

Article

Protective Effect of Resveratrol against Hepatotoxicity of Cadmium in Male Rats: Antioxidant and Histopathological Approaches

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Abstract: Cadmium (Cd) is widely used in some industries and emitted from fossil fuels. It is a heavy metal with a number of side effects, including hepatotoxicity. Resveratrol (Rs) is considered an important polyphenol, which is a secondary plant metabolite and has the ability to scavenge free radicals. The study was designed to evaluate the effects of resveratrol on Cd, which induced hepatotoxicity, by the assessment of some histopathological and biochemical alterations. Forty male albino rats were divided into four groups: the 1st group was the control group, the 2nd group was treated with Cd (5 mg/kg), the 3rd group was given Rs (20 mg/kg), and the 4th group was treated with Cd in combination with Rs intraperitoneally for 30 successive days. The results indicate that Cd increased liver enzymes alanine aminotransferase and aspartate aminotransferase (AST and ALT), alkaline phosphatase ALP and gamma-glutamyl transferase (γ -GT) while reducing the total protein level; Cd increased the malondialdehyde (MDA) level while decreasing the levels of other antioxidant enzymes super oxide dismutase, catalase and glutathione peroxidase (SOD, CAT and GPx). Serious congestion and hemorrhage related to the hepatic tissues were noticed in the Cd group, and Rs plays a major role in alleviating histopathological injuries and hepatic oxidative damage. It is clear that Rs has the ability to minimize the hepatotoxicity induced by Cd in male rats.

Keywords: cadmium; resveratrol; liver; oxidative stress; pollution; histopathology

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1. Introduction

The heavy metals toxicity is a universal problem for all livings. Toxicity of the heavy metals depends mainly on many factors, mainly the exposure route and nutrition. These industrial impacts have resulted in increasing environmental pollution and the production of free radicals and reactive oxygen species, such as cadmium (Cd) [1].

Cd is a highly common environmental pollutant and it is emitted into the atmosphere and aquatic environment, this heavy metal toxicity has increased dramatically during the 20th century [2]. The wide environmental distribution of Cd leads to elevated interest in its high toxicity and its harmful environmental effects [3]. Cd accumulates excessively in the hepatic and renal tissues and can cause severe cellular damages in different organs [4].

Cadmium is a toxic heavy metal which is commonly used in the industrial paints, electroplating, manufacturing of a wide range of batteries and the agricultural industry. In addition to its high used industrial applications, Cd is also found in soil, water, vegetables, aquatic foods and industrial workplaces [5]. Cigarette smoke is of the most public origin of Cd. Therefore, there is a high risk of possible exposure to Cd. Release of Cd into the environment resulting from its use poses a potential danger to the gen-

eral population and represents major health hazards. Exposure to Cd has harmful effects on the cells and tissues in a variety of organs, including the hepatic tissues [5].

When high amounts of Cd accumulate in the hepatic and renal tissues and exceed the binding limit, the compound-bound Cd ions are believed to cause high toxicity in the liver and kidney tissues of the body [5].

Cd, which is considered as one of the toxic heavy metals, poses several dangers to both animals and human health. Nearly 13,000 tons of Cd is produced each year, mainly from batteries, a lot of alloys, coatings and chemical stabilizers [6].

Cd-induced high oxidative damage leads to DNA genotoxicity and high lipid peroxidation. Several studies have demonstrated a positive relationship between induction of high lipid peroxidation and exposure to Cd [7].

Scientific studies have proven that excessive use of antioxidants is of high importance to our bodies. These substances interact, scavenge and stabilize the free radicals and, thus, prevent all cellular damage caused by oxygen species and free radicals [7].

CdCl₂ administration to animals induced hepatic lipid peroxidation, decreased Glutathione (GSH) content (to 65%) and inhibited catalase (CAT) (to 68%) and Glutathione peroxidase (GPx) (to 60%) in the hepatic tissues. Resveratrol prevented Cd-induced lipid peroxidation and Cd-induced inhibition of hepatic antioxidant enzymes. Rs were effective against Cd-induced hepatotoxicity and oxidative stress [8].

Resveratrol is obtained from the roots of the *Polygonum cuspidatum* plant used for the treatment of skin diseases, cardiovascular diseases and hepatic diseases. Rs is a phenolic phytochemical [9,10] found in grapes and cranberries. It is a potent antioxidant and it has been demonstrated in the brain [10] and the liver [11].

Resveratrol is a free radical scavenger that elevates the activity of several antioxidant enzymes, such as superoxide dismutase, catalase and glutathione [12].

Resveratrol can be obtained from natural foods such as fruits, juices and vegetables [13]. Recently, resveratrol, as well as bioactive compounds, have been recommended as dietary supplements due to their positive health effects [14].

Resveratrol is showing promising results due to its hepatoprotective therapeutic characteristics and its antioxidant activities in alleviating hepatotoxicity [15]. Sener et al. [16] demonstrated that Rs has a potent hepatoprotective effect against paracetamol in male mice due to its ability inhibit cytokines, decrease oxidative stress and high lipid peroxidation [16,17]. Resveratrol has resulted in vasodilation, so Rs mainly causes a lowering high blood pressure [18].

With regards to the effects of resveratrol in metal toxicity, only the influence of resveratrol on heavy metals induced oxidative DNA injury was studied in cell cultures. Resveratrol which is a naturally occurring polyphenol found in the skin of grapes, has been reported to exert protective effects in cancer, possibly due to antioxidant properties [10].

Additionally, resveratrol can enhance the production of cellular antioxidant enzymes in heart tissues and vascular cells, which leads to a decline in oxidative damage markers [19]. Resveratrol displays anti-aging activity, and this compound is useful in the treatment of highly neurodegenerative disorders.

Resveratrol is a polyphenolic compound, which mainly exists in grapes. It has antioxidant capacities and hepatoprotective effects [20].

Several previous studies have explored the protective effects of Rs against oxidative injury [21].

Resveratrol provides hepatoprotective effects against hepatotoxicity induced by many toxicants, such as carbon tetrachloride [22].

Most toxicants cause hepatic injury. Therefore, the current study aimed to evaluate the possible protection and antioxidant capacities of Rs against Cd hepatotoxicity; this aim was achieved through biochemical, histological level and antioxidant enzyme analyses of the liver.

2. Materials and Methods

2.1. Chemicals

CdCl₂ was used at a dose of 5 mg/kg, which is equal to the lethal dose (LD)₅₀ [23]. All the used chemicals were stored at temp. 2–8 °C. Cd was purchased from Sigma-Aldrich. Resveratrol was obtained from a Chinese company (Xi'an Natural Field Bio-Technique Co., Xi'an, China) for chemicals under code number 430075000.

2.2. Animals

Forty male albino Wistar rats, weighing about 150–160 g, were obtained from the king Fahad center at King Abdul-Aziz University, Jeddah, Saudi Arabia. Animals were adapted for two weeks before experimentation and provided with food and water; following the National Institutes of Health Guidelines for care and use. The experiment was carried out according to ethical committee approval in deanship of scientific research at the Taif University under approval number: 39-31-0034.

2.3. Experimental Design

The Cd and Rs doses were estimated according to Renugadevi and Prabu [23] and Oktem et al. [24]. About 40 male rats were split into 4 groups, 10 rats in each group as follows: Treatment with the tested compounds intraperitoneally (i.p) on one (CdCl₂ was firstly injected and after 10 min Rs was injected in another place of 1st injection) for 4 weeks was: the 1st group served as the control group and was administered normal physiological saline; the 2nd group was treated with CdCl₂ (5 mg/kg), with concentration (1×10^{-3} mol/L); and the 3rd group was treated with Rs at a dose of 20 mg/kg. Concentration of Rs was calculated as follows: (20×10^{-3}) g of Rs was dissolved in 100 mL saline, with concentration (1×10^{-3}). Finally, the 4th group was treated with CdCl₂ plus Rs. Treatment for all groups was intraperitoneally for 30 successive days as shown in the experimental protocol (Figure 1).

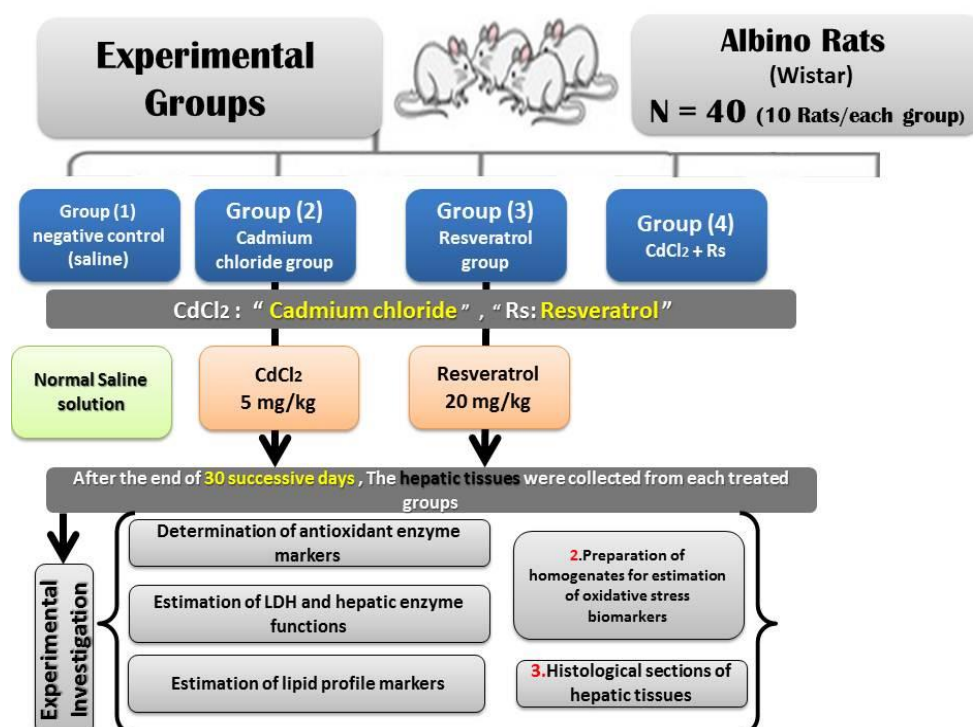


Figure 1. Graphical of experimental protocol.

2.4. Blood Samples Collection

Blood samples were harvested from the retro-orbital plexus according to Bousarie [25], and then the serum was used for biochemical analyses.

2.5. Biomarkers of Hepatic Functions

Lactic dehydrogenase (LDH) activity was determined by Human kits. Activities of ALT and AST were determined according to Reitman and Frankel [26]. ALP and γ -GT activities were estimated according to Young et al. [27] and Orlowski and Meister [28]. Albumin and total protein levels were evaluated according to Bowers and Wong [29] and Bradford [30], respectively.

Cholesterol and triglycerides levels were determined according to Carr et al. [31]. HDL-c and LDL-c were obtained by the method of Warnick et al. [32]. However, VLDL-c levels were determined according to Friedewald [33].

2.6. Tissue Preparation

Hepatic tissues (~0.35 g) were used for the analysis of oxidative stress parameters. Hepatic tissues were immersed and homogenized with a 50 mM ice-cold phosphate buffer (pH 7.4). The resulting supernatant was used for the assessment of antioxidant enzymes.

2.7. Determination of Antioxidant Markers

Lipid peroxidation was estimated according to Esterbauer and Cheeseman [20]. Superoxide dismutase (SOD) activity was measured according to Marklund and Marklund [34] by using Human kits (Germany). Catalase (CAT) activity was measured according to Aebi [27]. CAT activity was measured in tissues by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C, it was evaluated according to Aebi by using Human kits (Germany) [35]. Glutathione peroxidase (GPx) was determined according to Hafeman et al. [36] GPx activity was determined in the liver tissues. The peroxide substrate (ROOH) and total antioxidant capacity was determined according to Prieto et al. [37].

2.8. Histological Evaluation

Parts of the liver tissues were fixed in 10% neutral buffered formalin, and other processing was performed as described by Gabe [38]. Liver tissues were fixed in 10% formalin, and then it was removed by washing the samples with tap water overnight. The tissues were dehydrated using a series of alcohols and were embedded in paraffin. Sections were cut using a microtome at 5 μ m thickness. The thin sections were stained with hematoxylin and eosin. The slides were examined by light microscope and photographed by digital camera.

2.9. Statistical Analysis

The collected data was entered into and analyzed by computer using Statistical Package of Social Services (Chicago, IL, USA), version 25 (SPSS) (IBM, 2017) and Open Epi version 2.3.1 (Dean et al., 2013). A Shapiro–Wilk test was used to determine the distribution characteristics of variables and variance homogeneity. Data were summarized as mean and standard deviation. One way analysis of variance (ANOVA) and post hoc (LSD) were used to test differences between groups. Variances of p -value < 0.05 were taken as significant [39].

3. Results

In Table 1, ALT, AST, ALP, LDH and γ -GT were markedly elevated in the Cd group with a significant decline in total protein levels. Treatment with Rs afforded non-significant alterations in ALT, AST, ALP, LDH, γ -GT and total protein levels

compared to the control group. All liver parameters declined significantly in the hepatic tissues of the Cd/Rs group as compared to the Cd group in ALT, AST, LDH, ALP and γ -GT, respectively.

Table 1. Effect of either cadmium, resveratrol, or both, on some liver functions in male rats.

Groups	Total Protein (g/dL)	LDH (U/L)	AST (U/mL)	ALT (U/mL)	γ -GT (U/L)	ALP (U/L)
1—Control group	8.19 $\pm$ 0.78	115.25 $\pm$ 2.02	13.11 $\pm$ 1.01	12.36 $\pm$ 0.78	3.21 $\pm$ 0.25	10.12 $\pm$ 1.14
2—Cd group	3.48 $\pm$ 0.59	861.36 $\pm$ 1.25	152.36 $\pm$ 2.54	172.71 $\pm$ 2.31	7.65 $\pm$ 1.15	68.32 $\pm$ 2.55
3—Rs group	7.14 $\pm$ 1.15	110.25 $\pm$ 1.05	13.28 $\pm$ 1.72	12.83 $\pm$ 1.24	3.41 $\pm$ 0.37	9.98 $\pm$ 1.22
4—Cd + Rs group	5.18 $\pm$ 1.49	146.25 $\pm$ 1.75	43.12 $\pm$ 2.02	33.48 $\pm$ 2.11	3.87 $\pm$ 0.28	13.36 $\pm$ 1.27
Post hoc <i>p</i>-value						
Control vs. CdCl ₂	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Control vs. Rs	0.44	0.11	0.32	0.79	0.34	0.42
Control vs. CdCl ₂ + Rs	0.38	<0.001 *	<0.001 *	<0.001 *	0.30	<0.001 *
CdCl ₂ vs. Rs	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
CdCl ₂ vs. CdCl ₂ + Rs	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Rs vs. CdCl ₂ + Rs	0.10	<0.001 *	<0.001 *	<0.001 *	0.10	<0.001 *

* Statistically significant (*p*-value < 0.05). Cd: cadmium; Rs: resveratrol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; and γ -GT: gamma glutamyl transferase.

The antioxidant enzymes (SOD, GPx and CAT) declined significantly after Cd treatment (Table 2). The administration of Rs to the Cd-group restored these antioxidant enzymes when compared to the Cd-alone group. The Cd-treated group showed decreased SOD activity, which was elevated when the DC group was treated with Rs. CAT activity was decreased significantly in the hepatic tissues of the Cd group as compared to the control group (Table 2).

Table 2. Effect of either cadmium, resveratrol, or both, on some antioxidant/oxidative stress parameters in livers of male rats.

Groups	MDA (nmol/g Tissue)	GPx (mg/g Tissue)	CAT (U/g Tissue)	TCA %	SOD (U/g Tissue)
Control group	5.02 $\pm$ 1.01	16.15 $\pm$ 1.69	10.43 $\pm$ 1.58	96.8%	14.12 $\pm$ 1.23
CdCl ₂ group	115.28 $\pm$ 1.21ab	6.55 $\pm$ 1.19	1.51 $\pm$ 0.36	77%ab	3.26 $\pm$ 0.66ab
Rs group	5.01 $\pm$ 0.89	17.25 $\pm$ 1.29	10.41 $\pm$ 1.22	98%	14.15 $\pm$ 1.15
CdCl ₂ + Rs group	13.52 $\pm$ 2.12	14.53 $\pm$ 1.39	3.26 $\pm$ 0.78	89%	10.03 $\pm$ 1.31
Post hoc <i>p</i>-value					
Control vs. CdCl ₂	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Control vs. Rs	0.03 *	0.09	0.29	0.32	<0.001 *
Control vs. CdCl ₂ + Rs	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
CdCl ₂ vs. Rs	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
CdCl ₂ vs. CdCl ₂ + Rs	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Rs vs. CdCl ₂ + Rs	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *

* Statistically significant (*p*-value < 0.05). Cd: cadmium; Rs: resveratrol; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; MDA: malondialdehyde; and TAC: total antioxidant capacity.

The same finding was obtained for GPx activity, which declined in the Cd group and was elevated by the Rs treatment after Cd.

Regarding the oxidative damage in the hepatic tissues, Table 2 represents the MDA levels (the final marker of lipid peroxidation). MDA levels were elevated greatly in the DC group as compared to the control group.

Histopathological Finding

Histopathological findings showed normal hepatic structure in the control group, CdCl₂ induced hepatic damages, microvesicular steatosis hepatocytes with ballooning of hepatocytes and pericellular fibrosis. Meanwhile, the Rs group showed that hepatic tissues showing a central vein surrounded by normal cords of liver cells. While, groups treated with the CdCl₂ and Rs combination showed normal hepatocytes with the beginnings of mild hypertrophy; with the appearance of binucleated hepatocytes shown in Figure 2 and the histological score shown in Table 3.

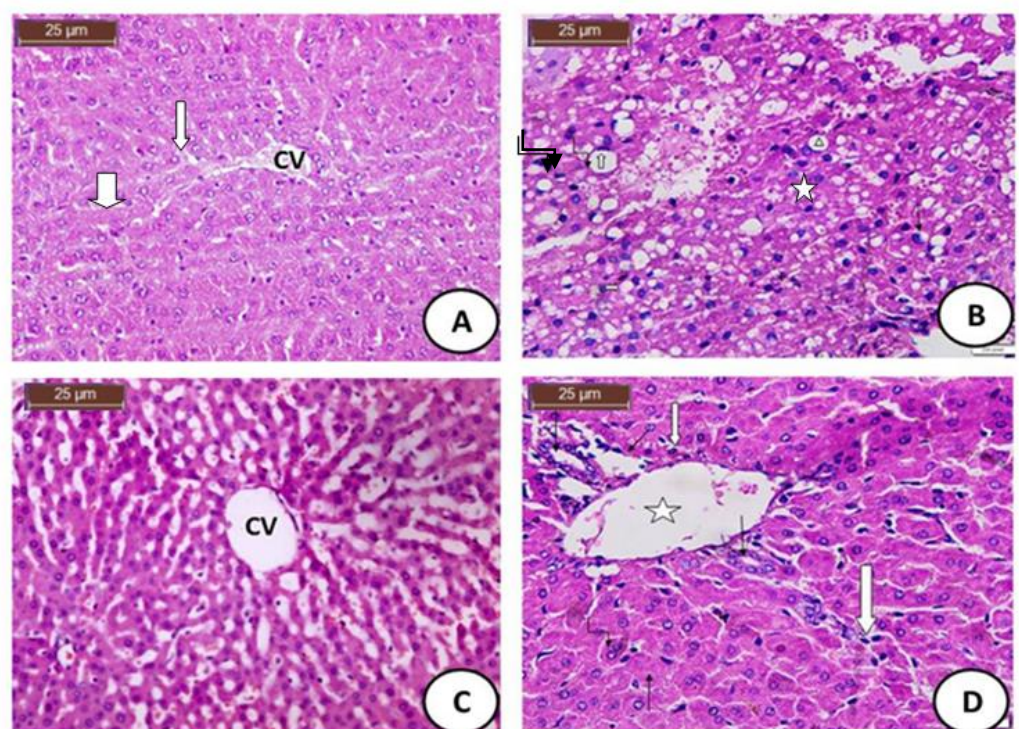


Figure 2. Effect of Cadmium and Resveratrol on hepatic tissues. (A) Control group: Photomicrograph of normal hepatic tissues showing central vein (CV) surrounded by rows and trabeculae of normal liver cells (H&E × 100); (B) Cadmium group: Photomicrograph of hepatic tissues treated with cadmium showing loss of normal hepatic architecture, macrovesicular (↑) microvesicular steatosis hepatocytes (⇐⇒) with ballooning of hepatocytes (↓) and cytoplasmic clearing (signet ring appearance (↓), hepatocellular damage, inflammatory infiltrate and pericellular fibrosis (△). (C) Resveratrol group: Photomicrograph of liver tissues treated with resveratrol showing central vein (CV) surrounded by normal cords of liver cells (H&E × 100). (D) Cadmium + Resveratrol group: Photomicrograph of hepatic tissues treated with Cadmium + Resveratrol showing normal hepatocytes with beginning of mild hypertrophy, appearance of binucleated hepatocytes (⇓), with vesicular nuclei and granular cytoplasm and formation of new bile ductules infiltrated by inflammatory cells (↘) at the periphery of mildly dilated portal vein (☆) With infiltration of blood sinusoids by inflammatory cells (⇓) (H&E × 100).

Table 3. Histopathological score findings in liver of rats treated with cadmium and resveratrol.

Findings	Groups			
	Control	Cadmium	Resveratrol	Cadmium + Resveratrol
Normal hepatic tissues	++++	-----	++++	+++–
Normal central vein	++++	+---	++++	+++–
Dilated congested central vein	----	++++	----	+++–
Degenerated liver cells	-----	++++	-----	+++–
Fatty change of liver cells	-----	++++	-----	+++–
Symbol	meaning			
-----	Absence of the change.			
++++	A change which was often found.			
+++–	A change which was observed in almost group.			
++–	A change so often not observed in all group.			
–+	A change that was rare in the group			

4. Discussion

Excessive exposure to Cd induced several health problems, mainly as cardiovascular dysfunction and hepatotoxicity [5]. The current study assessed the Cd effects on the liver function markers and the levels of the antioxidant enzymes, such as GPx, CAT and SOD. Histopathological variations were examined mainly in the Hepatic tissues.

Previous studies have mainly and excessively reported the potent antioxidant capacities of Rs. So, we aimed to assess the possible hepatoprotective effects of Rs against Cd hepatotoxicity. The results indicated a main decline in the total protein levels and mainly elevates the hepatic enzymes ALP, LDH, ALT and AST after the successive treatment with Cd.

Hypoproteinemia case was mainly showed in Cd group, which would be due to the dietary insufficiency and/or the high excessive excretion rate Chawla [40]. The observed decline in the total protein levels might be afforded by inflammation of the hepatic tissues as a result of treatment with Cd treatment and alters the biosynthesis of protein [40].

Gaskill et al. [41] reported severe hepatic toxicity reflected by significant elevations in the hepatic enzymes ALT, AST, ALP and GGT in the Cd-treated group as compared with those in the control group, which is in accordance with the results of the current study. The current results are also in accordance with those of Newairy et al. [42], who reported that Cd treatment elevated oxidative damage markers and that this oxidative stress could be the 1st sign of Cd for hepatotoxicity incidence.

Confirming the obtained results, Renugadevi and Prabu [23] reported Cd-induced oxidative stress and hepatotoxicity in liver tissues.

Hepatic injury caused by Cd treatment was clearly shown by the elevated levels of hepatic enzymes, concurrent with increased levels of the lipid peroxidation marker. Lakshmi et al. [43] also confirmed the obtained results by revealing that Cd exposure elevated the levels of the hepatic biomarkers and decreased the levels of total proteins.

The administration of Cd resulted in a significant increment in MDA levels. The current findings are in accordance with the results reported by Alghasham et al. [44], that Cd (40 mg) markedly elevated the stress marker (MDA) in male rats. Therefore, Cd is supposed to induce hepatotoxicity, which is associated with the incidence of oxidative stress.

The current results proved that levels of MDA were elevated markedly, Meanwhile the levels of antioxidant enzymes were declined markedly due to Cd treatment. The free radicals' triggering is the main mechanism of stimulation of the hepatic toxicity by Cd [45]. Oxidative stress is mainly characterized by the excessive triggering of the free radicals that result in induction of high lipid peroxidation levels and the high damaging of the cellular membranes [46].

Phospholipids of the cellular membrane are mainly oxidized and trigger MDA, which is considered as the main cause of inducing of the oxidative stress [47]. Glutathione is a key co-factor for triggering of the antioxidant enzymes, these antioxidant enzymes prevent the cellular injury from the production of the high levels of free radicals and helps mainly in the toxins removal [48].

There was a severe hepatic injury to the liver in the Cd-treated group. Hepatocytes were mainly observed as hepatic masses. The hepatic tissues were irregular. The hepatocytes have pyknotic nuclei with appearance of large foamy cytoplasm. The present results were in agreement with the previously obtained results, such as the loss in the hepatic architecture and changes in the lipid structures of the hepatic tissues.

Rs administration markedly reduced the cadmium-induced increase in the hepatocellular enzymes and severe hepatic injury, according to AST, ALT and ALP, which are significant markers of hepatic damage, and their serum elevation indicates a loss of cellular membrane integrity [46].

The hepatic enzymes AST, ALT and ALP declined after the administration of Rs, and this proved the improvement of the hepatocytes. AST is a marker of hepatic necrosis. ALT is a hepatic enzyme, which is elevated in the case of hepatic diseases associated with cellular death [49]. ALP is a hepatic enzyme that arises mainly from the bile duct [50].

ALP is a significant indicator of cholestasis [51–53]. Obstruction causes fats to be accumulated in the blood instead of being alleviated normally. Rs reduced the AST, ALT and ALP levels, suggesting that Rs can be a good agent for hepatoprotection [54,55].

Additionally, the declined total protein levels in the Cd group suggest a decrease in the protein synthesis level. On the other hand, protein levels were elevated in the Rs-treated group, suggesting hepatic cellular amelioration.

Oxidative stress damages and injuries result in the breakdown of the cellular membrane, which leads to the formation of end products of oxidation, such as MDA [56,57].

Free radicals are generated during Cd metabolism, have a potent effect on and interact with the triglycerides of the hepatic cellular membranes and can produce lipid peroxides.

The lipid peroxidation is a phenomenon that is common in toxic mechanisms. It occurs under normal physiological conditions [58,59].

The obtained results for the hepatic tissues of the Cd-treated group showed a significant elevation in MDA levels and a significant decline in glutathione levels. MDA is an oxidative stress marker. The significant decline in MDA levels and increment in the glutathione enzyme in Rs-treated animals may reflect a decrease in the lipid peroxidation and increment in the cellular antioxidant defense enzymes. This indicates that Rs reduces the generation of free radicals and thus reduces oxidative stress [52,60–62].

Rs has been proven to possess high antioxidant activity [46]. Animals treated with Rs only showed a normal physiological status, which proves the safety of using Rs. Rs induced a cellular restoration effect on the hepatic tissues [47,63].

The histological parameters were implemented for the evaluation of liver tissue damage. The necrosis in the Cd group indicated the death of the hepatic cells. The fatty change in the liver noticed in the Cd group reflects an imbalance of lipid metabolism due to the hepatic tissues.

5. Conclusions

Resveratrol has an enhancing effect on hepatotoxicity induced by cadmium. The obtained results indicate that the decline in antioxidant status contributes to cadmium toxicity, especially in the hepatic tissues. The significant increase observed in the reactive oxygen species in the hepatic tissues confirmed the occurrence of oxidative stress due to cadmium exposure; thus, the hepatic tissues are subjected to a high level of oxidative stress. Histological amelioration was observed after the administration of resveratrol to the cadmium-treated group. Resveratrol treatment removed the triggered free radicals induced by Cd.

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