

The Antioxidant Properties of Resveratrol Effectively Mitigate Cadmium-Induced Oxidative Stress by Enhancing the Endogenous Antioxidant Capacity in Wistar Rats

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Abstract Resveratrol is a phytochemical found in red grapes, berries, and certain microorganisms. This naturally occurring bioactive polyphenolic compound is known for its various biological activities. The current study aimed to evaluate resveratrol's in vivo antioxidant properties and its effectiveness in reducing oxidative stress caused by cadmium exposure. In the experiment, rats were divided into four groups (n=20): an untreated control group, a resveratrol-treated group, a cadmium-exposed group, and a cadmium-resveratrol group. Hematological and biochemical assays were conducted to meet the study's objectives. The haematological and biochemical profiles of the cadmium-exposed rats (Group 3) showed significant changes, including increases and decreases, compared to the untreated control group. In the biochemical profile analysis of serum, the resveratrol-treated animals in Group 2 displayed no significant differences relative to the untreated control. In contrast, rats in Group 4 (the cadmium-exposed and resveratrol-treated group) exhibited elevated levels of total proteins and marked increases in antioxidant markers, including total thiols, glutathione, total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase. Additionally, this group showed a substantial reduction in blood cadmium levels and lower concentrations of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, blood urea nitrogen (BUN), urea, bilirubin, and oxidative stress markers such as hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) compared to the cadmium-exposed animals (Group 3). Results from tissue homogenate analysis of the liver and kidneys in Group 3 were consistent with the serum biochemical findings. These results suggest that resveratrol possesses potent antioxidant properties that effectively mitigate oxidative stress resulting from cadmium exposure.

Keywords: Resveratrol, cadmium toxicity, oxidative stress, antioxidant, biochemical profile

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1. Introduction

Resveratrol (C₁₄ H₁₂ O₃) is a non-flavonoid polyphenol, specifically classified as a polyphenolic stilbenoid (3, 5, 4-trihydroxy-trans-stilbene). Polyphenols, which encompass both flavonoids and non-flavonoids, are important secondary metabolites found in a variety of vegetables, fruits, tea, and coffee. These compounds play

a crucial role in helping plants adapt to their environmental conditions [1].

Resveratrol is a naturally occurring bioactive polyphenolic compound that is particularly abundant in red grapes, berries, tomatoes, strawberries, and raspberries [2]. It is also present in certain microorganisms, such as *Cephalosporium* and *Penicillium* [3]. Notably, resveratrol is not synthesized by the human body, making dietary supplements an important source of this compound [4].

Research has demonstrated that resveratrol exhibits a

wide range of activities, primarily attributed to its anti-inflammatory and antioxidant properties [5,6]. Numerous experimental studies have focused on exploring the properties and potential health benefits of resveratrol, including its possible protective effects against various diseases.

The endogenous antioxidant system consists of both enzymatic and non-enzymatic components, such as thiols [7]. Key examples of antioxidant enzymes include catalase, glutathione peroxidase, and superoxide dismutase.

One of the primary sources of damage within the body comes from oxidative actions initiated by certain metabolites or external toxins. These harmful effects can be severe, potentially leading to cell death and significant tissue damage.

Reactive oxygen species (ROS) encompass both radicals—such as singlet oxygen, superoxide, hydroxyl radicals, and alkoxyl radicals—and non-radicals like hydrogen peroxide [1,8,9]. An imbalance between the production of oxidant molecules and the effectiveness of the antioxidant defence system results in oxidative stress. It is essential to keep ROS levels low under normal conditions, as intracellular concentrations must be regulated to maintain a balance between their generation and neutralization [10,11].

Oxidative stress is a significant factor in the development of various serious health conditions, including cardiovascular diseases and cancer. Excessive ROS can damage cellular components, including structural lipids, proteins, and DNA. This damage can contribute to various medical issues of great concern, such as neurodegenerative disorders, cardiovascular diseases, cancer, diabetes, accelerated aging, and immune dysfunction [12].

Counteracting these actions, either by prevention or inhibition, plays a crucial role in the relevant antioxidative mechanisms. The naturally operating and efficient endogenous antioxidant system detects oxidative metabolites, such as free radicals, and prevents their damaging effects [13]. The antioxidant system accounts for maintaining the balance status between the oxidative reactions and anti-oxidative activities in cells and tissues. The dysfunction and disturbances of this defense system contribute significantly to the development of disease conditions of medical concern such as diabetes, atherosclerosis, cancer, and neurodegenerative disorders [5,6].

Antioxidants refer to the compounds that can counteract the oxidative damage caused by ROS, and in this way combat oxidative stress [14].

The role of a potent antioxidant is to limit the extent of ROS-induced damage and contributes actively to maintaining Redox status as a cytoprotective mechanism [11].

The accumulated clinical and experimental evidence demonstrates the role of natural antioxidants in the prevention of disease conditions and the promotion of vital body functions including that of the heart, immune system, and brain [5]. In many cases, regular foods are deficient in the natural dietary antioxidants (antioxidant nutrients) that are best exemplified by vitamins C and E, selenium, zinc, polyphenols, and carotenoids. In such situations, there is an increasing demand for antioxidant supplements that support the body's bioactive processes and maintain the health status. The potent supplementary antioxidant can restore the balance between the oxidation

and reduction reactions (Redox status) [6].

Cadmium (Cd) is a highly toxic heavy metal that naturally exists in the environment and is involved in a wide spectrum of industries. Exposure to Cd is either occupational or non-occupational (environmental). Occupational exposure is linked to the inhalation of industrial fumes, while non-occupational exposure is related to the ingestion of polluted feed and water. The hazardous effects of chronic Cd toxicity are progressive since the accumulation of this toxicant heavy metal is gradual (cumulative) in various tissues [15]. Cadmium toxicity is implicated in the causation of profound oxidative stress associated with oxidative damaging effects in tissues and organs, particularly in the liver and kidney [16].

The focus is now on natural antioxidants that can be used as dietary supplements. Their use has been increasing as preventive and therapeutic measures for various significant health conditions including cardiovascular diseases, neurodegenerative disorders, cancer, arthritis, and age-related changes [5,17].

The study aimed to investigate the antioxidant properties of resveratrol in male Wistar rats. The antioxidative properties of resveratrol were tested in the presence of oxidative stress induced by cadmium toxicity.

2. Material and Methods

Ethical Considerations:

The guidelines for the care and use of laboratory rats, as per institutional and national regulations, set by the Research Ethics Committee of Imam Mohammad Ibn Saud Islamic University (IMSIU), were diligently adhered to (LAB-rats-2023-0239).

Type of Sampling and reasons for selection:

Blood, harvested serum, and tissue homogenates were selected as the investigated samples in the present study. These samples were chosen to reflect the alterations in the haematological and biochemical profiles of experimental rats.

Inclusion and Exclusion Criteria:

Inclusion Criteria

All rats in the different experimental groups were included in the conducted assays. Blood samples were collected from all experimental rats to carry out the haematological assay to assess erythrocytic and leucocytic counts, haemoglobin concentration, and packed cell volume. The harvested serum samples as well as the tissue homogenates obtained from all experimental rats were all subjected to the biochemical assay to estimate the levels of antioxidative and oxidative parameters.

Exclusion Criteria

No exclusion criteria were applied in the current study. In other words, all experimental rats were included in the collection of samples (blood and tissues). Any exclusion criteria can alter in different ways the accuracy of the performed analysis.

Experimental rats:

In this study, we used eighty adult male Wistar rats, which were 3 months old and weighed between 140-220 g. The rats were obtained from the inbred colonies at the animal house, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were kept under standard laboratory conditions, with an ambient temperature of 24

$\pm 1^{\circ}\text{C}$, a 12-hour dark-light cycle, and a relative humidity of 35-70%.

Resveratrol:

Resveratrol ($\text{C}_{14}\text{H}_{12}\text{O}_3$) 3,4',5-Trihydroxy-trans-stilbene, 5-[(1E)-2-(4-Hydroxyphenyl) ethenyl]-1,3-benzenediol (MW 228.24) was purchased from Sigma-Aldrich (Darmstadt, Germany) (CAS No.: 501-36-0).

Cadmium

Cadmium (Cd) was used as cadmium chloride (CdCl_2) of analytical grade (Merck, Darmstadt, Germany) (Product No. 655198).

Cadmium was dissolved in purified water to prepare the required aqueous solution.

Experimental design

Rats were acclimatized for one week and then randomly and evenly distributed into four groups, each consisting of 20 rats, designated as Groups 1, 2, 3, and 4. Rats in Group 1 served as the untreated control, meaning they were not exposed to cadmium and did not receive resveratrol. Group 2 received daily oral administration of resveratrol at a dosage of 170 mg/kg body weight in a volume of 1 mL/kg body weight. In Group 3, the rats were given an aqueous solution of cadmium chloride (CdCl_2) via oral gavage at a final concentration of 5 mg/kg body weight per day, also in a volume of 1 mL/kg body weight. The control rats received an equivalent volume of saline using the same method. Group 4 rats received both cadmium orally and resveratrol at the same aforementioned doses, with a 10-hour interval between the daily administration of cadmium and resveratrol.

The experimental period lasted for eight weeks, during which the rats had ad libitum access to dry feed (commercial pellets from Nesom Distributing, Envigo, USA) and drinking water. All experimental rats were monitored for behavioral activity, feed consumption, water intake, and any clinical signs.

Haematological and biochemical assays

On the termination day of the experiment, the rats were anaesthetized (3% isoflurane), and blood samples were collected via cardiac puncture from all the rats in different groups. Blood samples collected with anticoagulant (EDTA) were employed to estimate the various haematological indices. Serum harvested from the coagulated blood samples was removed immediately and stored at -20°C until a biochemical assay was performed. Rats were then killed by decapitation, liver and kidney tissues were removed and homogenized in 150 mM NaCl, and the homogenates were then centrifuged at 3000 Xg at 4°C for 10 min. The collected supernatants were used to determine the various biochemical parameters.

Blood cadmium level

To assess cadmium levels in the blood, 1 mL blood samples were subjected to digestion using a mixture of HClO_4^- and HNO_3 , and then blood cadmium levels were determined using an atomic absorption spectrophotometer (CBC 906 AA).

Haematological assay

Blood samples collected with an anticoagulant (EDTA) were used to estimate various haematological parameters, including RBCs and total WBC counts, and other erythrocytic indices, including haemoglobin (Hb) concentration and packed cell volume (PCV) %. Erythrocytic and total leukocyte counts were measured

using a convenient hemocytometer. The PCV% was determined using the micro-hematocrit method. Hb concentration was estimated using the Cyanmet-hemoglobin method, as described previously.

Biochemical assay

Serum separated from the coagulated blood samples was used to estimate the various biochemical parameters including; total proteins, albumin, globulin, creatinine, urea, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as the antioxidant markers including total thiols, catalase, glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total antioxidant capacity (TAC), and oxidation markers involving malondialdehyde (MDA) and hydrogen peroxide (H_2O_2).

Total thiols were measured using a total thiol colourimetric assay kit (Cell Biolabs Inc., USA) (MET-5053). GSH was estimated using a reduced glutathione colourimetric assay kit (ElabScience, USA) (E-BC-K030-S). Catalase levels were determined using a catalase activity colourimetric assay kit (BioVision; Abcam, UK) (ab 83464). Superoxide dismutase activity was estimated using a SOD activity assay kit (Sigma-Aldrich, Darmstadt, Germany). Glutathione peroxidase activity was assessed using a GSH-Px activity assay kit (Elabscience, Houston, Texas, USA).

TAC was assessed by employing of total antioxidant capacity assay kit (TAC assay kit) (Sigma-Aldrich, Germany) (MAK 187-1 KT). The principle of this kit is the determination of the concentration of combined protein and small-molecule antioxidants or the concentration of only small-molecule antioxidants. Cu^{2+} ions are converted to Cu^+ by small molecules and proteins. However, the insertion of a protein mask as a component of the kit prevents the reduction of Cu^{2+} by proteins, enabling the analysis of only small-molecule antioxidants. The Cu^+ ions (reduced by the small molecules) were chelated with a colourimetric probe, and the resultant absorbance peak was proportional to the antioxidant capacity.

The H_2O_2 levels were determined using an H_2O_2 colourimetric assay kit (Elabscience, USA) (E-BC-K102-S). MDA levels were estimated using a colourimetric assay kit for MDA (Elabscience, USA) (E-BC-K028-M).

ALT, AST, and ALP levels were measured using the relevant diagnostic kits (Abcam, UK) (Catalog Nos.: ALT ab105134, AST ab105135, ALP ab83369). The urea levels were determined using a colourimetric assay kit (BioVision, Biovision Incorporated, UK) (Catalog No. K375-100). BUN levels were estimated using a BUN colourimetric detection kit (ThermoFisher Scientific, USA) (Catalog No. EIABUN). Other biochemical parameters, including total proteins, creatinine, bilirubin, albumin and globulin were assessed using the relevant colourimetric diagnostic kits (Interchim Diagnostics Biochemistry kits, France) (Catalog Nos.: total proteins FT7250, creatinine FT7040, bilirubin FT 6920, albumin FT 6760, globulin FT 7253).

Liver and kidney homogenates were prepared to estimate the levels of total thiols, glutathione, catalase, H_2O_2 , MDA and TAC in the tissues. The same assay kits used to determine the serum levels of these parameters were used to assess their levels in the tissue homogenates.

3. Statistical Analysis

The data presented in this study are expressed as means $\pm$ standard deviation (S.D.). To compare means between multiple groups, one-way ANOVA and SPSS software (SPSS Inc. Chicago IL, USA) was used for statistical analysis. The normality and homogeneity of variances were checked, and the independence of observations was ensured. The normality of the data was verified using the Shapiro-Wilk test. Results with a *P*-value less than 0.05 ($P < 0.05$) were considered statistically significant.

4. Results

Rats that were given resveratrol, as well as rats that were exposed to cadmium and also given resveratrol, exhibited normal behaviour, activity, and food intake compared to the untreated control rats. However, rats exposed to cadmium but not treated with resveratrol showed a decrease in activity and reduced food intake, starting from week 3 of the experiment compared to the control rats. No deaths were observed among the rats in any of the experimental groups.

In control rats, the blood cadmium level was (0.0021 ± 0.0001 ppm) and significantly increased ($P < 0.05$) in rats exposed to cadmium (0.581 ± 0.020 ppm). Blood cadmium levels were comparatively lower in rats exposed to cadmium and received Resveratrol (0.231 ± 0.018 ppm) than in cadmium-exposed rats.

Regarding the assessed haematological parameters, rats that received Resveratrol (Group 2) showed no significant differences from the control levels. The haematological profile of rats exposed to cadmium and not receiving resveratrol (Group 3); showed varied decrements in its parameters compared to the control levels. The haemoglobin (Hb) concentration and packed cell volume (PCV) % were significantly altered. Compared to the parameter decrements recorded in Group 3, the haematological parameters estimated in rats exposed to cadmium and receiving resveratrol (Group 4) were relatively improved and became closer to the control

levels.

Table 1 shows the estimated haematological parameters in rats that received resveratrol, rats exposed to cadmium, and rats exposed to cadmium and administered with Resveratrol compared to the control rats.

Concerning the biochemical profile, rats that received resveratrol (Group 2) showed no significant biochemical alterations compared to the control levels. Comparable decrements in the estimated levels of total proteins, albumin, and globulin were recorded in rats of Group 3, which were exposed to cadmium and had no access to resveratrol. Rats in this group showed a significant increase in creatinine, urea, and BUN.

The serum and tissue levels (tissue homogenates) of total thiols, glutathione, superoxide dismutase, glutathione peroxidase, and catalase were significantly decreased in the group of rats exposed to cadmium and did not receive resveratrol (Group 3). Total antioxidant capacity (TAC), in serum and tissues, was significantly reduced in rats exposed to cadmium, and improved in rats exposed to cadmium and received Resveratrol. Levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in the serum and tissues of rats exposed to cadmium without access to resveratrol were significantly increased compared to the control levels.

The recorded parameters of the altered biochemical profile (serum and tissues) were brought closer to the control levels in rats exposed to cadmium and administered with resveratrol (Group 4).

Table 2 (a, b, c) shows the biochemical parameters (serum levels) in rats that received resveratrol, rats exposed to cadmium, and rats exposed to cadmium and administered with resveratrol compared to the control rats.

Table 2. Biochemical parameters of rats that received resveratrol, exposed to cadmium, exposed to cadmium and administered with resveratrol, and untreated control rats.

Table 3 shows the levels of total thiols, glutathione, catalase, glutathione peroxidase, superoxide dismutase, TAC, H_2O_2 , and MDA in the liver and kidney homogenates of rats that received resveratrol, rats exposed to cadmium, and rats exposed to cadmium and administered with resveratrol compared to the control rats.

Table 1. Haematological parameters of rats that received resveratrol, exposed to cadmium, and that were exposed to cadmium and administered with resveratrol, compared to the control rats. RBC count, total leucocytic count, haemoglobin concentration, and packed cell volume percentage of cadmium-exposed rats were significantly lower compared to the control rats. The measured haematological indices in resveratrol-administered rats exhibited no significant differences compared to the control rats. In cadmium-exposed rats and those administered with resveratrol, the estimated haematological parameters showed significant increments compared to cadmium-exposed rats and were closer to the control levels

Parameter	Untreated Control	Resveratrol	Cadmium	Cadmium and Resveratrol
Total leucocytic Count ($10^3/\text{mm}^3$)	6.44 ± 0.36	6.48 ± 0.21	$5.04^* \pm 0.25$	$6.21^{**} \pm 0.06$
RBCs count ($10^6/\text{mm}^3$)	5.53 ± 0.07	5.61 ± 0.09	$4.03^* \pm 0.11$	$5.43^{**} \pm 0.02$
Packed cell volume (PCV %)	44.26 ± 0.27	45.03 ± 0.27	$36.02^* \pm 0.53$	$43.54^{**} \pm 0.81$
Haemoglobin (Hb) concentration (g/dL)	12.61 ± 0.31	12.73 ± 0.26	$9.51^* \pm 0.34$	$12.13^{**} \pm 0.31$

Values are shown as means $\pm$ S.D., Number of rats/group = 20.

*Significantly different means from that of untreated control rats ($P < 0.05$)

**Significantly different means from that of cadmium-exposed rats.

Table 2a. Serum levels of albumin (g/dL), and globulin (g/dL), and total proteins (g/dL) were significantly lower in cadmium-exposed compared to the untreated control rats. Levels of creatinine (mg/dL), urea (mg/dL), blood urea nitrogen (BUN) (mg/dL), and bilirubin (mg/dL) in the cadmium-exposed rats were significantly increased compared to the untreated control rats. Rats administered with resveratrol demonstrated no significant differences in their biochemical parameters compared to the untreated control rats. The estimated biochemical parameters in cadmium-exposed and resveratrol-treated rats exhibited improvements towards the control levels and were significantly different from those estimated in the cadmium-exposed rats

Parameter	Untreated Control	Resveratrol	Cadmium	Cadmium and Resveratrol
Albumin	3.30 ± 0.02	3373 ± 0.16	2.32* ± 0.14	3.03** ± 0.15
Globulin	3.84 ± 0.08	3.83 ± 0.17	2.33* ± 0.16	3.54** ± 0.28
Total proteins	7.51 ± 0.15	7.54 ± 0.21	5.26* ± 0.16	7.06** ± 0.13
Creatinine	0.53 ± 0.13	0.55 ± 0.13	0.94* ± 0.07	0.63** ± 0.27
Bilirubin	40.62 ± 0.41	40.61 ± 0.38	74.19* ± 0.61	48.15** ± 0.64
Urea	6.39 ± 0.41	6.47 ± 0.37	11.54* ± 0.24	7.16** ± 0.41
BUN	15.34 ± 1.02	15.85 ± 1.05	29.33* ± 1.32	17.73 ± 1.46

Values are shown as means ± S.D., Number of rats/group =20.

*Significantly different means from that of untreated control rats ($P < 0.05$)

**Significantly different means from that of cadmium-exposed rats.

Table 2b. Serum levels of alanine transferase (ALP) (IU/L), aspartate transferase (ALT) (IU/L), and alkaline phosphatase (ASP) (IU/L) in the different groups. The cadmium-exposed rats had significantly increased levels of all measured biochemical parameters. No significant differences were recorded in the resveratrol group compared to the control group. In the group of rats exposed to cadmium and administered with resveratrol, the biochemical parameters displayed improvements towards the control levels and were significantly decreased compared to the cadmium-exposed rats

Parameter	Untreated Control	Resveratrol	Cadmium	Cadmium and Resveratrol
ALP	25.17 ± 1.21	25.16 ± 1.03	75.75* ± 1.38	28.16** ± 1.59
ALT	26.64 ± 1.12	26.53 ± 1.08	69.12* ± 1.13	30.51** ± 1.07
AST	41.33 ± 1.09	41.39 ± 1.07	139.17* ± 3.41	53.17** ± 1.19

Values are shown as means ± S.D., Number of rats/group =20.

*Significantly different means from that of untreated control rats ($P < 0.05$)

**Significantly different means from that of cadmium-exposed rats.

Table 2c. Serum levels of total antioxidant capacity (TAC) (nmol/mL), total thiols (mmol/L), glutathione (GSH) (μg/mL), catalase (IU/L), superoxide dismutase (SOD) (U/mL), glutathione peroxidase (GSH-Px) (U/mL), and , malondialdehyde (MDA) (nmol/mL), and hydrogen peroxide (H₂O₂) (mmol/L) in the different groups. Compared to the untreated control rats, the cadmium-exposed rats exhibited significantly decreased levels of total thiols, GSH, catalase, and TAC. The levels of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) were significantly increased in cadmium-exposed rats compared to the untreated control rats. Resveratrol-treated rats showed no significant differences in the measured biochemical parameters compared to the untreated control rats. In the rats exposed to cadmium and treated with resveratrol, the biochemical parameters showed improvements toward the control levels and were significantly different compared to those of the cadmium-exposed rats

Parameter	Untreated Control	Resveratrol	Cadmium	Cadmium and Resveratrol
TAC	35.42 ± 1.02	35.74 ± 1.09	16.76* ± 1.04	29.68** ± 1.08
MDA	311.15 ± 3.04	313.61 ± 3.12	445.31* ± 3.41	332.23** ± 3.51
H ₂ O ₂	40.73 ± 1.51	40.65 ± 1.43	90.17* ± 1.14	49.19** ± 1.03
Total thiols	2.44 ± 0.27	2.37 ± 0.21	0.27* ± 0.03	2.03** ± 0.81
Glutathione	40.95 ± 1.13	41.58 ± 1.08	13.93* ± 0.63	36.94** ± 1.29

Parameter	Untreated Control	Resveratrol	Cadmium	Cadmium and Resveratrol
Catalase	51.87 ± 1.52	52.69 ± 1.49	28.88* ± 1.05	46.81**± 1.21
SOD	6.32 ± 1.71	6.21 ± 1.61	3.44 ± 1.29	5.71 ± 1.34
GSH-Px	133.01 ± 2.40	128.03 ± 2.11	76.05 ± 1.67	120.07 ± 1.64

Values are shown as means ± S.D., Number of rats/group =20.

*Significantly different means from that of untreated control rats ($P < 0.05$)

**Significantly different means from that of cadmium-exposed rats.

Table 3. Levels of total antioxidant capacity (TAC) (nmol/mL), total thiols (mmol/L), glutathione (GSH) (μg/mL), catalase (IU/L), superoxide dismutase (SOD) (U/mL), glutathione peroxidase (GSH-Px) (U/mL), and total antioxidant capacity (TAC) (nmol/mL), malondialdehyde (MDA) (nmol/mL), and hydrogen peroxide (H₂O₂) (mmol/L) in the tissue homogenates of different groups. The levels of total thiols, GSH, catalase, and TAC were significantly decreased in the tissue homogenates of cadmium-exposed rats compared to untreated control rats. MDA and H₂O₂ levels were significantly increased in the tissue homogenates of cadmium-exposed rats compared to untreated control rats. Resveratrol -treated rats showed no significant differences compared to untreated control rats. The estimated biochemical parameters in tissue homogenates of rats exposed to cadmium and administered with resveratrol demonstrated improvements toward the control levels and were significantly different compared to those measured in the cadmium-exposed rats

Parameter	Untreated Control		Resveratrol		Cadmium		Cadmium and Resveratrol	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
TAC	15.72 ± 1.12	14.60 ± 1.09	15.18 ± 1.02	14.89 ± 1.03	7.38* ± 1.09	8.63* ± 1.08	13.77**±1.09	13.37**±1.01
Total thiols	1.93 ± 0.26	1.05 ± 0.26	1.77 ± 0.26	1.09 ± 0.27	0.22*±0.05	0.22*±0.04	1.84** ± 0.35	1.67** ± 0.45
Glutathione	16.51 ± 1.13	14.73± 1.13	16.55±1.07	14.77± 1.03	5.02*±0.61	4.98*±0.69	14.72**± 1.39	14.84**± 1.44
Catalase	20.21 ± 1.52	19.13 ± 1.21	19.85 ± 1.32	18.87 ± 1.11	9.19*±1.03	9.12*±1.07	17.74**± 1.11	17.46**± 1.56
SOD	6.23 ± 1.71	6.15 ± 1.74	6.17 ± 1.66	6.19 ± 1.61	3.42 ± 1.56	3.14 ± 1.58	5.26 ± 1.81	5.07 ± 1.64
GSH-Px	132 ± 2.41	127 ± 2.33	131 ± 2.21	114 ± 2.37	81 ± 1.48	76 ± 1.89	116 ± 1.78	113 ± 1.38
H ₂ O ₂	132 ± 2.41	127 ± 2.33	16.49± 1.17	14.41 ± 1.09	88.91* ± 1.17	98.11* ± 1.62	18.44**± 1.01	19.22**± 1.08
MDA	14.92 ± 1.49	14.84 ± 1.13	123.0 ± 13.13	122.11±3.15	412.61*±3.39	493.31*±3.64	147.28**±3.11	145.08**±3.61
	126.18± 3.18	122.11± 3.75						

Values are shown as means ± S.D., Number of rats/groups=20.

*Significantly different means from that of untreated control rats ($P < 0.05$)

**Significantly different means from that of cadmium-exposed rats.

5. Discussion

The current study used a resveratrol supplement along with cadmium, a highly toxic heavy metal that induces oxidative stress. The study aimed to test how well resveratrol could improve the haematological and biochemical changes caused by cadmium-induced oxidative stress.

Oxidative overload, also known as oxidative stress, happens when there is an imbalance between oxidation reactions and antioxidant activities (redox status). The oxidative stress index is referred to the ratio between the total oxidant status and the total antioxidant capacity [12]. In the presence of this metabolic stress, the effectiveness of the body's natural antioxidant system in combating the harmful effects of reactive oxygen species is remarkably reduced (ROS) [18]. Pathological conditions or

environmental factors that disrupt this balance are considered triggers for oxidative stress.

A balanced level of free radicals is important for normal metabolic processes such as cell respiration. Most of the cellular ROS are generated in mitochondria and their biosynthesis is related to oxidative enzymatic reactions [10,19]. ROS includes free radicals like hydroxyl and superoxide radicals, as well as non-radicals, primarily hydrogen peroxide. Oxidative stress is one of the key events in the progression of tissue damage and the development of diseases along with depletion of the antioxidant molecules. It is characterized by the overproduction of reactive oxygen species (ROS) [20]. Free radicals are unstable molecules that are featured by the presence of one or more unpaired electrons. Neutralization of free radicals by the endogenous antioxidants is mediated by hydrogen atom or single electron transfer mechanisms [14,21]. These neutralizing

mechanisms are among the pathways that the antioxidant system could regulate the excess free radicals to prevent, impede, or inhibit their harmful effect.

The antioxidant system consists of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic molecules [22]. Each of these antioxidants targets specified free radicals and works in various ways to prevent their damaging effects and maintain the balance between oxidative reactions and antioxidant activity [23]. Antioxidants block the reaction chain of free radicals and donate electrons to these radical metabolites to stabilize them.

Excess free radicals can be harmful whenever the antioxidant system is inefficient enough to combat them. These unstable molecules can oxidize other cellular biomolecules, leading to oxidative stress. This stress can damage cell proteins and DNA, potentially leading to cell death. Lipid peroxidation, which mainly affects cell membranes, is an important result of this oxidative damage [14].

Cadmium (Cd) is a highly toxic heavy metal that was studied as a cause of oxidative stress in our research. It specifically triggers the production of reactive oxygen species (ROS), leading to severe damage to the liver and kidneys [16]. The toxic effects of cadmium result in the oxidation of cell membrane lipids [24] and a significant reduction in the production of ATP and glutathione levels in the mitochondria [25]. Furthermore, cadmium toxicity impairs the function of antioxidant enzymes, resulting in heightened oxidative stress. Ultimately, cadmium-induced toxicity culminates in apoptosis due to caspase activation [26].

The ALT, AST, and ALP serum enzyme levels were significantly increased in the rats exposed to cadmium, indicating Cd-induced liver damage. This could be explained by the damage to the lysosomes caused by lipid peroxidation, which releases lysosomal enzymes into the blood, resulting in elevated enzyme levels. Additionally, cadmium exposure also increased blood urea and creatinine levels, indicating kidney damage in the exposed rats.

The markers presently used to measure antioxidative status, such as total thiols, glutathione, superoxide dismutase, glutathione peroxidase, catalase, and total antioxidant capacity (TAC), were found to be significantly decreased in rats that were given cadmium. This indicates that the antioxidants were affected by an excessive amount of oxidative stress. It is suggested that ROS generated during this process caused the oxidation of these antioxidant molecules, leading to their altered functions and reducing their capacity to combat oxidative damage.

Some endogenous antioxidants, such as glutathione and catalase, work to directly eliminate free radicals. When these antioxidants become oxidized, it leads to an increase in free radicals and exacerbates oxidative stress. In the case of catalase, it breaks down hydrogen peroxide (H_2O_2), which is responsible for lipid peroxidation. Reduced catalase activity allows hydrogen peroxide-induced powerful oxidizing effects in rats. The action of hydrogen peroxide forms the basis of the Fenton reaction, which produces the highly damaging hydroxyl radical (OH).

The current significant increase in the levels of malondialdehyde (MDA) in cadmium-intoxicated rats is attributed to lipid peroxidation of the cell membranes.

MDA is one of the final oxidation products of polyunsaturated fatty acids and it serves as a marker for lipid peroxidation [27].

The present findings indicate that cadmium toxicity has significantly impacted the body's natural antioxidant system, as well as causing changes in blood biochemical profile. This has resulted in a depletion of antioxidant molecules, a reduction in overall antioxidant capacity, and disrupting the natural balance between oxidants and antioxidants in the body. In situations where the body's natural antioxidant system is compromised, it becomes crucial to introduce external sources of antioxidants to restore this balance.

The supplementary antioxidants are expected to counteract oxidative damage by rapidly eliminating and neutralizing excess free radicals and strengthening the endogenous antioxidant system. It is noteworthy that the byproducts resulting from the reaction of antioxidant molecules with free radicals have the potential to eliminate more radicals, thereby enhancing the overall antioxidant capacity. As a result, the supplemented antioxidants work together with internal antioxidants to neutralize and remove free radicals.

The properties of resveratrol make it an effective exogenous antioxidant as it actively enhances the antioxidant system to counteract oxidative stress [28]. Previous studies have focused on the potential of resveratrol in combating oxidative stress and associated damage while maintaining redox status [29].

In general, the phenolic OH groups of polyphenols enable them to neutralize ROS and converting them to stable molecules. The antioxidant property of resveratrol is strongly linked to its phenolic OH groups that provide its paramount redox activity [30]. Resveratrol exert its antioxidant bioactivities through inhibition of ROS production and reducing biosynthesis of superoxides [10,31,32]. Moreover, resveratrol suppresses lipid peroxidation as evidenced by the decreased lipid hydroperoxides, upregulates the antioxidant enzymes, neutralizes ROS, and regulate redox systems [33,34].

Briefly, the current results are in line with the previous studies as regard to the ability resveratrol to enhance the antioxidant system and upregulate the expression of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (antioxidant markers), decrease the expression of oxidative factors involving pro-oxidative enzymes, and reduce the level of malondialdehyde (MDA) (lipid peroxidation marker).

The present findings are compliant with the aforementioned published studies as evidenced by the currently recorded improved levels of antioxidant markers (total thiols, glutathione, catalase, and TAC in rats exposed to Cd and had access to Resveratrol. These findings may confirm the positive effect of resveratrol on the expression of these antioxidant molecules and, consequently, its reinforcing significant effect on endogenous antioxidant capacity.

Resveratrol provides antioxidant protection through diverse mechanistic pathways. These pathways include donating electrons to neutralize and scavenge free radicals, interrupting free radical-induced lipid peroxidation, upregulation of antioxidant enzymes and inhibiting pro-oxidant activity are among the antioxidative mechanisms

of resveratrol.

Furthermore, the significant decrease in cadmium blood levels in presently cadmium-intoxicated rats and administered resveratrol may be interpreted by the supposed cadmium chelation. Polyphenols as a potential antioxidant act as metal chelators and in this way eliminate the toxic pro-oxidant metals that are incriminated in generation of free radicals and inducing lipid peroxidation [35]. Cadmium is the trigger of oxidative overload and decreasing its level in the blood greatly limits the initiation of oxidative stress and thus helps recover the endogenous antioxidant system.

The current haematological and biochemical assays in rats exposed to Cd and treated with Resveratrol exhibited improved parameters that were relatively reversed and became closer to the control levels. These findings may reinforce the proposed synergistic antioxidant role of resveratrol in alleviating cadmium-induced oxidative stress.

6. Conclusion

The current study offers evidence of the antioxidant properties of resveratrol in countering the pronounced oxidative stress and resulting damage caused by cadmium toxicity. However, further in-depth research is recommended to understand the precise molecular mechanisms involved in the antioxidant effects of resveratrol in cases of heavy metal toxicity.

Limitation of the Study

The current findings provide evidence of the efficient antioxidant activity of resveratrol; however, further investigation is recommended to reveal the detailed molecular mechanisms through which resveratrol exerts its antioxidant properties.

Authors' Contribution

M Z, H R, and AAD performed the laboratory work. MAE supervised the experiment. LMA, NHA, KNY, EAA, SA, SAA and FAN were responsible for the lab work, software, and statistical data analysis. MM designed the study, supervised the methodology, and wrote the manuscript.

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Institutional Review Board Statement

The guidelines for the care and use of laboratory rats, as per institutional and national regulations, set by the Research Ethics Committee of Imam Mohammad Ibn Saud Islamic University (IMSIU), were diligently adhered to (LAB-rats-2023-0239).

Data Availability Statement

Data will be made available on request

Conflicts of Interest

There are no conflicts of interest.

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