub>4</sub> levels in Male (A) and Female (B) rats. Values are expressed as Mean ± SEM at P<0.05 relative to control. Different letters indicate significance while similar letters represent non-significance.



**Figure 3:** Effect of Ascorbic acid on GV-induced changes in serum TSH levels in Male (A) and Female (B) rats. Values are expressed as Mean ± SEM at <0.05 relative to control. Different letters indicate significance while similar letters represent non-significance.

and the large intrathyroidal and extrathyroidal storage pools of thyroid hormone. The thyroid gland is susceptible to damage by a great number of exogenous and endogenous chemicals (goitrogens). These same agents can cause alterations in various aspects of thyroid hormone economy [6] including inhibition of thyroid hormone synthesis. For instance, many goitrogenic xenobiotics that increase the incidence of thyroid cancers in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis and secretion of thyroid hormones [7]. They may inhibit the iodine trapping mechanism (thiocyanate or perchlorate), block organic binding of iodine and coupling of iodothyronines to form thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>), (eg. sulfonamides), and/or inhibit thyroid hormone secretion by an effect on proteolysis of active hormone from the colloid (lithium or an excess of iodide). Another large group of goitrogenic chemicals can disrupt thyroid hormone economy by increasing or decreasing the peripheral metabolism

of thyroid hormones through an induction of hepatic microsomal enzymes [8]. Gasoline is one such substance with documented toxic effects on thyroid gland, and leading to thyroid dysfunction mainly due to its ability to induce Oxidative Stress (OS), inflammation and immune system dysfunction. This notion is supported by GV-induced changes in markers of OS [9,10] inflammation and immune system dysfunction. Given this, the aim of the present study was to assess the effect of ascorbic acid supplementation on GV-induced thyroid dysfunction in rats.

## Materials and Methods

### Animal care and use

Thirty-five (18 male and 17 female) Wistar albino rats weighing between 200 gm to 250 gm were obtained from the Animal house of the department of Pharmacology, Faculty of Pharmacy, University of Uyo, Nigeria. They were kept in well-ventilated cages for seven (7)

days to acclimatize and given humane care in accordance with the Principle of Laboratory Animal Care and Use [11]. Prior to onset of the experiment, the median lethal dose ( $LD_{50}$ ) of vitamin C was determined using standard methods. The rats were segregated into five groups ( $n=7$  per group), and housed in well ventilated wooden cages and kept under controlled environmental conditions of temperature/humidity and 12-hr light/dark cycle. They were given standard animal chow (Vital Feeds Grand Cereals Ltd., JOS). Water was provided ad libitum throughout the 6-weeks study period. Group 1 served as control and orally gavaged 2 ml of normal saline, Group 2 was exposed to gasoline alone group. Groups 3, 4 and 5 were exposed to gasoline and orally gavaged low (100 mg/kg) (0.5 ml) medium dose (200 mg/kg) (1 ml) and high dose (280 mg/kg) (2.4 ml) of vitamin C, a known antioxidant.

### Exposure of animals to gasoline vapor

All rats in groups 2, 3, 4 and 5 were exposed to gasoline vapor in the exposure chambers ( $60 \times 80 \times 100 \text{ cm}^3$ ) for 4 weeks (8 hrs daily). 200 ml of gasoline was poured into a 250 ml beaker and the quantity evaporated after 8 hrs was calculated by subtracting the final volume from the initial volume on daily basis.

### Sample collection and analysis

After two weeks of vitamin C administration, the rats were again weighed and anaesthetized with chloroform soaked in a swap of cotton wool in desiccators. The blood samples were collected and immediately transferred into properly labeled 5 ml plain sample bottles for the determination of  $T_3$ ,  $T_4$  and TSH activity.

The sample 20  $\mu\text{l}$  was collected using a micropipette from the 5 ml plain bottles and added to new plain bottles containing 10 ml of cold distilled water for the estimation of red cell Catalase (CAT) and Malondialdehyde (MDA) activities.

### Hormonal assays

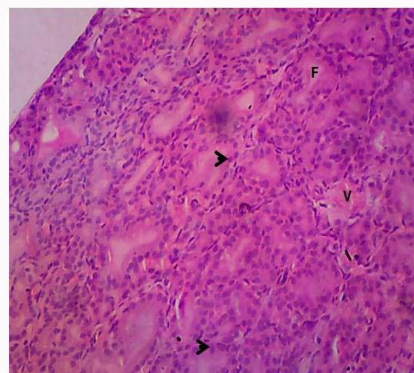
Blood samples were collected into plain sample bottles and centrifuged at 3000 rpm for 15 min using a bench centrifuge and the plasma stored at  $4^\circ\text{C}$  for subsequent assay of triiodothyronine ( $T_3$ ), thyroxine ( $T_4$ ) and thyroid stimulating hormone (TSH) using the enzyme-linked immunosorbent assay (Accu-Bind ELISA microwells) kits from Monobind Inc. Lake Forest, CA 92630, USA. Assay was carried out in the morning to avoid temperature influence on hormones.

The red cell CAT activity was estimated using Titrimetric method [12-14]. Serum  $T_3$ ,  $T_4$  and TSH were assayed using the REITMAN and FRANKEL calorimetric method [15,16]. Test for TSH was based on the principle of a Sandwich Enzyme-Linked Immune-Sorbent Assay (ELISA) which utilizes a unique monoclonal antibody directed against a distinct antigen determinant on the intact TSH molecule [17,18].

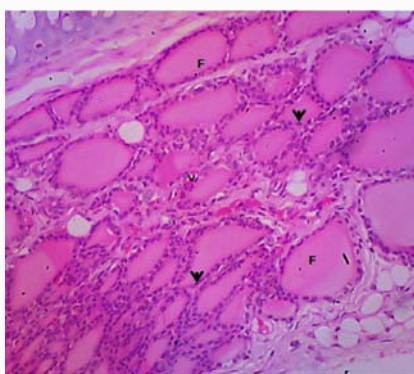
### Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS), version 20.0. The one-way analysis of variance (ANOVA) and post-hoc Tukey least significant difference (LSD) test were used to analyze the data and to determine the significant levels respectively.

Data were expressed as Mean  $\pm$  Standard Error of Mean (SEM) and tables were used to illustrate the variations in the numerical values across the experimental groups. P values  $<0.05$  were considered statistically significant.



**Figure 4:** Histological section of thyroid gland (Haematoxylin & Eosin Stain) of gasoline alone group (at magnification X400) demonstrating thyroid follicles (F) lined by cubical follicular cells that exhibit rounded nuclei (arrow head), congested blood vessels (V) present. The section displays hypertrophy evidence with numerous compacted follicles with almost disappearing colloid.



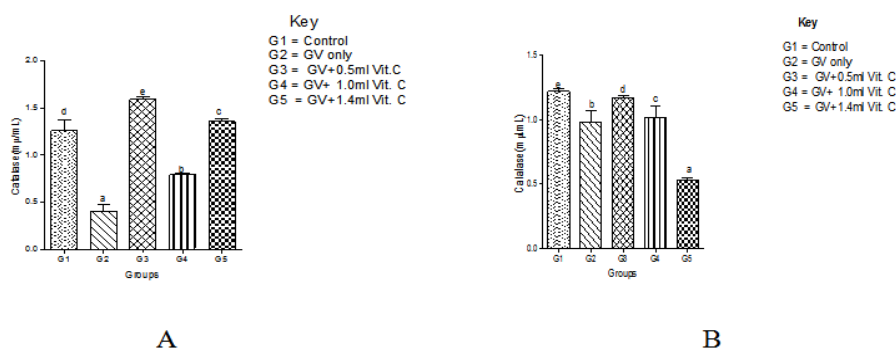
**Figure 5:** Histological section of thyroid gland (Haematoxylin & Eosin Stain) of control group at magnification (X400) demonstrating thyroid follicles (F) lined by cubical follicular cells that exhibit rounded nuclei (arrow head), tiny blood vessels (V) present.

## Results

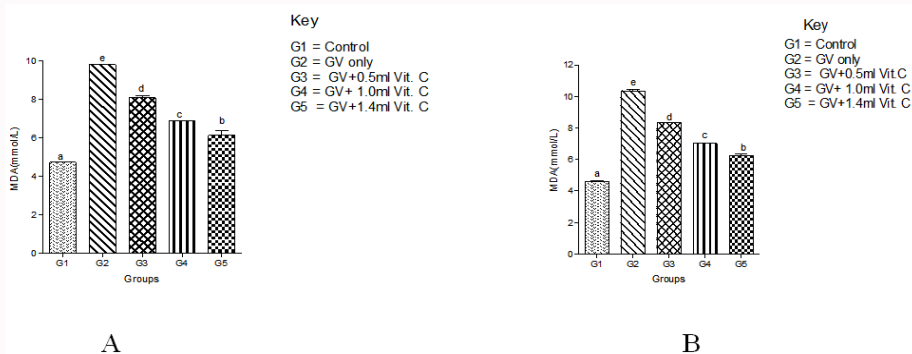
Environmental pollution by gasoline constituents is a particular public health problem in most parts of the world. Several millions of people are exposed to the toxic constituents of gasoline daily [19], and multiple organ pathologies have been reported following exposures [5]. However, few reports have been documented for endocrine glands [3] including thyroid gland despite the significant role of this gland in human development and metabolism, and the knowledge that various environmental chemicals can disrupt thyroid hormonal balance and normal architecture.

The results of the present study showed that exposure to GV could be associated with a significant risk for thyroid gland dysfunction mediated through oxidative damage to thyroid tissues. Also, the administration of synthetic antioxidant such as ascorbic acid could attenuate the damaged thyroid tissues.

Evidence for this notion is two folds; first, animals exposed to GV alone had significant alteration in serum levels of thyroid hormones (decreased  $T_3$  and increased  $T_4$  and elevated TSH) (Figures 1-3), and marked alteration in normal thyroid architecture (rounded follicular nuclei, congested blood vessels, hypertrophic nuclei with numerous compacted follicles with almost disappearing colloid) (Figure 4), compared to animals in the unexposed group (thyroid follicles with cubical follicular cells that exhibit rounded nuclei and tiny blood



**Figure 6:** Effect of Ascorbic acid on GV-induced changes in serum Catalase levels in male (A) and female (B) rats. Different letters indicate significance while similar letters represent non-significance.



**Figure 7:** Effect of Ascorbic acid on GV-induced changes in serum MDA levels in Male (A) and Female (B) rats. Values are expressed as Mean ± SEM at P<0.05 relative to control. Different letters indicate significance while similar letters represent non-significance.

vessels) (Figure 5).

Second, significant changes in serum levels of oxidative stress markers (decreased CAT and increased MDA) (Figure 6,7) were observed in the GV alone group compared to the levels in the control group.

Also, supplementation with ascorbic acid (a known antioxidant) reversed these changes (Figures 8-10).

These observations indeed confirm previous studies that acknowledged the endocrine disrupting potential of gasoline constituents [3]. Although the mechanisms underlining these effects (GV-induced thyroid disorders) are poorly understood, recent evidence suggests three patho-physiologic pathways including GV-induced oxidative stress, inflammation and immune system dysfunction [20-24]. However, OS has received growing interest since it is the central driving force in the signaling cascade linking inflammation, immune system disorder with thyroid gland dysfunction.

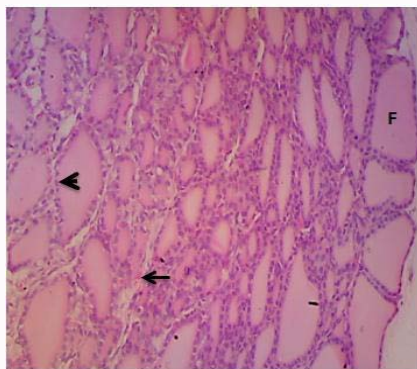
It is interesting to note that the GV-induced thyroid disorders observed in the present and previous studies (hypothyroidism and hyperthyroidism) are by themselves implicated in the induction of OS, although with different mechanisms. For instance, while lower than normal level of thyroid hormones (hypothyroidism) is associated with reduction in antioxidant enzyme levels/activities, higher than normal level of thyroid hormone (hyperthyroidism) is known to cause increase generation of ROS, in either case, further promoting GV-induced OS. Without any contrary evidence, we speculated a villain-victim relationship between thyroid disorders

and oxidative stress.

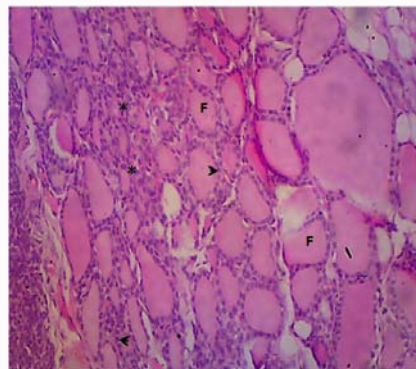
Several studies observed that the MDA concentrations were significantly increased in patients with hypothyroidism and hyperthyroidism [25-28]. Likewise lower plasma levels of SOD and CAT were found in hypothyroidism and hyperthyroidism. Mancini et al. [23] noted that all the antioxidant enzymes including SOD, CAT and vitamin E correlated well with plasma level of T<sub>3</sub>, and that the correlation between T<sub>3</sub> and CAT remained significant even after corrected for total cholesterol. Taking together, these observations indicate that the above mentioned thyroid disorders are OS-mediated. However, it is noteworthy that OS-mediated thyroid disorders are tissue dependent.

In the present study, we observed a concurrence decreases in serum levels of T<sub>3</sub> and CAT and increases in serum levels MDA and TSH. Collectively, these findings confirmed that the biochemical and histo-morphological aberrations observed in the thyroid gland of animals in the GV alone group were OS-mediated. These notion is corroborated by previous studies that demonstrated OS-induced reduction in the peripheral conversion of T<sub>4</sub> to T<sub>3</sub>, and leading to low serum T<sub>3</sub> and elevated T<sub>4</sub> (Low-T<sub>3</sub> syndrome) as observed in the present study [23,29,30]. Accumulated evidence indicates that low serum T<sub>3</sub> is associated with elevated serum MDA and TSH levels as observed in this study.

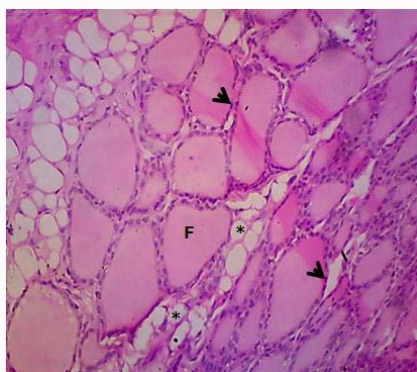
We also speculated that GV-induced thyroid disorders could have been mediated through induction of inflammation and immune system dysfunction. Available data indicate that persistent low serum level of T<sub>3</sub> (as in low-T<sub>3</sub> syndrome) is associated with OS which is a major upstream component in the signaling cascade involved in



**Figure 8:** Histological section of thyroid gland (Haematoxylin & Eosin Stain) of vitamin C (low dose) group 2 at magnification (X400) demonstrating slightly enlarged compressed thyroid follicles (F) lined by cuboidal follicular cells that exhibit rounded nuclei (arrow head). Note the minute blood capillaries (arrow).



**Figure 10:** Histological section of thyroid gland (Haematoxylin & Eosin Stain) of vitamin C (high dose) group 4 at magnification (X400) demonstrating numerous compressed thyroid follicles (F) due to cellular hyperplasia (\*), the follicles are lined by cubical follicular cells that exhibit rounded nuclei (arrow head).



**Figure 9:** Histological section of thyroid gland (Haematoxylin & Eosin Stain) of group 3 at magnification (X400) demonstrating numerous enlarged thyroid follicles (F) lined by cubical follicular cells that exhibit rounded nuclei (arrow head), there is also increased fatty changes (asterisk).

inflammation and immune system responses [31].

Therefore, GV-induced OS can lead to thyroid gland dysfunction, which further promote OS, inflammation and immune system dysfunction in a vicious cycle pattern. We also observed the significant increase in serum level of TSH in male rats suggesting the negative feedback effect of low serum  $T_3$ . However, in female rats, serum levels of TSH rather decreased indicating poor feedback response, probably due to the opposing effect of estrogen (E2) on the molecules (uncoupling protein-2 and 3) known to fine regulate the oxidative status of thyroid gland through auto loop feedback system [23]. This can also account for the gender-related differences in other parameters observed in the present study including oxidative stress makers and serum levels of thyroid hormones ( $T_3$  and  $T_4$ ).

The decrease in serum  $T_3$ ,  $T_4$  and TSH levels in female rats suggests a more severe effect of GV-induced thyroid dysfunction including the low- $T_3$  syndrome in female than male rats probably because of the effect of E2. This observation is consistent with the higher prevalence of thyroid disorders in female than male humans [32] Co-administration of ascorbic acid to the animals in GV-exposed group caused improvement in the deranged functional and histopathological parameters. Ascorbic acid is a water soluble vitamin that acts as first-line antioxidant in the plasma [33]. It has the potential to directly scavenge free radicals, quench singlet oxygen, donate

hydrogen atom to lipid radicals, remove molecular oxygen and cause regeneration of  $\alpha$ -tocopherol from the tocopheroxyl radical species [34,35]. Besides the antioxidant effect, ascorbic acid also has anti-inflammatory and immune modulatory actions [36].

Recent research indicates that ascorbic acid administration can modify and prevent the detrimental effects of gasoline compounds on various organs including the reproductive, renal, hematopoietic, hepatic and cardiovascular systems [37-40]. Here we have also shown that ascorbic acid can mitigate GV vapor-induced thyroid gland dysfunction.

## Conclusion

Exposure to GV could be associated with oxidative stress and thyroid gland dysfunction, which can be mitigated by the administration of a potent synthetic antioxidant such as ascorbic acid.

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