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Original Article

The Effects of Gasoline Vapor Inhalation on Serum Level of Testosterone and Testes Tissue in Male Rats

Rahim Ahmadi[1*], Seyedeh Nasim Mirbahari[2], Masumeh Ghorbani-Vahed[3]

[1] *Avicenna International College, Budapest, Hungary*
[2] *Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran*
[3] *Department of Biology, School of Basic Sciences, Hamadan Branch, Islamic Azad University, Hamadan, Iran*

[*] *Corresponding author: Rahim Ahmadi*
Email: rahahmadi2001@yahoo.com

ABSTRACT

Background: Studies show that gasoline inhalation has severe damage on certain tissues in body. This study aimed to determine the effects of gasoline vapor inhalation on serum testosterone level and testes tissue in male rats.
Methods: Male Wistar rats were randomly divided into control and experimental groups exposed to gasoline vapor for 1, 2 or 3 h/day of 5 rats in each group. After 12 weeks, blood samples were collected using the cardiac puncture method. Serum testosterone was measured using the radioimmunoassay method. Testes tissue was examined histologically using Hematoxylin and Eosin staining method. Data were analyzed using one way- ANOVA.
Results: Serum testosterone level significantly increased in rats exposed to gasoline vapor for 1, 2, 3 hours/day compared with control group. The number of spermatogonia cells significantly decreased in experimental groups compared to control rats; However, the number of spermatocytes and Sertoli cells, and the seminiferous tunnel diameter did not significantly change in groups exposed to gasoline vapor compared with control rats.
Conclusion: Inhalation of gasoline vapor had deleterious effects on testis tissue, resulting in decreased number of spermatogonia cells and increased serum testosterone level.
Keywords: Gasoline vapor, Testosterone, Testis, Rat, Spermatogenesis
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Introduction

Testes are the main reproductive organs in males that play the role of producing sperm and sex hormones. The testes secrete several male sex hormones, including testosterone, dihydrotestosterone and androstenone, which are called androgens. Testosterone is higher than the others and is considered the main testicular hormone (1). On average, each male produces 20 times more testosterone than a female, butdue to increased metabolism, plasma levels of this hormone in men are only seven times higher than in women (2). Testosterone is the most important male hormone and is very crucial in the growth and development of sexual organs and the occurrence of secondary sexual traits such as facial hair growth, hoarseness, male hair loss, and anabolic properties such as muscle growth, and bone mass. This hormone causes growth spurts in adolescents and stops growth by closing growth plates at both ends of the bones and also builds tissues (3-5). At the onset of puberty, testosterone secretion is rapidly increased under the influence of anterior pituitary gonadotropic hormones. This secretion persists throughout life. But after half-life, it decreases rapidly, reaching a maximum of 20% of its maximum secretion during elderly (6). It has recently been established that testicular tissue is very sensitive to toxins, and chemicals. At least 2 million workers die each year as a result of work-related injuries, and about 160 million suffer

from work-related illnesses. About $ 1.25 trillion a year is spent on workplace problems. Exposure to toxic gasoline vapors is one of the work-related problems (7). Epidemiological data show that the petrochemical industry has increased the vulnerability of workers in the workplace. Toxic chemicals from these industries, in addition to workers, threaten the health of other people in large, busy cities (8).

Crude oil gasoline contains more than 500 aliphatic, aromatic, and other types of saturated and unsaturated branched hydrocarbons with 3 to 12 carbon atoms. These hydrocarbons include n-pentane, and n-hexane. These hydrocarbon elements from gasoline disrupt the endocrine system of animals. These compounds are often present in the environment but are not seen due to volatility (9). Benzene, toluene, and xylene have been reported to be the most dangerous aromatic hydrocarbon compounds in gasoline (10). The result of burning gasoline in vehicles is carbon dioxide, nitrogen oxides, and carbon monoxide (11). Organic volatile compounds from gasoline vapors (VOCs) pose a risk to human health that will have a variety of effects depending on how they come into contact with different parts of the human body. Research shows that excessive respiration of this substance causes an irregular heartbeat, and abnormalities in central nervous system stimulation such as dizziness, headache, nausea, and some other disorders which in the wore condition will cause death (12). Frequent inhalation of gasoline has many pathophysiological effects on the endocrine glands, cardiovascular system, nervous system, liver, kidneys, and reproductive system (11, 12). Exposure to high concentrations of benzene vapor will cause non-lymphocytic leukemia (13), abortion in the implantation stage, and congenital anomalies (14). On the other hand, it has been shown that the effects of inhaling gasoline vapors on hormones depend on sex, and the females are more at risk (15). Despite many studies on the physiological effects of fuels on the reproductive system, studies on the effect of fuels, especially gasoline on the reproductive system have been very limited and sometimes the results are contradictory (16). As far as we know, there are few reports on the effect of gasoline vapor on testicular tissue in animal model. In this study we investigated the effect of inhaling gasoline vapor on serum level of testosterone and testicular tissue in male rats.

Methods

Animals

In this experimental-laboratory experiment study, 20 Wistar male rats with an average weight of 100-110 g were purchased from the Pasteur Institute of Iran. The animals were kept in special glass cages (200 × 75 × 100 cm^3). The cages were covered with a metal net and a mesh frame. The room temperature was about 22 ± 2°C, and the 12-hour lighting program started at 8 am. Water and food (mouse-prepared pellets) were purchased from Pars company (Teharn, Iran) and were available to animals ad libitum. This study was conducted according to guidelines of the Ethics Committee on the use and care of laboratory animals (17).

Gasoline vapor exposure

The animals were randomly divided into control (untreated) group and experimental groups exposed to gasoline vapor for 1, 2, 3 hours/day of 5 rats in each group. A total voulume of 500 ml liquid gasoline was supplied every day from the gas station. One hundred ml liquid gasoline was aliquoted into a test tube and placed in the cage of each group. The cages were covered to prevent air exchanging inside the cages.

Blood sampling

After 12 weeks, the rats were anesthetized using ether for blood sampling. Blood samples were collected at 8 am – 8:30 am. The blood samples were collected through cardiac puncture using a 1ml syringe (Nipro Syringe, 26G) and transfered to a 1.5 ml microcentrifuge tube (Eppendorf) and allowed to coagulate for 30 minutes in room temperature. The coagulated blood samples were centrifuged at 3000 rpm for 10 minutes and serum samples were separated and kept in refrigerator until hormon assay.

Testosterone measurements

The serum level of testosterone was measured by ELFA (enzyme-linked fluorescence immunoassay) by VIDAS in biolabroty of Islamic Azad University of Hamedan. This procedure involves a two-stage enzymatic reaction with a sandwich method that produces a fluorescent product instead of a colored product, at the end of the experiment.

Histological studies

The testes were carefully removed from the scrotal sac and fixed in Bowen solution to prepare tissue for staining and routinely processed for paraffin embedding. Slices of 5 µm were obtained with rotary microtome, stained with hematoxylin-eosin and examined under a light microscop. To measure the diameter of the seminiferous tubes and the number of spermatogonia, spermatocyte and Sertoli cells, images of testes histology were prepared and analyzed using a computer.

Statistical analysis

The data were analyzed using SPSS software (PASW Statistics 19). Kolmogorov-Smirnov test was used for normal distribution assessment and. Analysis of variance (ANOVA) and Benferroni post-hoc test were used to compare groups. A P-value less than 0.05 were considered as sugnicant.

Results

Serum testosterone level

Serum testosterone level significantly increased in rats exposed to gasoline vapor for 1, 2, and 3 hour/day compared to the control group (Figure 1).

Testes histology

There was no significant difference between seminiferous tubules diameters, spermatocyte and Sertoli cell count in rats exposed to gasoline vapor and control group. The number of spermatogonia cells was significantly lower in animals exposed to gasoline vapor for 1, 2, and 3 hours/day compared to control group (Table 1). Figure 2 (a-c) shows histology of testes from control animals and rats exposed to gasoline vapor for 1h/day, 2h/day, and 3h/day, respectively.

Table 1. Diameter of the Seminiferous Tubules and Spermatogonia Cells, Spermatocytes, and Sertoli Cells Count in Tubular Tunnel in Control and Experimental Groups

Groups	Seminiferous tubules diameter (µm)	P-value	Number of spermatogonia cells	P-value	Number of spermatocyte cells	P-value	Number of Sertoli cells	P-value
Control	26.83 ± 4.03	- NS	60.7 ± 11.40	- 0.001	128.2 ± 45.25	- NS	25.3 ± 9.47	- NS
1h/day	27.64 ± 3.21		36.5 ± 8.2*		148.2 ± 38.6		23.2 ± 5.9	
2h/day	27.04 ± 2.99		44.3 ± 15.4*		126.5 ± 28.9		25.7 ± 3.9	
3h/day	27.64 ± 4.33		52.7 ± 11.2*		109.7 ± 38.3		26.1 ± 4.5	

NS; Non-significant. *Represent Significant Differences with the Control Group

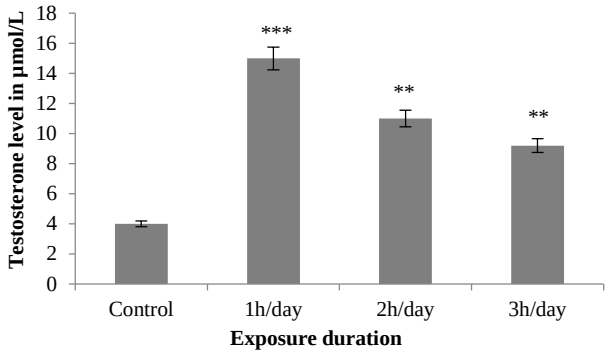

Figure 1. Serum Levels of Testosterone (µmol/L) in Male Rats by Study Group.
The Symbols ** and *** Represent Significant Differences with the Control Group (P-value < 0.01 and P-value < 0.001 respectively).

The more exposure period to gasoline vapor in animlas was associated with higher destructive effects on testes tissue and destructive effects of testes tissue was observed in rats exposed to gasoline vapor.

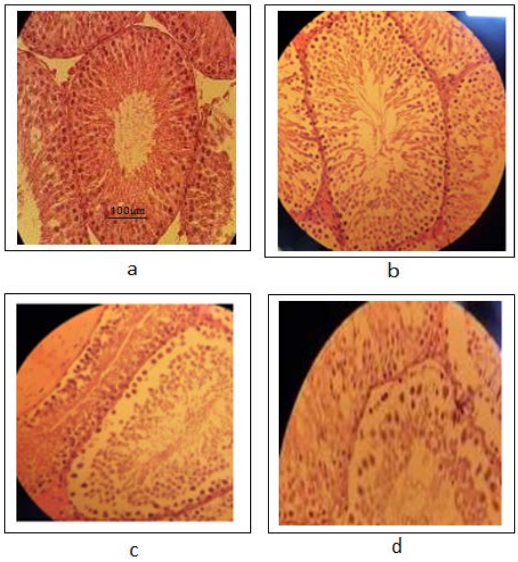

Figure 2. Micrographs (400X) of Testes from Control Animals (a) and Rats Exposed to Gasoline Vapor for 1h/day (b), 2h/day (c) and 3h/day (d).

Discussion

Although many studies have shown that inhaling gasoline has detrimental effects on male fertility, the type and mechanism of this effect are still challenging. The present study, aimed to investigate the alteration in testosterone levels and testicular tissue in rats exposed to daily gasoline vapor for a long period (12 weeks). The results of this study shows that exposure to gasoline vapor has destructive effects on testicular tissue and the destruction magnitude is proportionally associated with the period of exposure to gasoline vapor. Although Spermatogina cells decreased in animals exposed to gasoline vapor, spermatocytes and Sertoli cells count did not significantly changed. Serum testosterone level increased in rats exposed to gasoline vapor.

Testosterone in the testes is regulated by a negative pituitary feedback mechanism, so that an increase in serum testosterone levels decreases serum LH (luteinizing hormone) levels, while a decrease in serum testosterone leads to an increase in serum LH. In this study, it seems that inhalation of gasoline damages LH receptors in the pituitary gland, so increasing testosterone no longer reduces LH and therefore does not reduce testosterone secretion (18).

Elevated testosterone resulting from exposure to gasoline vapor or other environmental pollution can lead to baldness as well as the risk of heart disease and prostate cancer and other problems including sleep apnea, acne, breast enlargement in men, and even testicular shrinkage (19). Decreased spermatogonia also causes male infertility. In line with our findings, a study on 40 albino mice found that exposure to liquid gasoline for six hours a day for 12 weeks reduced sperm motility as well as increased the number of abnormal sperms (no head, twisted tail, damaged head) (20). It was also shown that oral exposure to gasoline, decreased daily sperm production, sperm number in the caput/corpus epididymis, progressive motility, mitochondrial activity, and acrosomal membrane integrity (21). In this regard, another research suggests that exposure to gasoline vapors in workers working at gas station stations can affect the profile of sex hormones (22). Research findings suggest that inhaling diesel vapors, which have very similar gasoline compounds, can cause disorders in the reproductive system (23). A tissue study was performed on the carcinogenicity of gas-emitted gases on various tissues of the mouse body. In this study, it was shown that exposure to 10 g/m^3 of gasoline vapor for 5 hours per day for 104 weeks caused significant tissue changes in the testicles and malignant mesothelioma tumors (24). Previous research also shows that exposure to gasoline vapors can disrupt other tissues, which can indirectly affect the functioning of the reproductive system. In this regard, it has been suggested that inhaling gasoline vapor can affect the kidneys (25) as well as the nervous system (26). The compounds in gasoline, including benzene and lead, have cytotoxic effects on many organs (27). The benzene compounds that enter the blood easily pass through the blood-brain and blood-testicular barriers, causing the destruction and degeneration of testicular tissue cells (28). Exposure to toluene and benzene can kill spermatogonia cells and reduce spermatocyte cells in the testicles. These

compounds also reduce spermatogenic cells by damaging the sodium-potassium pump, causing them to die (35). Reducing the expression level of two genes: "α6 -integrin and β1 - integrin" has been shown to play a regulatory mechanism for reducing spermatogenesis in gasoline inhaling subjects (29). In contrast to our findings there are studies suggesting that exposure to gasoline vapors can reduce spermatocytes and Leydig cells count (30).

Due to some limitations this study was unable to examine the tissue alteration at molecular level including evaluation of proteins and gene expression levels in testicular tissue. Further research is required to reveal molecular alterations in testicular tissue following exposure of animals to gasoline vapor.

Conclusion

The results of the present study show that inhalation of gasoline vapor has significant destructive effects on testicular tissue resulting in decreased spermatogonial cells and increase in serum levels of the testosterone. Considering the gasoline vapor as testicular damaging environmental pollutant, further research is needed to perform for evaluating the effect of gasoline vapor in high-risk occupational groups.

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Ethical **consideration**

The current study was approved by the Ethics Committee of Islamic Azad University, Hamadan Branch, Hamadan, Iran.

Conflicts of **interests**

Authors declared no conflict of interest.

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