

Biochemical Studies on Rosemary and Licorice against Lead -Induced Oxidative Stress in Rats

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Abstract

The present study was carried out to explore the effect of rosemary and licorice alone or in combination with lead acetate on the biochemical parameters of adult male albino rats. A120 rats were equally divided into 6 groups. Group1 was kept as a control group (negative control). Groups 2 and 3 received aqueous extract of rosemary (100 mg/kg BW/day) and licorice (150 mg/kg BW/day) alone, respectively. Group 4 was given lead acetate (0.2%) alone; Group 5 was administered lead acetate (0.2%) in combination with the aqueous extract of rosemary and Group 6 received lead acetate (0.2%) in combination with licorice. All treatments were given orally in drinking water for 6 weeks. Blood samples were collected at 2nd, 4th and 6th weeks post-treatment. Results showed that chronic lead toxicity caused a significant disturbance in liver and kidney functions, as well as serum antioxidants and immunoglobulins. However, rosemary or licorice extracts restored such changes. This study revealed that lead exposure induced severe oxidative stress and disturbed simultaneously the serum biochemical profile of albino male rats and rosemary or licorice administration improved these disturbances.

Keywords: Liver enzymes, Rosemary, Licorice, Creatinine, Catalase.

Introduction

Oxidative stress is a natural activity occurring due to an imbalance between the antioxidants and production of free radicals. It is one of the most important problems affecting the animals causing serious problems affecting health and production [1]. Insufficient amounts of antioxidants or antioxidant enzymes result in oxidative stress that causes massive reactive oxygen containing molecules, severe inflammation and cell damage that affected the cell [2]. Antioxidants play role in the treatment of lead poisoning [3]. Lead toxicity encourages severe oxidative stress, which causes tissue damage to the exposed individuals [4]. It occurs due to continuous exposure to lead compounds through air, water and food.

Medicinal plants with antioxidant effects such as rosemary and licorice are excellent choice to protect against oxidative stress with least side effects. The antioxidant effect of rosemary (*Rosmarinus officinalis L.*) is due to its content of flavonoids and phenolic compounds [5]. Licorice (*Glycyrrhiza glabra L.*) has many biological and pharmacological effects, as antioxidant, immunoregulatory activities and atherosclerosis inhibition that

attributed to its content of glycyrrhizin, triterpenoid saponin and various phenolics [6]. The protective role of rosemary and licorice extracts on lead acetate-induced oxidative stress is not entirely investigated. Thus, the current study was aimed to assess the antioxidant effect of rosemary and licorice on biochemical parameters of rats with oxidative stress or toxicity induced by lead.

Material and methods

Preparation of plants extracts

Rosemary aqueous extract: the air-dried leaves (obtained from Mansoura University garden in the winter) were coarsely powdered. The powder (8g) were dissolved in 100 mL of distilled water then boiled for 5 min and infused for 10 min. Subsequent to cooling and passing via the filter paper, a clear solution was acquired [7]. Licorice aqueous extract: the dipping method was used for extraction with 10 g of licorice powder (licorice roots were purchased from private market at Damietta) extracted with 500 mL ethanol/water (30:70, v/v). The powder of licorice was mixed, stirred and dipped for 50 minutes at 60 °C [8].

Experimental design

A total of 120 clinically healthy male albino rats weighing 200-250g were obtained from the Animal Laboratory House, Faculty of Veterinary Medicine, Zagazig University, Egypt. All animals were kept in clean metal cages under hygienic conditions and fed with balanced ration and provided with *ad-libitum* water. The ethical regulations for the protection of animals' welfare were in accordance with the guidelines of Faculty of Veterinary Medicine, Zagazig University, Egypt. The rats were randomly divided into six groups (each of 20). Group 1 kept as control group (negative control). Group 2 received rosemary aqueous extract (100 mg/kg BW/day) orally in drinking water for 6 weeks [9]. Group 3 received licorice aqueous extract (150 mg/kg BW/day) orally in drinking water for 6 weeks [10]. Groups 4 (positive control) received lead acetate 2% (99.6% purity, purchased from El-Gomhoria Pharmaceutical Chemical Co. Zagazig, Egypt) in drinking water for 6 weeks [11]. Group 5 received lead acetate 2% in combination with the aqueous extract of rosemary, while group 6 received lead acetate 2% in combination with the licorice with the same dose, route and duration as previously mentioned. The rats were cautiously monitored during the study for signs of toxicity, morbidity and mortality. At the end of 2nd, 4th and 6th weeks of the experiment, blood samples were collected from 5 rats of each group from the retro-orbital venous plexus into clean, dry and labeled centrifuge tubes without anticoagulant. The tubes were left to clot and serum was carefully separated after centrifugation at 3000 rpm for 10 minutes for biochemical analysis.

Biochemical analysis

The obtained serum was used to assess serum markers of hepatic and renal disorders. Serum aminotransferases (AST [12] and ALT [13] and ALP activities [14] as well as total protein (TP) and albumin levels [15] were colorimetrically determined using kits (BioMérieux, Marcy l'etoile, France). The

serum globulin level was estimated by subtracting the albumin from TP level [16]. Serum IgG, IgM and IgA were determined using a radial immunodiffusion (RID) assay [17]. Serum levels of urea [18] and creatinine [19] were evaluated using colorimetric kinetic methods. Oxidative stress was assessed through estimating the enzymatic antioxidant biomarkers; i.e. CAT [20] and SOD [21]. Lipid peroxidation was evaluated by measuring MDA content in tissues [22].

Statistical analysis

Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests [23].

Results

Clinical observations and mortality rate

Rats in groups one, two and three appeared healthy without any clinical signs of illness or mortalities during the experimental periods. On the other hand, rats in group 4 showed ruffled coat and hairs, pale mucous membrane, weakness and the mortality rate was 25%. Moreover, the clinical signs were reduced post treatment in groups 5 and 6 with mortality rate of 5% and 10%, respectively.

Serum biochemical parameters

Effect on liver enzymes

Rats in groups 2 and 3 showed a nonsignificant change in ALT, AST and ALP activities in all experimental periods when compared with the control group. On the other hand, groups 4, 5 and 6 showed a significant increase in serum ALT, AST, and ALP at the end of the 2nd and 4th week of the experiment. The highest values were detected in group 4. Non significant changes in serum enzymes of groups 5 and 6 were observed at the end of the 6th week when compared with the control group. Rats in groups 5 and 6 showed a significant decrease in ALT, AST and ALP in comparison to rats in group 4 in all the experimental periods.

Table 1: Serum liver enzymes of albino rats at 2, 4 and 6 weeks post-treatment (Means±SE)

Parameters	Times post-treatment								
	2 weeks			4 weeks			6 weeks		
	ALT (IU/L) ¹	AST (IU/L) ²	ALP (IU/L) ³	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
G₁	26.12±0.24 ^c	41.26±0.44 ^c	151.30±0.54 ^c	26.00±0.35 ^c	41.00±0.71 ^d	150.00±0.71 ^c	25.16±0.41 ^b	43.00±0.52 ^b	149.00±1.22 ^b
G₂	25.70±0.20 ^c	41.44±0.22 ^c	153.74±0.31 ^c	26.40±0.54 ^c	44.02±0.51 ^d	152.70±1.18 ^c	25.60±0.36 ^b	42.98±0.38 ^b	151.10±0.72 ^b
G₃	25.80±0.15 ^c	41.18±0.26 ^c	153.20±1.07 ^c	27.00±0.40 ^c	43.52±0.53 ^d	151.40±1.04 ^c	26.28±0.35 ^b	42.76±0.64 ^b	151.48±0.97 ^b
G₄	43.60±1.36 ^a	86.20±1.36 ^a	181.00±3.11 ^a	48.60±1.03 ^a	93.00±1.84 ^a	182.80±2.06 ^a	50.88±0.92 ^a	106.78±1.95 ^a	189.32±3.07 ^a
G₅	34.00±0.71 ^b	65.20±1.77 ^b	167.70±1.43 ^b	38.60±1.21 ^b	58.00±1.00 ^c	162.40±0.83 ^b	28.00±0.71 ^b	48.20±0.86 ^b	155.80±1.56 ^b
G₆	35.00±0.71 ^b	67.80±1.16 ^b	168.80±2.27 ^b	38.80±1.16 ^b	63.40±1.08 ^b	167.62±0.96 ^b	29.40±1.03 ^b	49.00±2.10 ^b	156.16±1.79 ^b

¹ALT: alanine aminotransferase; ²AST: aspartate aminotransferase and ³ALP: alkaline phosphatase.

G₁: control group (negative control); G₂: received rosemary aqueous extract (100 mg/kg BW/day) orally in drinking water for 6 weeks; G₃: received licorice aqueous extract (150 mg/kg BW/day) orally in drinking water for 6 weeks; G₄: received lead acetate (0.2%) in drinking water for 6 weeks (positive control); G₅: received lead acetate in combination with the aqueous extract of rosemary (0.2%) in drinking water for 6 weeks and G₆: received lead acetate in combination with the licorice in drinking water for 6 weeks.

Means within the same column carrying different superscript letters were significantly different at $p \leq 0.05$

Effect on protein profile

Rats in group 2 revealed a significant increase in serum TP and globulin, while albumin and A/G ratio didn't show a significant change in comparison to the control group. Group 3 showed a non significant change in proteinogram during different experimental periods. Rats in group 4 showed a significant reduction in TP, albumin and globulin, while A/G ratio showed a non significant change. A significant reduction in TP and albumin with non significant change in globulin and A/G ratio was noticed in groups 5 and 6 at the end of the 2nd and 4th weeks of the experiment. However, non significant change in proteinogram was recorded at the end of the 6th week in comparison to the control group. Rats in groups 5 and 6 revealed a significant increase in TP, albumin and globulin in comparison to group 4 during all the experimental periods.

Effect on some renal function tests

The serum creatinine and urea levels showed non significant change in groups 2 and 3, while a significant increase was recorded in groups 4, 5 and 6 when compared with the control group during the experimental periods. On the other hand, groups 5 and 6 showed a significant decrease in serum creatinine and urea levels when compared with group 4 in all experimental periods.

Effect on immunoglobulins levels

Seum IgG level showed a significant increase in rats of groups 2 and 3. It showed a

significant decrease in group 4 in all experimental periods and only at the 2nd and 4th week post-treatment in groups 5 and 6 when compared with the control group. Serum IgM and IgA levels were significantly increased in group 2 and nonsignificantly changed in group 3 in all experimental periods when compared with the control rats. However, they revealed a significant decrease in all experimental periods in group 4 and only at the end of 2nd and 4th week in groups 5 and 6. The decrease in serum IgG, IgM and IgA values was more in group 4 than the others. Rats in groups 5 and 6 showed a significant increase in all immunoglobulins when compared with the group 4 during all experimental periods

Effect on antioxidant activities and MDA

Rats in groups 2 and 3 revealed a significant increase in the serum CAT and SOD activities with a significant reduction in MDA level when compared with the control group during all the experimental periods. However, the serum antioxidants activities showed a significant decrease, while MDA revealed a significant increase in all the experimental periods in rats of group 4 and only at the 2nd and 4th week of the experiment in groups 5 and 6 when compared with the control group. In comparison with group 4, rats in groups 5 and 6 showed a significant increase in serum CAT and SOD activities with a significant decrease in MDA level during all the periods of the experiment.

Table 2: Serum proteins' profile of albino rats at 2, 4 and 6 weeks post-treatment (Means±SE)

Parameters	Times post-treatment											
	2 weeks				4 weeks				6 weeks			
	TP (g/dL) ¹	Alb (g/dL) ²	Glob (g/dL) ³	A/G Ratio ⁴	TP (g/dL)	Alb (g/dL)	Glob (g/dL)	A/G ratio	TP (g/dL)	Alb (g/dL)	Glob (g/dL)	A/G ratio
G₁	6.50±0.10 ^b	3.16±0.05 ^a	3.34±0.05 ^b	0.95±0.01 ^{ab}	6.60±0.11 ^b	3.14±0.04 ^a	3.50±0.07 ^b	0.90±0.01	6.56±0.13 ^b	3.12±0.07 ^{ab}	3.44±0.07 ^b	0.90±0.00
G₂	6.94±0.07 ^a	3.28±0.06 ^a	3.66±0.04 ^a	0.90±0.02 ^b	7.26±0.18 ^a	3.30±0.09 ^a	4.16±0.22 ^a	0.80±0.05	7.70±0.07 ^a	3.36±0.07 ^a	4.34±0.12 ^a	0.78±0.03
G₃	6.56±0.11 ^b	3.30±0.07 ^a	3.26±0.05 ^b	1.01±0.02 ^a	6.46±0.14 ^b	3.14±0.06 ^a	3.32±0.08 ^b	0.95±0.01	6.44±0.10 ^b	3.04±0.05 ^b	3.46±0.07 ^b	0.88±0.01
G₄	4.14±0.11 ^d	2.00±0.07 ^c	2.14±0.07 ^c	0.94±0.04 ^{ab}	4.12±0.16 ^d	1.96±0.09 ^d	2.16±0.09 ^c	0.91±0.04	3.82±0.11 ^c	1.72±0.10 ^c	2.10±0.19 ^c	0.87±0.14
G₅	5.20±0.11 ^c	2.06 ±0.05 ^{bc}	3.14±0.09 ^b	0.66±0.02 ^c	5.84±0.11 ^c	2.78±0.09 ^b	3.06±0.11 ^b	0.92±0.05	6.14±0.14 ^b	2.90±0.07 ^b	3.24±0.18 ^b	0.91±0.07
G₆	5.40±0.07 ^c	2.26±0.05 ^b	3.14±0.04 ^b	0.72±0.02 ^c	5.48±0.10 ^c	2.40±0.12 ^c	3.08±0.06 ^b	0.78±0.05	5.92±0.11 ^b	2.88±0.06 ^b	3.04±0.08 ^b	0.95±0.03

¹TP: Total protein; ²Alb: Albumin, ³Glob: Globulin and ⁴A/G ratio: Albumin globulin ratio. G₁: control group (negative control); G₂: received rosemary aqueous extract (100 mg/kg BW/day) orally in drinking water for 6 weeks; G₃: received licorice aqueous extract (150 mg/kg BW/day) orally in drinking water for 6 weeks; G₄: received lead acetate (0.2%) in drinking water for 6 weeks (positive control); G₅: received lead acetate in combination with the aqueous extract of rosemary (0.2%) in drinking water for 6 weeks and G₆: received lead acetate in combination with the licorice in drinking water for 6 weeks.

Means within the same column carrying different superscript letters were significantly different at $p \leq 0.05$

Table 3: Some renal function tests of albino rats at 2, 4 and 6 weeks post-treatment (Means± SE)

Parameters	Times post-treatment					
	2 weeks		4 weeks		6 weeks	
	Creatinine (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)
G₁	0.46±0.02 ^c	32.80±0.86 ^d	0.43±0.01 ^c	36.40±1.08 ^c	0.44±0.02 ^b	36.38±0.60 ^b
G₂	0.43±0.01 ^c	33.80±0.86 ^d	0.42±0.01 ^c	34.20±0.82 ^c	0.44±0.01 ^b	37.34±0.35 ^b
G₃	0.44±0.02 ^c	33.00±0.71 ^d	0.45±0.01 ^c	35.60±0.86 ^c	0.46±0.01 ^b	36.72±0.27 ^b
G₄	1.46±0.09 ^a	67.00±0.71 ^a	1.91±0.07 ^a	70.80±0.86 ^a	2.82±0.21 ^a	80.60±1.08 ^a
G₅	0.75±0.02 ^b	52.80±0.86 ^c	0.60±0.02 ^b	45.82±0.42 ^b	0.50±0.01 ^b	38.76±0.75 ^b
G₆	0.82±0.03 ^b	56.40±1.21 ^b	0.62±0.02 ^b	48.80±0.86 ^b	0.55±0.02 ^b	39.98±0.37 ^b

G₁: control group (negative control); G₂: received rosemary aqueous extract (100 mg/kg BW/day) orally in drinking water for 6 weeks; G₃: received licorice aqueous extract (150 mg/kg BW/day) orally in drinking water for 6 weeks; G₄: received lead acetate (0.2%) in drinking water for 6 weeks (positive control); G₅: received lead acetate in combination with the aqueous extract of rosemary (0.2%) in drinking water for 6 weeks and G₆: received lead acetate in combination with the licorice in drinking water for 6 weeks.

Means within the same column carrying different superscript letters were significantly different at $p \leq 0.05$.

Table 4: Immunoglobulins (mg/dL) of albino rats at 2, 4 and 6 weeks post-treatment (Means± SE)

Parameters	Times post-treatment								
	2 weeks			4 weeks			6 weeks		
	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM	IgA
G₁	744±4.30 ^b	168±2.81 ^b	52.40±1.63 ^b	744.0±2.98 ^b	170.40±1.89 ^b	54.00±1.95 ^b	746.00±3.05 ^b	168.60±1.60 ^b	52.20±1.66 ^b
G₂	804±9.27 ^a	191±2.92 ^a	61.40±1.21 ^a	828.0±14.63 ^a	204.20±3.97 ^a	65.80±1.88 ^a	819.8±15.64 ^a	209.00±6.40 ^a	62.00±1.00 ^a
G₃	795±5.70 ^a	172±2.50 ^b	50.00±1.00 ^b	802.60±6.69 ^a	175.00±2.77 ^b	54.20±1.66 ^b	815.00±7.07 ^a	169.00±2.35 ^b	52.80±1.85 ^b
G₄	528±11.58 ^c	127±2.50 ^d	30.20±1.77 ^d	505.0±12.25 ^d	120.20±1.77 ^d	28.80±0.80 ^d	411.20±11.70 ^c	115.20±2.15 ^c	24.80±1.77 ^c
G₅	654±9.27 ^c	145±1.03 ^c	41.60±1.21 ^c	695.20±5.16 ^c	155.00±1.70 ^c	45.40±0.51 ^c	728.60±4.63 ^b	158.00±1.79 ^b	51.60±1.63 ^b
G₆	618±12.41 ^d	142±1.50 ^c	39.80±1.43 ^c	676.80±2.08 ^c	151.00±1.14 ^c	42.20±0.73 ^c	732.00±4.64 ^b	157.00±1.73 ^b	50.20±1.98 ^b

G₁: control group (negative control); G₂: received rosemary aqueous extract (100 mg/kg BW/day) orally in drinking water for 6 weeks; G₃: received licorice aqueous extract (150 mg/kg BW/day) orally in drinking water for 6 weeks; G₄: received lead acetate (0.2%) in drinking water for 6 weeks (positive control); G₅: received lead acetate in combination with the aqueous extract of rosemary (0.2%) in drinking water for 6 weeks and G₆: received lead acetate in combination with the licorice in drinking water for 6 weeks.

Means within the same column carrying different superscript letters were significantly different at $p \leq 0.05$.

Table 5: Some antioxidant enzymes activities and malondialdehyde in serum of albino rats at 2, 4 and 6 weeks post-treatment (Means±SE)

Parameters	Times post-treatment								
	2 weeks			4 weeks			6 weeks		
	CAT (U/mL) ¹	SOD (U/mL) ²	MDA (mmol/L) ³	CAT (U/mL)	SOD (U/mL)	MDA (mmol/L)	CAT (U/mL)	SOD (U/mL)	MDA (mmol/L)
G₁	60.40±0.93 ^b	91.40±0.93 ^c	8.34±0.18 ^c	63.50±1.23 ^b	91.98±1.13 ^c	8.64±0.10 ^c	62.38±1.46 ^c	91.00±0.81 ^c	8.90±0.15 ^b
G₂	76.40±1.81 ^a	116.2±1.77 ^a	6.64±0.09 ^e	79.58±0.68 ^a	117.7±0.88 ^a	6.52±0.11 ^e	86.70±1.12 ^a	120.0±1.71 ^a	5.92±0.09 ^d
G₃	74.00±1.22 ^a	105.6±1.23 ^b	7.40±0.13 ^d	76.72±0.70 ^a	108.1±.55 ^b	7.10±0.22 ^d	79.32±0.69 ^b	115.4±1.16 ^b	7.80±11.30 ^c
G₄	46.40±0.93 ^d	74.80±1.36 ^f	11.94±0.26 ^a	39.52±0.63 ^d	70.20±0.86 ^e	12.62±0.20 ^a	33.70±1.17 ^d	61.44±1.06 ^d	13.14±0.28 ^a
G₅	54.40±1.50 ^c	86.00±1.14 ^d	10.50±0.10 ^b	56.02±1.59 ^c	84.94±1.83 ^d	9.88±0.09 ^b	61.96±0.96 ^c	90.44±1.17 ^c	9.04±0.23 ^b
G₆	52.80±1.46 ^c	81.00±0.71 ^e	10.68±0.21 ^b	55.56±0.63 ^c	83.28±1.17 ^d	10.18±0.18 ^b	60.44±1.42 ^c	88.68±1.35 ^c	9.64±0.25 ^b

¹CAT: catalase activities; ²SOD: superoxide dismutase activities and ³MDA: malondialdehyde. G₁: control group (negative control); G₂: received rosemary aqueous extract (100 mg/kg BW/day) orally in drinking water for 6 weeks; G₃: received licorice aqueous extract (150 mg/kg BW/day) orally in drinking water for 6 weeks; G₄: received lead acetate (0.2%) in drinking water for 6 weeks (positive control); G₅: received lead acetate in combination with the aqueous extract of rosemary (0.2%) in drinking water for 6 weeks and G₆: received lead acetate in combination with the licorice in drinking water for 6 weeks.

Means within the same column carrying different superscript letters were significantly different at p ≤ 0.05.

Discussion

Oxidative stress is one of the major mechanisms of lead toxicity. Medicinal plants with antioxidant effects are excellent choice to protect against oxidative stress with least side effects. Rosemary and licorice have an antioxidant action. Rats received rosemary or licorice alone appeared healthy without mortalities. This may be attributed to the useful role of rosemary in controlling nutrients uptake through GIT and the immunomodulatory effect of rosemary [24] and licorice [25] that improved the health status of rats. On the other hand, inappetence, general weakness and weight loss with high mortality rate were observed in the lead group may be attributed to the direct effect of lead on the GIT initiating deficient absorption of nutrients or on protein synthesis [26]. The severity of clinical signs was improved in groups 5 and 6, which agreed with earlier studies [24,25].

Administration of rosemary or licorice to normal rats didn't induce significant changes in liver or kidney functions except an increase in serum total protein and globulin levels in rosemary group. This may be due to that rosemary has a more beneficial immune-improving effect than licorice and both rosemary and licorice have no deleterious effects on liver and kidney. These results are in agreement with others who reported that administration of rosemary extract [27] or licorice [28] didn't induce any change in serum ALT, AST and ALP activities.

Activities of AST and ALT are most commonly used as biochemical markers for monitoring chemically induced liver damage. In the present study, administration of lead (G_4) caused a significant increase in the levels of liver enzymes (ALT, AST and ALP activities). These results might be due to organ failure or damage, as the excessive generation of ROS as well as depletion of the antioxidant reserves, cause accumulation of ROS in hepatocytes leading to hepatotoxicity and liberation of ALT from damaged hepatocytes into the systemic circulation [29]. Similar changes in the values of ALT and AST were associated with lead acetate intoxication in rats [24,30]. On the other hand, the increases in the previous enzymes activities were significantly

suppressed by administration of rosemary (G_5) or licorice extracts (G_6) at all experimental periods when compared with the positive control (G_4). Treatment with rosemary or licorice of lead-induced oxidative stress rats were able to alleviate hepatic damage by lowering their biomarkers through scavenging the free radicals and terminating the membrane LPO by improving cellular antioxidant defense system. Glicyrrhizic acid showed hepatoprotective effect through preventing changes in the cell membrane permeability and increasing the survival rate of hepatocytes. These outcomes agreed with previous results [31,32].

Rosemary treated rats (G_2) revealed a significant increase in serum TP and globulins and this agreed with others [33], who reported that rosemary extract had a beneficial effect on immunity by developing and protecting cells and inhibiting non-enzymatic oxidation. On the other hand, rats received licorice (G_3) showed non-significant changes in TP, albumin, globulin and A/G ratio during all the experimental periods, which is comparable to the findings of Jagadeeswaran and Selvasubramanian [34]. Lead-treated rats (G_4) showed a significant decrease in all proteinogram which may be due to the catabolic effects of lead [35] or decrease the number of hepatocytes that may cause a reduction of hepatic capacity to synthesize protein [29]. Similar changes in serum TP and albumin were previously obtained by El-Tantawy [36]. But, the restoration of TP and albumin levels after the administration of rosemary (G_5) or licorice extracts (G_6) confirmed the hepatoprotective nature of both extracts and agreed with those obtained in rat of lead induced toxicity that treated with rosemary or licorice root extract [9,28].

Administration of rosemary (G_2) or licorice alone (G_3) showed no significant change in serum urea and creatinine levels. This indicated that both plants are safe and had no deleterious effect on kidney. On the other hand, chronic lead exposure (G_4) induced a significant increase in serum urea and creatinine in all experimental periods. This elevation was reflected the state of glomerular filtration and indicated kidney damage. Lead-induced renal dysfunction may be associated

with the attenuation of cell membrane integrity with cell damage or the effect of excessive production of ROS. Also, decreased renal perfusion may be due to the metabolic disorders caused by lead [35]. Combination of lead with rosemary (G₅) or licorice (G₆) improved the kidney functions in a duration dependent effect as creatinine and urea levels returned to the normal level by the 6th week of the experiment. These results were in harmony with others [25,37].

The current results showed that rosemary (G₂) increased all types of immunoglobulins, while licorice (G₃) increased only serum IgG. The increase in the globulin fractions indicated the effective role of rosemary in increasing immunity due to its role in developing and protecting cells and inhibiting nonenzymatic oxidation [38]. Conversely, lead acetate administration (G₄) caused a significant decrease in serum immunoglobulins (IgG, IgM and IgA) during the different experimental periods. These results indicated that lead had a deleterious effect on humeral immunity. The effect of lead was modulated by the addition of rosemary (G₅) or licorice (G₆). These results indicated that rosemary or licorice extracts improved the immunoglobulins state and rosemary was more potent than licorice as an immunomodulating agent [38].

Oxidative stress is induced by excessive production of superoxides, hydrogen peroxides and hydroxyl radicals and/or by insufficient antioxidative resistance [39]. Regarding the antioxidant activities, our study revealed that rosemary and licorice extracts have antioxidative properties that appeared as a significant increase in serum CAT and SOD levels with a significant decrease in MAD (G_{3,4}). The powerful antioxidant activity of rosemary may be due to the presence of phenolic compounds [40] and that of licorice to its content of flavonoids [41]. However, rats given lead acetate alone (G₄) affected the antioxidant activities by a significant reduction in CAT and SOD with a significant increase in MDA due to lipid peroxidation in a duration dependent effect. From our opinion, this may be related to the effect of lead toxicity that increased the oxidative stress with excessive production of free radicals and exhausted the antioxidant defense system leading to decrease

CAT and SOD with increased lipid peroxidation (MDA). Lead toxicity leads to free radical damage by two pathways; the first is the generation of reactive oxygen species (ROS) including hydroperoxides and hydrogen peroxides evaluated by MDA levels as the final product of lipid peroxidation, while the second is the direct depletion of antioxidant reserves [3,4]. These results agreed with other previously reported findings [11, 30, 36, 37]. Addition of rosemary (G₅) or licorice (G₆) extracts to rats exposure to lead, improved the antioxidant activities that returned to the normal level after the 6th week from the beginning of the experiment, which was evidenced by a significant increase in CAT and SOD levels with a significant decrease in MAD level when compared with lead received group. This may be due to the active natural components present in rosemary and licorice, which have antioxidant activities through the elimination of free radicals and subsequently prevent the cellular destruction [41,42]. Similar results concerning the increase of CAT and SOD with significant decrease of MDA levels were obtained in rabbits received rosemary in combination with lead acetate [33] and in rats administered licorice with lead acetate [30].

Conclusion

From these obtained findings, we can conclude that rosemary and licorice extracts have a protective role in alleviating the harmful effects of lead on the hepato-renal functions, immunity and antioxidants activities.

Conflict of interest

The authors declare that they have no conflict of interest.

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الملخص العربي

دراسات بيوكيميائية على إكليل الجبل و عرق السوس في الجرذان المصابة بالإجهاد التأكسدي المستحدث بالرصاص

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بعض النباتات في الطبيعة تحتوي على مضادات الاكسدة ويعتبر تناولها مفيد للكائنات الحية لما تقوم به من دور هام في ايقاف الاكسدة الضارة. أجريت هذه الدراسة لتقييم تأثير مضادات الأكسدة في إكليل الجبل أو عرق السوس وانعكاسها على المؤشرات البيوكيميائية في الجرذان المصابة بالإجهاد التأكسدي الناجم عن الرصاص. تمت الدراسة على عدد مائة وعشرون جرذاً أبيض يتراوح وزن الجرذ منهم من ٢٠٠ الى ٢٥٠ جرام وقد قسمت هذه الجرذان الى ستة مجموعات. كل مجموعة تحتوي على عدد ٢٠ جرذاً وتمت الدراسة على النحو التالي: المجموعة الاولى: تركت بدون معاملة كمجموعة ضابطة للتجربة وبدون اصابة. المجموعة الثانية: تم اضافة مستخلص مائي من نبات اكليل الجبل ١٠٠ مجم/كجم من وزن الجسم لمياه الشرب يوميا لمدة ٦ اسابيع. المجموعة الثالثة: تم اضافة مستخلص مائي من نبات العرق سوس ١٥٠ مجم/كجم من وزن الجسم لمياه الشرب يوميا لمدة ٦ اسابيع. المجموعة الرابعة: تم اضافة خللات الرصاص بتركيز ٠.٢% لمياه الشرب يوميا لمدة ٦ اسابيع. المجموعة الخامسة: تم اضافة مستخلص مائي من نبات اكليل الجبل ١٠٠ مجم/كجم من وزن الجسم بالاضافة الى خللات الرصاص بتركيز ٠.٢% لمياه الشرب يوميا لمدة ٦ اسابيع. المجموعة السادسة: تم اضافة مستخلص مائي من نبات العرق سوس ١٥٠ مجم/كجم من وزن الجسم بالاضافة الى خللات الرصاص بتركيز ٠.٢% لمياه الشرب يوميا لمدة ٦ اسابيع. تم تجميع عينات بدون استخدام مضادات التجلط لفصل السيرم لاجراء الفحوص البيوكيميائية في نهاية الأسبوع الثاني والرابع والسادس من التجربة بواقع خمس جرذان من كل مجموعة. وقد اظهرت نتائج بعض وظائف الكبد زيادة معنوية في كل من انزيم الالنين أمينوترانسفيريز والاسبرنتيت أمينوترانسفيريز والفوسفاتيز القاعدي في المجموعة الرابعة والخامسة والسادسة ولوحظ في المجموعة الخامسة والسادسة ان هذه الانزيمات تعود لمستواها الطبيعي في نهاية الاسبوع السادس. بينما لوحظ في المجموعة الثانية والثالثة عدم حدوث تغيرات معنوية في هذه الانزيمات طوال فترات التجربة. كما أظهرت تغيرات واضحة في البروتين ووظائف الكلى و IgA و IgM - IgG والانشطة المضادة للاكسدة (MDA+ (catalase, SOD خاصة في المجموعة الرابعة طول فترات التجربة. كما لوحظ عودة لهذه التغيرات الى الطبيعي في المجموعة الخامسة والسادسة خاصة في نهاية الاسبوع السادس. ومن هذه الدراسة يمكن استخلاص التالي التسمم بخلات الرصاص سبب اضرار كبدية وكلى بالاضافة الى تثبيط المناعة وزيادة الاكسدة الداخلية. أظهر تناول مستخلص اكليل الجبل و العرق سوس فاعلية في تقليل التأثيرات الضارة لخللات الرصاص. اظهر اكليل الجبل و العرق سوس بعض التغيرات الايجابية في وظائف الكبد والكلى. لذلك يوصى باستخدام كلا النباتين كمضادات طبيعية للاكسدة وخاصة اكليل الجبل.