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Protective Role of Resveratrol Against Toluene-Induced Oxidative Stress in Rats

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ABSTRACT

Toluene is a toxic substance commonly used in the industry that leads to oxidative stress in the body and causes in the formation of reactive oxygen species. In addition to the antioxidants produced by our body, we also obtain antioxidants through foods. Resveratrol is a polyphenol found in many plant species and possesses antioxidant properties. The effects of oxidative stress induced by toluene and the protective effect of resveratrol were investigated through changes in malondialdehyde (MDA) and glutathione (GSH) levels. Male rats were administered different doses of toluene and resveratrol (5 mg/kg, 10 mg/kg, 20 mg/kg), while control groups were given physiological saline and ethanol. At the end of the experiment, blood and lung tissues of the animals were examined, and MDA and GSH levels were measured. According to the obtained data, a significant increase in GSH activity was observed after the administration of toluene. However, in groups treated with combinations of toluene and various doses of resveratrol, a significant decrease in GSH levels was detected. While a significant increase in MDA levels in lung tissues was observed between the control groups and the toluene group, no significant change was observed in blood samples.

Keywords: Toluene, Resveratrol, Malondialdehyde, Glutathione.

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Introduction

Toluene is an important solvent widely used in various industrial applications and consumer products. Exposure to toluene can lead to oxidative stress. Among the primary reasons for this interaction is the activation of toluene through the metabolic process [1]. Toluene is metabolized in the body, resulting in the formation of various by-products. Among these metabolic products are compounds produced when toluene is metabolized by monooxygenase enzymes. The emergence of these metabolic products can trigger oxidative stress. Exposure to toluene can contribute to the formation of free radicals. Free radicals, which can damage macromolecules such as lipids, proteins, and DNA, can increase oxidative stress in cells [2]. Exposure to toluene can affect the body's natural defense against free radicals by impacting the antioxidant system. Antioxidants neutralize free radicals to protect cells [3,4]. Exposure to toluene can disrupt the balance of the antioxidant system, leading to increased oxidative stress. It can contribute to increased oxidative stress by promoting inflammation in the body, resulting in damage to cells and tissues. Additionally, it can cause damage to mitochondria. Mitochondria are vital structures in cells that produce energy, and when damaged, they can produce free radicals. In conclusion, exposure to toluene can contribute to increased oxidative stress and cellular damage [5].

Phytoalexins are effective compounds naturally produced by plants to combat infections and harmful microorganisms and are often referred to as "plant antibiotics" [6]. Among the most effective phytoalexins of the stilbenoid type is resveratrol, which is a polyphenolic compound [7]. Specifically in grapevines, an infection by a pathogen called Botrytis cinerea leads to the specialized production of resveratrol in the leaves and skin of the grapes [8]. This particular compound is found more abundantly in the skins and seeds of grapes and other fruits. Known for its antifungal, antiviral, anti-inflammatory, antioxidant, and anti-aging properties, resveratrol also stands out for its cardiovascular health-supporting, phytoestrogenic, vasorelaxant, neuroprotective, and anticancer attributes. Possessing antioxidant properties, resveratrol has the ability to effectively neutralize free radicals. Various oxidants, such as O_2^-, •OH, H_2O_2, and $ONOO^-$, can be directly deactivated by resveratrol [9]. Many people prefer to consume this beneficial compound through fruits or as a supplement. However, there are warnings that consuming large amounts of resveratrol can lead to stomach and intestinal issues in some individuals [10].

Glutathione (GSH) is a tripeptide found within cells. It plays a critical role in neutralizing free radicals and reactive oxygen species (ROS), thereby preventing oxidative stress and maintaining cellular homeostasis. It is a central component in preserving redox homeostasis (the oxidative state within cells) [11]. The balance between oxidized glutathione (GSSG) and reduced glutathione (GSH) determines a cell's capacity to cope with oxidative stress. This ensures that cells function healthily and prevent the onset of pathological conditions. Therefore, GSH and its metabolism are considered potential

therapeutic targets in many diseases associated with oxidative stress [12, 13].

Malondialdehyde (MDA), a by-product of lipid peroxidation, is also considered an indicator of oxidative damage. It is used to assess the extent and effects of oxidative damage to membrane lipids [14, 15].

In particular, in research and clinical studies, the examination of GSH and MDA aids in understanding oxidative damage and elucidating cellular mechanisms at the cellular level. In this study, the effects of toluene exposure-induced oxidative stress in rats were investigated. Additionally, it was assessed whether resveratrol plays a protective role against these adverse effects. Levels of MDA, an indicator of lipid peroxidation, and GSH, which plays a significant role in cellular antioxidant defense, were analyzed.

Materials and Methods

Animal Experiments and Procedures

Within the scope of the experiment, 36 adult Wistar Albino male rats weighing between 200–350 grams were selected. The rats were housed in an environment with a room temperature of 20-24ºC where they could freely access food and water. The animals were divided into two main groups: control and experimental. The experimental group was prepared based on exposures to toluene and toluene+resveratrol. Over a span of 6 days, the animals received intraperitoneal (i.p) injections. The first experimental group was administered toluene at a dose of 900mg/kg, the second group received the same dose of toluene combined with 5mg/kg of resveratrol, the third group was given 10mg/kg of resveratrol, and the fourth group received 20mg/kg of resveratrol, all via the i.p route. Resveratrol was dissolved in 10% ethanol before injection. For the control groups, the first group was administered with physiological saline, and the second group received an injection of 10% ethanol.

Collection of Tissue and Blood Samples

After the 6-day experiment, the rats were euthanized in accordance with ethical guidelines using the cervical dislocation method. Lung tissue and blood samples were rapidly collected from the animals. Once obtained, the tissues were rinsed and cleaned in a phosphate buffer with a pH value of 7.4, and their weights were determined using a sensitive balance. While the blood samples were centrifuged at 1000g for 15 minutes at 0-4ºC, the lung tissues were prepared in a 1/9 (w/v) ratio with cold buffer solution on ice and homogenized at a speed of 3000 revolutions per minute. The homogenates were then centrifuged at 10,000g for 15 minutes at 0-4ºC. These procedures were meticulously performed in accordance with the instructions recommended by the analytical kits used. The obtained samples were stored at -80°C."

Determination of MDH Levels

MDH levels were calculated using the formulations recommended by the MDH kit. The standard operational table of the MDA kit used in the study is shown in Table 1.

Table 1. MDA standard operational table.

	blank tube	standard tube	sample tube	Control tube
absolute ethanol (mL)	a*			
reactive 4 (mL)		a*		
samples (mL)			a*	a*
reactive 1 (mL)	a*	a*	a*	a*
reactive 2 application solution (mL)	3.0	3.0	3.0	3.0
reactive 3 application solution (mL)	1.0	1.0	1.0	1.0
50% glacial acetic acid				1.0

a* The volume represents the volumes of the sample, standard, absolute ethanol, and reagent 1, and they are equal. For instance, if the sampling volume is 0.1 mL, then the volume for the standard, absolute ethanol, and reagent 1 is also 0.1 mL each. If the sampling volume is 0.2 mL, then the volume for the standard, absolute ethanol, and reagent 1 is also 0.2 mL each.

Determination of GSH Levels

The thiol group of the cysteine amino acid, which functions in the structure of GSH, reacts with dithionitrobenzoic acid (DTNB) to produce a yellow-colored thio-nitrobenzoic (TNB) compound. The concentration of GSH was determined based on the optical density (OD) values of TNB at a wavelength of 420nm. The standard operational table of the GSH kit used in this study is shown in Table 2.

Table 2. GSH Standard operation table.

	blank tube	standard tube	sample tube
reactive 1 (mL)	1.0		
20 µmol/L GSH standard solution (mL)		1.0	
supernatant (mL)			1.0
reactive 2 application solution (mL)	1.25	1.25	1.25
reactive 3 (mL)	0.25	0.25	0.25
reactive 4 application solution (mL)	0.05	0.05	0.05
It was thoroughly mixed and left at room temperature for 15 minutes. The OD values of each tube were measured at a wavelength of 420nm using a spectrophotometer.			

Statistical Analysis

The statistical analysis of the data was conducted using GraphPad Prism 9.3.0 software. Statistical differences between the control and experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by the posthoc Tukey test. The results were presented as mean values and standard error (SE). For statistical significance, the values *p<0.05, **p<0.01, ***p=0.001, and ****p<0.001 were used.

Results

GSH findings

In the lung tissue samples, GSH levels showed a significant increase of 57% when compared to the control groups in the group that received toluene injections (p<0.001). When the toluene+resveratrol treatments were compared to the control groups, statistically significant increases of 40% (p<0.05) for 5mg/kg, 57% for 10mg/kg, and 63% for 20mg/kg were observed (p<0.001).

A decrease of 28% was observed in the group treated with toluene and toluene+resveratrol at a dose of 5mg/kg. However, no significant difference was detected at a resveratrol dose of 10mg/kg. In the 20mg/kg dose of resveratrol, an 18% increase was observed, which was statistically insignificant (p>0.05).

Upon examining serum samples; a 5% increase was observed between the saline control group and the toluene group, while a 12% decrease was seen between the ethanol control group and the toluene experimental group. These findings did not show a statistically significant difference. When compared to the saline control group, a statistically significant decrease of 36% was recorded in the toluene+resveratrol (5mg/kg, 10mg/kg) treatments (p<0.01). However, despite a 5% decrease between the saline control group and the toluene+resveratrol (20mg/kg) group, this difference was considered statistically insignificant (p>0.05).

A statistically significant decrease of 46% was observed between the ethanol control group and the toluene+resveratrol (5mg/kg, 10mg/kg) group (p<0.001). Similarly, an insignificant decrease of 21% was seen between the ethanol control group and the toluene+resveratrol 20mg/kg group. A statistically significant decrease of 39% was recorded between the toluene group and the toluene+resveratrol (5mg/kg, 10mg/kg) groups (p<0.001). However, a statistically significant increase of 32% was observed between the toluene+resveratrol (5mg/kg, 10mg/kg) groups and the toluene+resveratrol 20mg/kg group (p<0.05). GSH levels in the lungs and serum are presented in Table 3 and Figure 1.

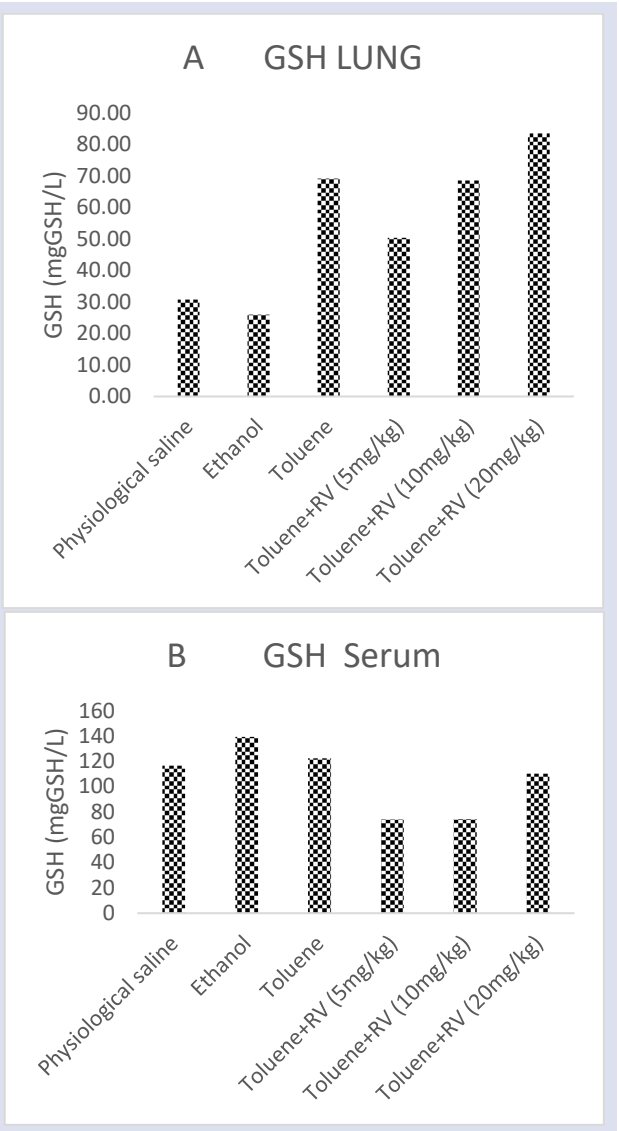


Figure 1 (A) GSH levels in rat lung. (B) GSH levels in rat serum

Table 3. GSH levels in lung and serum (mean ± standard error).

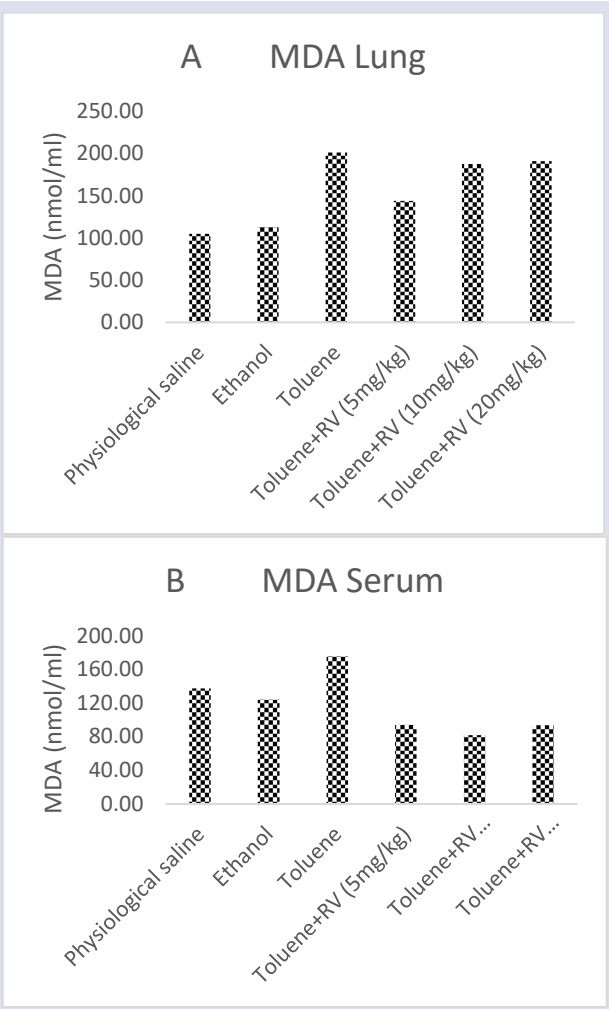
Groups	GSH Lung (µmol/L) Ort ± SE	GSH Serum (µmol/L) Ort ± SE
Control physiological saline	30.77±7.55	116.59±0.25
Control ethanol	26.00±4.45	139.35±6.57
Toluene	69.19±15.39	122.38±13.64
Toluene+Resveratrol (5 mg/kg)	50.32±4.32	74.14±8.07
Toluene +Resveratrol (10 mg/kg)	70.32±6.91	74.39±11.68
Toluene +Resveratrol (20 mg/kg)	83.43±3.77	110.21±19.36

MDA Findings

In lung tissue samples, statistically significant increases of 48% were recorded between the control saline solution group and the toluene group, and 43% between the ethanol group and the toluene group ($p<0.001$).

An increase of 35% was observed between the control saline solution group and the toluene+resveratrol 5mg/kg group, and 27% between the ethanol control group and the same group; however, these increases were not statistically significant ($p>0.05$). A statistically significant increase of 44% was detected between the control saline solution group and the toluene+resveratrol 10mg/kg group, and a 45% increase was observed between the control saline solution group and the toluene+resveratrol 20mg/kg group ($p<0.001$). Furthermore, when compared to the ethanol control group, a 39% increase was noted between the ethanol control group and the toluene+resveratrol 10mg/kg group, and a 40% increase was recorded between the ethanol control group and the toluene+resveratrol 20mg/kg group, both of which were statistically significant ($p<0.01$).

In serum samples, an increase of 21% was observed between the control saline solution group and the toluene group, but this increase was not statistically significant ($p>0.05$). Conversely, a statistically significant increase of 29% was seen between the ethanol control group and the toluene group ($p<0.01$). Significant decreases of 31%, 40%, and 32% were noted between the control saline solution group and the toluene+resveratrol groups at doses of 5mg/kg, 10mg/kg, and 20mg/kg, respectively ($p<0.01$ and $p<0.05$, respectively). When compared to the ethanol control group, decreases of 24%, 34%, and 25% were recorded in the toluene+resveratrol groups at doses of 5mg/kg, 10mg/kg, and 20mg/kg, respectively. While there was a statistically insignificant decrease in the levels of resveratrol at doses of 5mg/kg and 20mg/kg ($p>0.05$), a significant decrease was identified at a dose of 10mg/kg ($p<0.05$). Finally, statistically significant decreases of 46%, 53%, and 46% were observed between the toluene group and the toluene+resveratrol groups at doses of 5mg/kg, 10mg/kg, and 20mg/kg, respectively ($p<0.001$). The MDA levels in the lungs and serum are presented in Table 4 and Figüre 2.



Figüre 2. (A) MDA levels in the lungs. (B) MDA levels in the serum

Table 4. MDA levels in lung and serum (mean ± standard error).

Gruplar	MDA Lung (µmol/L) Ort ± SE	MDA Serum (µmol/L) Ort ± SE
Control physiological saline	104.88±25.92	137.29±16.30
Control ethanol	113.13±7.25	124.06±13.45
Toluene	201.50±38.35	175.63±19.25
Toluene+Resveratrol (5 mg/kg)	144.17±36.22	94.06 ±13.21
Toluene+Resveratrol (10 mg/kg)	187.75±28.64	81.72 ±14.53
Toluene+Resveratrol (20 mg/kg)	191.00±14.51	93.44 ±21.58

Discussion

After injecting toluene into the rats, redness in the eyes, bleeding from the mouth and nose, and unsteady movements were observed. It is believed that these symptoms stemmed from the toxic effects of toluene. When resveratrol was administered, it was observed that the bleeding symptoms ceased and the rats regained their normal mobility. These findings support the protective properties of resveratrol. In previous studies, symptoms

induced by toluene in rabbits included vomiting within 2-3 hours, bleeding from the mouth and nose, and signs of imbalance [16].

According to the MDA analyses, it is observed that toluene injection leads to a significant increase in MDA levels. The reason for this increase may be due to toluene's lipophilic properties, allowing it to easily interfere with lipid structures in the cell membrane and interact with proteins. In a previous study, it was determined that prolonged inhalation of thinner increased lipid peroxidation in lung and liver tissues. One study suggests that inhalation of thinner can accelerate ROS formation, triggering lipid peroxidation [17].

In another study, it was noted that exposure to high doses of toluene triggers oxidative stress, increasing the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This condition was stated to directly cause tissue damage and have an impact on antioxidant systems [18].

In the tissue and serum samples we examined in our study, significant differences in GSH levels were identified in the group treated with toluene. It is known that in an organism exposed to organic solvents and smoking, diseases like cancer develop due to the increase of ROS and free radicals. In this context, GSH, which has antioxidant properties, plays a critical role in neutralizing accumulated free radicals. This process can lead to a decrease in the body's antioxidant capacity [19]. Additionally, GSH has various critical functions in biological processes such as DNA synthesis, repair of damaged DNA, execution of metabolic functions, neutralization of toxins, and protection against potential harms of free radicals [20].

In our study, a decrease in GSH and MDA levels was observed following the application of resveratrol. We believe that this change is due to resveratrol neutralizing ROS. Some studies indicate that resveratrol is highly effective in removing reactive oxygen species from the cell, such as O_2- anions, ROS, and H_2O_2, by preventing cellular damage and apoptosis during oxidative stress [21, 22].

Toluene has been observed to increase GSH and MDA levels in both lung tissue and serum. This increase may be attributed to toluene triggering oxidative stress and subsequently initiating lipid peroxidation. When examining lung tissue samples, although there were significant differences between the control and experimental groups, no significant change in GSH values was detected between toluene and toluene+resveratrol injections. However, in serum samples, it is evident that toluene and varying doses of toluene+resveratrol injections significantly influenced GSH activity. These findings demonstrate the enhancing effect of resveratrol, especially at high doses, on GSH activity in lung tissue. Elevated antioxidant levels can reduce the amount of ROS. This condition can lead to a decrease in lipid peroxidation by clearing free radicals, consequently resulting in a reduction in MDA levels.

When MDA levels were carefully examined, it was observed that the MDA levels in the lung tissue of groups treated with toluene increased compared to the control groups. On the other hand, a different picture emerges in serum samples; a significant difference in MDA levels was detected between groups receiving toluene and those receiving toluene+resveratrol injections.

According to the results of this study, a protective effect of resveratrol on oxidative stress and antioxidant activity has been determined. An increase in MDA levels in the lung tissue and serum due to the effect of toluene was observed, while an increase in GSH levels in the lung tissue was also noted.

These findings indicate the need for further research to more comprehensively understand the protective mechanisms of resveratrol and its regulatory effects on GSH. Evaluating the experimental results by administering resveratrol via gavage at 40 mg/kg/day and 80 mg/kg/day for 10 weeks may create new perspectives.

Conflict of interest

There is no conflict of interest among the authors.

Acknowledgement

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