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Rosmarinic Acid Supplementation Acts as an Effective Antioxidant for Restoring the Antioxidation/Oxidation Balance in Wistar Rats with Cadmium-Induced Toxicity

Mohammed Al-Zharani[1], Eman Almuqri[1], Mohammed Mubarak[1,*], Hassan Rudayni[1], Nada Aljarba[2], Khadijah Yaseen[3], Shaikha A. Albatli[1], Saad Alkahtani[3], Fahd A. Nasr[1], Amin A. Al-Doaiss[4], Mohammed S. Al-eissa[1]

[1]Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh 11623, Saudi Arabia
[2]Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh 11671, Saudi Arabia
[3]Department of Zoology, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia
[4]Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia
*Corresponding author: mohammedahmed_62@yahoo.com

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Abstract Rosmarinic acid exists prominently in plant species in the Boraginaceae and Lamiaceae families. It is a natural bioactive polyphenolic compound with a wide range of bioactivities. The present study aimed to evaluate the in vivo antioxidant properties of rosmarinic acid and to investigate the effectiveness of exogenous rosmarinic acid in mitigating cadmium-induced oxidative stress. The experimental rats were allotted into four groups (n=20), designated as untreated control, rosmarinic acid-treated, cadmium-exposed, and cadmium-rosmarinic acid groups. The haematological and biochemical assays were performed to achieve the study's aim. Both the haematological and biochemical profiles of cadmium-exposed rats (Group 3) manifested significant alterations, including increments and decrements, compared to that of untreated control. Concerning the biochemical profile (serum profile), Group 2 animals (rosmarinic acid-treated) demonstrated no significant changes compared to the untreated control. Rats in Group 4 (cadmium-exposed and Rosmarinic acid-accessed) exhibited increased levels of total proteins, a significant increase in the levels of antioxidant markers including total thiols, glutathione, total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase, and a significant decrease in the levels of blood cadmium, ALP, ALT, AST, creatinine, blood urea nitrogen (BUN), urea, bilirubin, and oxidation markers (H2O2, and malondialdehyde MDA) compared to the animals exposed to cadmium (Group 3). Tissue homogenates of liver and kidney prepared from Group 3 animals demonstrated parallel results to that revealed by serum biochemical analysis. It was concluded that rosmarinic acid possesses effective antioxidant properties that significantly help attenuate the oxidative stress induced by cadmium.

Keywords: *Rosmarinic acid, cadmium toxicity, oxidative stress, antioxidant, biochemical profile*

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1. Introduction

Rosmarinic acid (C_{18} H_{16} O_8) is an ester of caffeic acid and 3 (3,4-dihydroxyphenyl) lactic acid and is named after rosemary (*Rosmarinus officinalis*) from which it was originally extracted as a secondary metabolic phytochemical. [1,2,3]. It is a natural bioactive polyphenolic compound that is built up of two phenolic rings with two hydroxyl groups in both of them and an unsaturated double bond joining the structural rings. Rosmarinic acid exists prominently in the plant species belonging to the families Boraginaceae and Lamiaceae including *Ocimum Toasilicum, Melissa officinalis, Rosmarinus officinalis, Coleus blumei, Perilla fritescens,* and *Saliva officinalis* [3,4].

Rosmarinic acid (RA) has been shown to exert remarkably diverse activities related mainly to its anti-inflammatory, anticancer, and antioxidant properties [5,6,7]. A considerable number of experimental studies have been focused on the properties and potential bioactivities of RA, as well as its possible protective effects against various disease conditions. The reported wide range of health benefits of RA are attributed to its anti-cancer, anti-depressive, antiallergic, anti-inflammatory, anti-angiogenic, cardioprotective, hepatoprotective, nephroprotective, neuroprotective,

antimicrobial, hypoglycemic, and hypolipidemic bioactivities [4] [8,9,10,11] [12,13] [14,15,16].

The endogenous antioxidant system encompasses a variety of antioxidant enzymes exemplified by catalase, glutathione peroxidase, and superoxide dismutase. Oxidative actions induced by certain metabolites or external toxins are among the main damaging insults that occur inside the living body. These undesired actions may be severe enough to eventually cause cell death and extensive tissue damage. The extraordinary production rate of reactive oxygen species creates oxidative stress and subsequent oxidative damage [17,18]. Oxidative stress is the status of imbalance between the rate of reactive oxygen species (ROS) production and the efficacy of the endogenous antioxidant system to overcome the detrimental effects of ROS. Oxidative stress is one of the major contributors to the development of significant disease conditions such as cardiovascular diseases and cancer [18]. Excess ROS can damage the cellular structural lipids, proteins, and DNA, and eventually lead to disease conditions of significant medical concern including neurodegenerative disorders, cardiovascular diseases, cancer, diabetes, accelerated ageing, and immune dysfunction [19].

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 [20,21]. 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 [4].

Antioxidants refer to the compounds that can counteract the oxidative damage caused by ROS, and in this way combat oxidative stress [6]. The accumulated clinical and experimental evidence demonstrates the role of natural antioxidants in promotion of the vital body functions including that of the heart, immune system, and brain as well as slowing signs of ageing. 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) [17].

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 [22,23,24].

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 [25]. 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 [26].

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 [27,28,29,30].

The study aimed to investigate the antioxidant properties of rosmarinic acid in male Wistar rats. The antioxidative properties of rosmarinic acid 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-2023-0213).

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 ± 1^{o}C, a 12-hour dark-light cycle, and a relative humidity of 35-70%.

Rosmarinic acid:

Rosmarinic acid (C_{18} H_{16} O_8) *R*) -*O*- (3,4-Dihydroxycinnamoyl)-3-(3,4- dihydroxyphenyl) lactic acid, 3,4-Dihydroxycinnamic acid (*R*)-1-carboxy-2-(3,4-di

hydroxyphenyl) ethyl ester, (MW 360.31) was purchased from Sigma-Aldrich (Darmstadt, Germany) (CAS No. 20283-92-5).

Cadmium:

Cadmium (Cd) was used as cadmium chloride (Cd Cl_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 equally allotted into four groups; of 20 rats each, designated as 1, 2, 3, and 4. Rats in Group 1 served as the untreated control that is they were not exposed to cadmium and did not receive rosmarinic acid. Rats in Group 2 were administered daily with rosmarinic acid via oral route at 200 mg/kg b.w. in 1 mL/kg b.w. Rats in Group 3 received the aqueous solution of Cd Cl_2 by oral gavage at a final concentration of 5 mg/kg b.w. / day in 1 mL/kg b.w. The control rats received an equal volume of saline via the same route. Rats in Group 4 received Cd orally and administered with rosmarinic acid at the same aforementioned doses. There was a 10-hour gap between the daily administration of Cd and rosmarinic acid.

The experimental period was 8 weeks, during which dry feed (commercial pellets, Nesom Distributing Envigo, USA) and drinking water were supplied *ad libitum.*

All experimental rats were observed for behavioural activity, feed consumption, water intake, and 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^{o}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^{o}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 $HCIO_4^-$ 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 is 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

± 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 rosmarinic acid, as well as rats that were exposed to cadmium and also given rosmarinic acid, exhibited normal behaviour, activity, and food intake compared to the untreated control rats. However, rats exposed to cadmium but not treated with rosmarinic acid 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 Rosmarinic acid (0.231 ± 0.018 ppm) than in cadmium-exposed rats.

Regarding the assessed haematological parameters, rats that received Rosmarinic acid (Group 2) showed no significant differences from the control levels. The haematological profile of rats exposed to cadmium and not receiving rosmarinic acid (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 rosmarinic acid (Group 4) were relatively improved and became closer to the control levels.

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

Concerning the biochemical profile, rats that received rosmarinic acid (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 rosmarinic acid. 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 rosmarinic acid (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 Rosmarinic acid. Levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in the serum and tissues of rats exposed to cadmium without access to rosmarinic acid 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 rosmarinic acid (Group 4).

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

Table 3 shows the levels of total thiols, glutathione, catalase, glutathione peroxidase, superoxide dismutase, TAC, H2O2, and MDA in the liver and kidney homogenates of rats that received rosmarinic acid, rats exposed to cadmium, and rats exposed to cadmium and administered with rosmarinic acid compared to the control rats.

Table 1. Haematological parameters of rats that received rosmarinic acid, exposed to cadmium, and that were exposed to cadmium and administered with rosmarinic acid, 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 rosmarinic acid-administered rats exhibited no significant differences compared to the control rats. In cadmium-exposed rats and those administered with rosmarinic acid, the estimated haematological parameters showed significant increments compared to cadmium-exposed rats and were closer to the control levels

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

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 2a. Serum levels of total proteins (g/dL), albumin (g/dL), and globulin (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 rosmarinic acid demonstrated no significant differences in their biochemical parameters compared to the untreated control rats. The estimated biochemical parameters in cadmium-exposed and rosmarinic acid-treated rats exhibited improvements towards the control levels and were significantly different from those estimated in the cadmium-exposed rats

Parameter	Untreated Control	Rosmarinic acid	Cadmium	Cadmium and Rosmarinic acid
Total proteins	7.51 ± 0.15	7.54 ± 0.21	5.26* ± 0.16	7.06** ± 0.13
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
Creatinine	0.53 ± 0.13	0.55 ± 0.13	0.94* ± 0.07	0.63** ± 0.27
Urea	40.62 ± 0.41	40.61 ± 0.38	74.19* ± 0.61	48.15** ± 0.64
BUN	15.34 ± 1.02	15.85 ± 1.05	29.33* ± 1.32	17.73 ± 1.46
Bilirubin	6.39 ± 0.41	6.47 ± 0.37	11.54* ± 0.24	7.16** ± 0.41

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 (ALT) (IU/L), aspartate transferase (AST) (IU/L), and alkaline phosphatase (ALP) (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 rosmarinic acid group compared to the control group. In the group of rats exposed to cadmium and administered with rosmarinic acid, the biochemical parameters displayed improvements towards the control levels and were significantly decreased compared to the cadmium-exposed rats

Parameter	Untreated Control	Rosmarinic acid	Cadmium	Cadmium and Rosmarinic acid
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
ALP	25.17 ± 1.21	25.16 ± 1.03	75.75* ± 1.38	28.16** ± 1.59

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 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), hydrogen peroxide (H_2O_2) (mmol/L), and total antioxidant capacity (TAC) (nmol/mL) 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 (H_2O_2) were significantly increased in cadmium-exposed rats compared to the untreated control rats. Rosmarinic acid-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 rosmarinic acid, the biochemical parameters showed improvements toward the control levels and were significantly different compared to those of the cadmium-exposed rats

Parameter	Untreated Control	Rosmarinic acid	Cadmium	Cadmium and Rosmarinic acid
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
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
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_2O_2	40.73 ± 1.51	40.65 ± 1.43	90.17* ± 1.14	49. 19** ± 1.03

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 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), hydrogen peroxide (H_2O_2) (mmol/L), and total antioxidant capacity (TAC) (nmol/mL) 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 H_2O_2 levels were significantly increased in the tissue homogenates of cadmium-exposed rats compared to untreated control rats. Rosmarinic acid-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 rosmarinic acid demonstrated improvements toward the control levels and were significantly different compared to those measured in the cadmium-exposed rats

Parameter	Untreated Control		Rosmarinic acid		Cadmium		Cadmium and Rosmarinic acid	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
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
MDA	126.18± 3.18	122.11± 3.75	123.0 ±13.13	122.11±3.15	412.61*±3.39	493.31*±3.64	147.28**±3.11	145.08**±3.61
H_2O_2	14.92 ± 1.49	14.84 ± 1.13	16.49± 1.17	14.41 ± 1.09	88.91* ± 1.17	98.11* ± 1.62	18.44**± 1.01	19.22**± 1.08
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

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 rosmarinic acid supplement along with cadmium, a highly toxic heavy metal that induces oxidative stress [31]. The study aimed to test how well rosmarinic acid 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). This leads to reduced effectiveness of the body's natural antioxidant system in handling the harmful effects of reactive oxygen species (ROS) [32]. Pathological conditions or environmental factors that disrupt this balance are considered triggers for oxidative stress [33].

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. Oxidative stress is characterized by the overproduction of reactive oxygen species (ROS). ROS includes free radicals like hydroxyl and superoxide radicals, as well as non-radicals, primarily hydrogen peroxide [34]. A balanced level of free radicals is important for normal metabolic processes such as cell respiration. The body's internal antioxidant system regulates excess free radicals to prevent, impede, or inhibit their harmful effects [35].

The antioxidant system consists of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic molecules [36]. 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 [37]. Antioxidants block the reaction chain of free radicals and donate electrons to these radical metabolites to stabilize them [7].

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 [38]. Lipid peroxidation, which mainly affects cell membranes, is an important result of this oxidative damage [39].

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 [26]. The toxic effects of cadmium result in the oxidation of cell membrane lipids [40] and a significant reduction in the production of ATP and glutathione levels in the mitochondria [41]. 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 [42].

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 [43]. 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 [44]. 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) [45].

The current significant increase in the levels of malondialdehyde (MDA) in cadmium-intoxicated rats is attributed to lipid peroxidation of the cell membranes. MDA serves as a marker for lipid peroxidation, which is a severe consequence of oxidative damage [46].

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 [28]. 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 [47]. As a result, the supplemented antioxidants work together with internal antioxidants to neutralize and remove free radicals [40].

The properties of rosmarinic acid (RA) make it an effective exogenous antioxidant as it actively enhances the antioxidant system to counteract oxidative stress [6]. Previous studies have focused on the potential of rosmarinic acid in combating oxidative stress and associated damage while maintaining redox status [4,5] [48,49,50].

The antioxidant property of RA is strongly linked to its ability to scavenge free radicals and H_2O_2 and inhibit lipid peroxidation (51,52,53,54]. The phenolic structure of RA contributes to its antioxidant property since it encompasses 4 hydrogens and two catecholic groups [4].

Rosmarinic acid enhances the activity and expression of the key antioxidant enzymes including catalase, glutathione peroxidase, and superoxide dismutase, and maintains glutathione reserves and the total antioxidant capacity [5,8].

and decreased ROS generation in mitochondria [51,52,53,54,55,56]. The antioxidant property of rosmarinic acid is partially through suppression of lipid peroxidation as evidenced by the decreased lipid hydroperoxides [5].

Briefly, the current results are in line with the previous studies that concluded that rosmarinic acid enhances the antioxidant system and possesses the capability to significantly upregulate the expression of antioxidant enzymes such as glutathione peroxidase (antioxidant markers), decrease the expression of oxidative parameters involving pro-oxidative enzymes, and reduce the level of malondialdehyde (MDA) (lipid peroxidation marker) [58,59,60,61]. Moreover, RA restores mitochondrial functions including the electron transport chain and mitochondrial membrane potential [15,57].

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 Rosmarinic acid. These findings may confirm the positive effect of rosmarinic acid on the expression of these antioxidant molecules and, consequently, its reinforcing significant effect on endogenous antioxidant capacity.

Rosmarinic acid 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 rosmarinic acid.

Furthermore, the significant decrease in cadmium blood levels in presently cadmium-intoxicated rats and administered rosmarinic acid may be interpreted by the supposed cadmium chelation. Chelation of pro-oxidant metals and protection against metal-induced lipid peroxidation is one of the suggested antioxidative activities of rosmarinic acid [2]. 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 Rosmarinic acid exhibited improved parameters that were relatively reversed and became closer to the control levels. These findings may reinforce the proposed synergistic antioxidant role of rosmarinic acid in alleviating cadmium-induced oxidative stress.

6. Conclusion

The current study offers evidence of the antioxidant properties of rosmarinic acid 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 rosmarinic acid in cases of heavy metal toxicity.

Limitation of the Study

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

Authors' Contribution

M Z, H R, and AAD performed the laboratory work. MAE supervised the experiment. NHA, KNY, EAA,

SA,SAA and FAN were responsible for the lab work, software, and statistical data analysis. M M 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-2023-0213).

Data availability Statement

Data will be made available on request

Conflicts of Interest

There are no conflicts of interest.

References

[1] Tupas GD, Catherine B, Otero MC, Israel IE, Egbuna C, Aslam M. Antidiabetic lead compounds and targets for drug development. In: Editor(s): Phytochemicals as Lead Compounds for New Drug Discovery. Eds, Chukwuebuka Egbuna C, Shashank Kumar S, Jonathan C. Ifemeje J, Shahira M. Ezzat SM, Saravanan Kaliyaperumal S. Elsevier; 2020, PP.: 127-141.

[2] Noor S., Mohammad T, Rub M.A. Biomedical features and therapeutic potential of rosmarinic acid. Arch Pharm Res; 2022; **45**, 205–228.

[3] Singh D, Kumari K, Ahmed S. Natural herbal products for cancer therapy. In: Understanding Cancer. Eds. Jain B, Pandey S. 2022; Academic Press.

[4] Nadeem M, Imran M, Aslam Gondal T, Imran A, Shahbaz M, Muhammad Amir R, Wasim Sajid M, Batool Qaisrani T, Atif M, Hussain G, et al. Therapeutic Potential of Rosmarinic Acid: A Comprehensive Review. App Sci. 2019; 9(15): 3139.

[5] Taiwo O. Elufioye TO, Solomon Habtemariam S. Hepatoprotective effects of rosmarinic acid: Insight into its mechanisms of action. Biomed Pharmacoth; 2019: 112.

[6] Basu, S, Bank BK. Natural species in medicinal chemistry: properties and benefits. In: Green Approaches in Medicinal Chemistry for Sustainable Drug Design. 2020; Pp.739-758. Elsevier Inc.

[7] Tijjani H, Olatunde A, Zangoma MH, Egbuna C, Danyaro AM, Abdulkarim H, Mahmoud FA, Muhammad M. Nanoformulation of antioxidant supplements. In: Drug Discovery Update,Applications of Nanotechnology in Drug Discovery and Delivery. Eds, Egbuna C, Găman MA, Jeevanandam J; 2022, Pp. 45-70. Elsevier.

[8] Bachar SC, Ritesh Bachar R, Khoshnur Jannat K, Jahan R, Rahmatullah M. Hepatoprotective natural products. Eds; Satyajit D. Sarker SD, Lutfun Nahar L. Annual Rep Med Chem, 2020; Pp. 131-155.

[9] Değer U, Çavuş Y (2020) Investigation of the role of rosmarinic acid treatment in regulating inflammation, cell damage, and angiogenesis in rat ovarian torsion and detorsion models. Acta Cir Bras; 2020, 35:e202000304.

[10] Kim HK, Hwang S, Sung B, Kim YH, Chang Y (2020) Gd-Complex of a rosmarinic acid conjugate as an anti-inflammatory theranostic agent via reactive oxygen species scavenging. Antioxidants 9: 744.

[11] Jeong JH, Hwang JH (2021) Herbal medicine for the prevention of sarcopenia: a protocol for systematic review. Medicine 100: e25474.

[12] Jin BR, Chung KS, Hwang S, Hwang SN, Rhee KJ, Lee M, An HJ (2021) Rosmarinic acid represses colitis-associated colon cancer: a pivotal involvement of the TLR4-mediated NF-κB-STAT3 axis. Neoplasia 23: 561–573.

[13] Kim TH, Bormate KJ, Custodio RJP, Cheong JH, Lee BK, Kim HJ, Jung YS (2022) Involvement of the adenosine A1 receptor in the hypnotic effect of rosmarinic acid. Biomed Pharmacother 146: 112483.

[14] Lee BK, Jee HJ, Jung YS (2021) Aβ1-40-induced platelet adhesion is ameliorated by rosmarinic acid through inhibition of NADPH oxidase/PKC-δ/integrin αIIbβ3 signaling. Antioxidants 23(10):1671.

[15] Zhang W, Cheng C, Sha Z, Chen C, Yu C, Lv N, Ji P, Wu X, Ma T, Cheng H, Shi L (2021b) Rosmarinic acid prevents refractory bacterial pneumonia through regulating Keap1/Nrf2-mediated autophagic pathway and mitochondrial oxidative stress. Free Radic Biol Med 168: 247–257.

[16] Koprivica I, Jonić N, Diamantis D, Gajić D, Saksida T, Pejnović N, Tzakos AG, Stojanović I (2022) Phenethyl ester of rosmarinic acid attenuates autoimmune responses during type 1 diabetes development in mice. Life Sci 288: 120184.

[17] Shamsi A, Ahmed A, Khan MS, Husain FM, Bano B (2020) Rosmarinic acid restrains protein glycation and aggregation in human serum albumin: multi spectroscopic and microscopic insight—possible therapeutics targeting diseases. Int J Biol Macromol 161: 187–193.

[18] Ilhan N, Bektas I, Susam S, Ozercan IH (2022) Protective effects of rosmarinic acid against azoxymethane-induced colorectal cancer in rats. J Biochem Mol Toxicol 36: e22961.

[19] Andresa Marques de Mattos, José Abrão Cardeal da Costa, Alceu Afonso Jordão Júnior, Paula Garcia Chiarello. Omega-3 Fatty Acid Supplementation is Associated With Oxidative Stress and Dyslipidemia, but Does not Contribute to Better Lipid and Oxidative Status on Hemodialysis Patients,Journal of Renal Nutrition,Volume 27, Issue 5,2017, Pp. 333-339.

[20] Graham Mazereeuw, Nathan Herrmann, Ana C. Andreazza, Gustavo Scola, David W.L. Ma, Paul I. Oh, Krista L. Lanctôt, Oxidative stress predicts depressive symptom changes with omega-3 fatty acid treatment in coronary artery disease patients.Brain, Behavior, and Immunity, 60, 2017, Pages 136-141,

[21] Augus,S.T.; Eka, M.; Oh, L.K.; Keizo, H. Isolation and characterization of antioxidant protein functions from melinjo (*Gnetum gnemon*) seeds. *J. Agric. Food Chem.*, 2011, 59, 5648-5656.

[22] Yigit, A.A.; Panda, A.K.; Cherian, G. The avian embryo and its antioxidant defence system. *World Poultry Sci.*, 2014, 70, 563-574.

[23] Heshmati J, Morvaridzadeh M, Maroufizadeh S, Akbari A, Yavari M, Amirinejad A, Maleki-Hajiagha A, Sepidarkish M. Omega-3 fatty acids supplementation and oxidative stress parameters: A systematic review and meta-analysis of clinical trials. Pharmacol Res. 2019 Nov; 149: 104462.

[24] Areej Mohamed Ateya, Nagwa Ali Sabri, Ihab El Hakim, Sara M. Shaheen. Effect of Omega-3 Fatty Acids on Serum Lipid Profile and Oxidative Stress in Pediatric Patients on Regular Hemodialysis: A Randomized Placebo-Controlled Study, Journal of Renal Nutrition,Volume 27, Issue 3 Pages 169-174.

[25] S. Saboori, F. Koohdani, E. Nematipour, E. Yousefi Rad, A.A. Saboor-Yaraghi, M.H. Javanbakht, M.R. Eshraghian, A. Ramezani, M. Djalali. Beneficial effects of omega-3 and vitamin E coadministration on gene expression of SIRT1 and PGC1α and serum antioxidant enzymes in patients with coronary artery disease.Nutrition, Metabolism and Cardiovascular Diseases,Volume 26, Issue 6,2016,Pages 489-494.

[26] Li, S.; Tan, H.; Wang, N.; Zhang, Z.; Lao, L.; Wong, C. The role of oxidative stress and antioxidants in liver diseases. Int. J. Med. Sci., 2015, 16: 26087-26124.

[27] Sharma, H.; Rawal, N.; Mathew, B.B. The characteristics, toxicity and effects of cadmium. Int. J. Nanotech. *Nanosci.*, 2015, 3, 1-9.

[28] Feng, P.; Ding, H.; Lin, H., Chen, W. AOD: the antioxidanrt protein database, *Sci. Rep.*, 2017, 7, 7449.

[29] D. Yong, S.; Lulu, W.; Ying, W.; Xiaqian, O.; Zhaoyuan, S. Purification and identification of a natural antioxidant protein from fertilized eggs. *Korea* J. Food sci. Anim. Resource., 2017, 37, 764-772.

[30] Case, A.j. On the origin of superoxide dismutase: an evolutionary perspective of superoxide-mediated redox signaling. *Antioxidants*, 2017, 6, 82.

[31] Sley EG, Rosen EM, van 't Erve TJ, Sathyanarayana S, Barrett ES, et al. Omega-3 fatty acid supplement use and oxidative stress levels in pregnancy. PLOS ONE.

[32] Effect of omega-3 fatty acid plus vitamin E Co-Supplementation on oxidative stress parameters: A systematic review and meta-analysis. Sepidarkish M, Akbari-Fakhrabadi M, Daneshzad E, Yavari M, Rezaeinejad M, Morvaridzadeh M, Heshmati J. Clin Nutr. 2020 Apr; 39(4): 1019-1025.

[33] Effect of omega-3 fatty acids supplementation on cardio-metabolic and oxidative stress parameters in patients with chronic kidney disease: a systematic review and meta-analysis. Fazelian S, Moradi F, Agah S, Hoseini A, Heydari H, Morvaridzadeh M, Omidi A, Pizarro AB, Ghafouri A, Heshmati J. BMC Nephrol. 2021 May 1; 22(1): 160

[34] Staudacher, V.; Trujillo, M.; Diederichs, T.; Dick, T.P.; radi, R. Redox-sensitive GFP fusions for monitoring the catalytic mechanism and inactivation of peroxiredoxins in living cells. *Redox Biol.*, 2018, 14, 549-556.

[35] Pisoschi, A.M. and Negulescu, G.P. Methods for total antioxidant activity determination: A review. *Bioch. Analyt. Bioch*, 2011, 1.

[36] Temleton, D.M. and Liu, Y. Multiple roles of cadmium in cell death and survival. Chemo-Biol. *Interactions*, 2010, 188, 267-275.

[37] Klaudia, J. and Marian, V. Advances in metal-induced oxidative stress and human disease. *Toxicology*, 2011, 253, 65-87.

[38] Urso, M.L.; and Clarkson, P.M. Oxidative stress, exercise, and antioxidant supplementation. *Toxicol.*, 2003, 189, 41-54.

[39] Klaus, A. and Heribert, H. reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 2004, 55, 373-399.

[40] Mruk, D.D., Silvestrinin, B.; Mo, M.Y.; Cheng, C.Y. Antioxidant superoxide dismutase- a review: its function, regulation in the testis, and role in male fertility. *Contraception*, 2002, 65, 305-311.

[41] Alfonso-Prieto, M.; Biarnes, X.; Vidosschi, P.; Roviva, C. The molecular mechanism of the catalase reaction. *J.Am.Chem.Soc.*, 2009, 131, 11751-11761.

[42] Pham-Huy, Z.A.; He, H. and Pham-Huy, C. Free radicals and antioxidants in disease and health. *Int. J. Biomed. Sci.*, 2008, 4, 89-96.

[43] Dorta, D.J.; Leite, S.; McMarco, K.C.; Prado, M.R.; Rodrigues, T. et al. A proposed sequence of events for cadmium induced mitochondrial impairment. *J. Inorganic Biochem.*, 2003, 97, 251-257.

[44] Dhu, P.; Gorg, M.L.; Dhawn, D.K. Protective role of zinc in nickel induced hepatotoxicity in rats. *Chemici-Biol. Interaction*, 2004, 150, 199-209.

[45] Feng, P.; Chen, W.; Lin, H. Identifying antioxidant proteins by using optimal dipeptide compositions. *Interdiscipl. Sci. Comput. Life Sci.*, 2016, 8,186-191.

[46] Nichole, C.; Ying, Z.; Marian, N.; Fereidoon, S. Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food Chem.*, 2008, 109, 144-148.

[47] Huang, W.; Deng, Q.; Xie, B., Shi, J.; Huang, F. Purification and characterization of an antioxidant protein from *Grigo biloba* seeds. *Food Res. Int.*, 2009, 43, 86-94.

[48] Guan M, Guo L, Ma H, Wu H, Fan X (2021) Network pharmacology and molecular docking suggest the mechanism for biological activity of rosmarinic acid. Evid Based Complement Altern Med 2021:5190808.

[49] Marchev AS, Vasileva LV, Amirova KM, Savova MS, Koycheva IK, Balcheva-Sivenova ZP, Vasileva SM, Georgiev MI (2021) Rosmarinic acid-from bench to valuable applications in food industry. Trends Food Sci Technol 117:182–193.

[50] Pattananandecha T, Apichai S, Julsrigival J, Ungsurungsie M, Samuhasaneetoo S, Chulasiri P, Kwankhao P, Pitiporn S, Ogata F, Kawasaki N, Saenjum C (2021) Antioxidant activity and anti-photoaging effects on UVA-irradiated human fibroblasts of rosmarinic acid enriched extract prepared from *Thunbergia laurifolia* Leaves. Plants 10: 1648.

[51] Ghorbani A, Sadeghnia HR, Afshari AR, Hosseini A (2019) Rosmarinic acid protects adipose tissue-derived mesenchymal stem cells in nutrient-deficient conditions. Prev Nutr Food Sci 24: 449–455.

[52] Zych M, Wojnar W, Borymski S, Szałabska K, Bramora P, Kaczmarczyk-Sedlak I (2019a) Effect of rosmarinic acid and sinapic acid on oxidative stress parameters in the cardiac tissue and serum of type 2 diabetic female rats. Antioxidants 8: 579.

[53] Sadeghi A, Bastin AR, Ghahremani H, Doustimotlagh AH (2020) The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Mol Biol Rep 47: 3557–3566.

[54] Wang Y, Meng J, Men L, An B, Jin X, He W, Lu S, Li N (2020) Rosmarinic acid protects mice from concanavalin A-induced hepatic injury through AMPK signaling. Biol Pharm Bull 43: 1749–1759.

[55] Khalaf AA, Hassanen EI, Ibrahim MA, Tohamy AF, Aboseada MA, Hassan HM, Zaki AR (2020) Rosmarinic acid attenuates chromium-induced hepatic and renal oxidative damage and DNA damage in rats. J Biochem Mol Toxicol 34:e22579.

[56] Tsai C-F, Wu J-Y, Hsu Y-W (2019) Protective effects of rosmarinic acid against selenite-induced cataract and oxidative damage in rats. Int J Med Sci 16: 729.

[57] Ma Z, Yang J, Yang Y, Wang X, Chen G, Shi A, Lu Y, Jia S, Kang X, Lu L (2020b) Rosmarinic acid exerts an anticancer effect on osteosarcoma cells by inhibiting DJ-1 via regulation of the PTEN-PI3K-Akt signaling pathway. Phytomedicine 68: 153186.

[58] Hassanzadeh-Taheri M, Ahmadi-Zohan A, Mohammadifard M, Hosseini M (2021) Rosmarinic acid attenuates lipopolysaccharide-induced neuroinflammation and cognitive impairment in rats. J Chem Neuroanat 117: 102008.

[59] Komeili-Movahhed T, Bassirian M, Changizi Z, Moslehi A (2021) SIRT1/NFκB pathway mediates anti-inflammatory and anti-apoptotic effects of rosmarinic acid on in a mouse model of nonalcoholic steatohepatitis (NASH). J Recept Signal Transduct Res 31: 1–10.

[60] Luo C, Zou L, Sun H, Peng J, Gao C, Bao L, Ji R, Jin Y, Sun S (2020a) A review of the anti-inflammatory effects of rosmarinic acid on inflammatory diseases. Front Pharmacol 11: 153.

[61] Touiss I, Ouahhoud S, Harnafi M, Khatib S, Bekkouch O, Amrani S, Harnafi H (2021) Toxicological evaluation and hepatoprotective efficacy of rosmarinic acid-rich extract from *Ocimum basilicum* L. Evid Based Complement Altern Med 2021: 6676998.