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Protective Effect of Rosemary (*Rosmarinus officinalis*) Extract on Lithium-Induced Renal and Testis Toxicity in Albino Rats

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Abstract

Lithium is used common as an effective drug for the medication of many psychiatric disorders in human. The current study was proceed to analysis the protective effect of rosemary leaves extract on renal and testis toxicity induced by Lithium in rats. Forty adult Albino male rats were divided into four equal groups. Group (I) was kept on standard rodent chow and served as healthy control group, Group (II) were orally administrated with 220 mg/kg/day of rosemary extraction, group (III) were orally administrated with 100 mg/kg/day of lithium carbonate (Li_2CO_3) and Group (IV) were orally administrated with both 220 mg/kg/day rosemary extraction and 100 mg/kg/day (Li_2CO_3). All groups were treated for 4 weeks. Lithium caused significant increase in plasma Malondialdehyde (MDA), creatinine, urea, Neuron Specific Enolase (NSE) and Human Chorionic Gonadotropin (HCG) levels, and while, significant decrease in plasma free and total testosterone, Superoxide Dismutase (SOD) and Glutathione (GSH) levels has been reported as compared to healthy control group. On the other side, treatment with rosemary extraction was significantly improving the level of oxidative stress, biomarkers (HCG), (NSE) and routine blood test of kidney function. Conclusion, rosemary leaves extract had a protective effect against renal and testis toxicity induced by lithium in rats.

Keywords: Lithium; *Rosmarinus officinalis*; Antioxidant activity; Steroidogenic enzymes

Introduction

In the past fifty year ago, a number of chemical salts of lithium have been applied as a first drug for the treatment of mental diseases like mania, depression and manic-depressive psychosis. Despite its clinical importance, finding suggests that lithium has side effects on thyroid function, intestine, liver, brain, reproductive and endocrine systems and other organs [1]. Lithium chloride when given decreases many parameters including ovarian steroidogenic enzymes and folliculogenesis in the adult female rats [2].

One of the main targets of Lithium is the kidney and long-term treatment may induce advanced nephrotoxicity. Where lithium interferes with the capacity of the cortical portion of the collecting tubule in the kidneys to produce cyclic adenosine monophosphate in response to antidiuretic hormone. Many researches have been demonstrated to pathogenesis of a great effect of toxic substances in free radicals [3].

In rats and humans, lithium mostly causes tubule interstitial nephropathy; however, severe destruction of the mitochondria and endoplasmic reticulum in electron microscope examinations has revealed renal ischaemia [3]. It has been stated that lithium induced kidney damage parallels to alterations in renal hemodynamics and the normally higher cortical blood flow distribution shifted to the medullary regions as similar to ischaemia model [4].

Studies of the male reproductive toxicity of lithium not many published studies available. Previous result indicated that lithium treatment causes sexual impairment in male humans [5]. Lithium chloride-exposed rats appear inhibition of testicular hydroxysteroid dehydrogenase activity, testicular steroidogenesis and spermatogenesis [6]. In male birds, lithium chloride induces remarkable degenerative changes in germ cells in that most of the spermatids and mature spermatozoa illustrated necrotic changes and sloughed off from the seminiferous tubular epithelium [7]. In a long-term exposure study, oral exposure to lithium carbonate was found to cause reproductive toxicity [8].

Rosemary, *Rosmarinus officinalis* L. (*Labiatae*), is an evergreen perennial shrub in overripe in many parts of the world. It has been documented to possess a number of therapeutic applications in medicine for curing or managing of different diseases such as DM, respiratory, gastrointestinal disorders, and inflammatory diseases [9].

The aim of this study is to investigate the protective effect of rosemary leaves extract against renal and testis toxicity caused by lithium chloride in rats.

Materials and Methods

Chemicals

Lithium carbonate (Li_2CO_3) purchased from Merck-Schuchardt Chemical, Hohenbrunn, Germany.

Plant materials

The Egyptian cultivated herbs were purchased from local market Harraz Herbal Drugstore, Cairo, Egypt.

Preparation of rosemary extract

The extraction of rosemary was carried out according to the method of Dorman et al. Briefly, 25 g fine powdered were mixed with 250 ml distilled water in rounded flask where it connected to a hydrodistillator instrument and the mixture was left to boil for 2 h. The water extract was removed and another 150 ml of distilled water were added and left to boil for 1 h. All water extract were collected and filtered using Whatman filter papers. The aqueous extract was then ready for lyopholyzation process through freeze drier under pressure, 0.1 to 0.5 mbar and temperature -35°C to - 40°C conditions. The dry extraction was saved at 4C until used. The used dose was 220 mg/ kg body weight [10].

Experimental animal design

Forty male albino rats weighting 120 g were kept in the laboratory under constant conditions of temperature (24 ± 2°C) at minimum one week before and through the experimental work, being maintained on a standard diet and water were ready ad-libitum. The animals were maintained in accordance with the guidelines prescribed by the faculty of Science and study was approved by Animal Ethics Committee of the University of Fayoum, Egypt. The experimental rats were being divided into four groups:

Group 1: Healthy control group which was fed *ad-libitum* with an isocaloric regular rat chow for 4 weeks.

Group 2: Animals were orally administrated with 220 mg/kg/day rosemary leaves extract for 4 weeks [10].

Group 3: Animals were treated orally by stomach tube with 100 mg/kg/day of lithium for 4 weeks [11].

Group 4: Animals were treated orally by stomach tube with both. 220 mg/kg/day rosemary extraction and 100 mg/kg/day Li_2CO_3 for 4 weeks.

Sample collection

At the end of the experimental interval, the animals were kept fasting for 12 hrs and the blood samples were collected of the retro-orbital venous plexus under diethyl ether anesthesia. The blood samples from each animal were added in tubes containing EDTA and plasma was separated after centrifugation at 4000 rpm for ten minutes at 5°C. The clear plasma samples were stored at -20°C until analysis. After blood collection, all animals were sacrificed by cervical dislocation and testis and kidney of each rat was rapidly excised, washed in isotonic saline, then cut into small pieces (0.5 × 0.5 cm) and fixed in 10% formalin saline solution for histological examination.

Biochemical analysis

Plasma MDA was carried out by thiobarbituric acid according to the method of Zima et al. [12]. Plasma total Superoxide Dismutase (SOD) activity was estimated colorimetrically using kit purchased from Quimica Clinica Aplicada S.A. Spain, depending on the method of minami and yoshikawa [13]. Plasma glutathione (GSH) concentration was determinate colorimetrically using kit purchased from Stanbio Laboratory, Boerne, Texas, USA, depending on the method of Beutler et al. [14]. Plasma Creatinine concentration was weighted colorimetrically using kit purchased from Stanbio Laboratory, Texas, USA, depending on the method of Bowers and Wong [15]. Plasma urea was determined colorimetrically using kit purchased from Stanbio Laboratory, Boerne, Texas, USA, depending on the method of Tietz [16]. Plasma-free testosterone concentration was weighted by enzyme-linked immunosorbent assay (ELISA) technique utilizing kit purchased from Diagnostics Biochem, Canada, according to the method of Nazian [17]. Total testosterone concentration was measured by ELISA technique using kit purchased from Dima Gesellschaft Fur Diagnostika, Germany, depending on the method of Chen et al. [18]. Plasma human chorionic gonadotropin (HCG) concentration was measured via ELISA technique using kit purchased from ALPCO Diagnostics, USA, depending on the method of Lenton et al. [19] and plasma Neuron Specific Enolase (NSE) concentration was measured by ELISA technique using kit purchased from DRG International Inc., USA, depending on the method of Fossa et al. [20].

Histopathological examination

After fixation of kidneys and testis tissues in 10% saline buffered formalin, the kidneys and testis tissues were dried in ascending grades of ethanol, cleared in xylol and then immersed in paraffin. Impregnated kidneys and testis tissues were treated three times in pure paraffin to be established in blocks. Sections (5 µm thick) were preparatory using Leica microtome and stained by hematoxylin and eosin (H&E) for histopathological investigation [21].

Statistical analysis

In the present study, all results were showed as Mean ± S.E of the mean. Data were analyzed by one way Analysis Of Variance (ANOVA) using the (SPSS) program, version 16 followed by Minimal Significant Difference (LSD) to compare significance between groups. Difference of what was significant when P value >0.05. The percent of difference was calculated according to the following equation:

$$\% \text{ of difference} = \frac{Treatedvalue - Controlvalue}{Controlvalue} \times 100$$

Results

Biochemical Results

The effect of treatment with *Rosmarinus officinalis* extract on plasma MDA, SOD and GSH levels in on Lithium toxicity in rat model is illustrated in Table 1. A significant increase (P<0.05) in plasma MDA (46.44%) level was recorded in (Li_2CO_3) treated group. While, There is significant decrease (P<0.05) in both plasma SOD and GSH (-66.4% and -73.85%) respectively levels in (Li_2CO_3) treated group as compared to the healthy control group. On the other hand, significant decrease (P<0.05) has been recorded in plasma MDA (-17.36%) and significant increase (P<0.05) in both plasma SOD and GSH (16.39%, 18.44%) respectively levels in extraction/(Li_2CO_3) treated group as compared to the (Li_2CO_3) treated group.

The data depicted in Table 2 represent the effect of treatment with *Rosmarinus officinalis* extract on plasma creatinine and urea levels in (Li_2CO_3)-toxicity rat model. In comparison with the healthy control group, significant reduction (P<0.05) in plasma creatinine and urea levels (392.9% and 25.51%) respectively were recorded in (LiCl) treated

group. In contrast, treatment of (Li_2CO_3) group with *Rosmarinus officinalis* extract resulted in significant increase (P<0.05) in plasma creatinine and urea levels (-39.96% and -12.53%) respectively as compared to the untreated (Li_2CO_3) group.

The Data in Table 3 show that (Li_2CO_3) treated elicited significant decline (P<0.05) in plasma total and free testosterone levels (-70.1% and - 60.21% respectively) as compared to the healthy control group. Meanwhile, treatment of extraction/ (Li_2CO_3) treated group resulted in significant elevation (P<0.05) in plasma total and free testosterone levels (13.30 % and 64.85% respectively) as compared to the (Li_2CO_3) treated group.

The data in Table 4 determined the effect of treatment with *Rosmarinus officinalis* extract on plasma Human Chorionic Gonadotropin (HCG) and NSE levels in (Li_2CO_3)-toxicity rat model. The (Li_2CO_3) group displayed a significant increase (P<0.05) in plasma HCG and NSE levels (477.2% and 227.3% respectively) when compared with the healthy control group. On the other side, treatment of (Li_2CO_3) group with *Rosmarinus officinalis* extract induced significant decrease (P<0.05) in plasma HCG and NSE levels (-30.32% and -41.67% respectively) in comparison with the untreated (Li_2CO_3) group.

There were no significant correlation between NSE and other parameters are detected in (Li_2CO_3) treated group, but there were positive significant correlation between HCG and both SOD and creatinine (Figures 1A and 1B).

Histopathological results

Microscopic investigation of kidneys tissue sections of rats in the healthy control group showed the normal structure of the kidneys (Figure 2A), Similarly, histopathological investigation of kidneys tissue sections of rats treated with 220 mg/kg/day extract of rosemary extract groups showed no significant abnormality could be detected in the glomeruli, proximal tubules or descending loops of Henle (Figure 2B). Meanwhile, histopathological examination of kidneys tissue sections of rats that were treated with Li showed necrotizing cell in damaged tubules with hemorrhagic glomerulitis (Figure 2C), while, microscopic examination of kidneys tissue sections of rats in the (Li_2CO_3) group treated with rosemary extract improved histopathology of kidneys (Figure 2D).

Microscopic investigation of testis tissue sections of rats in the healthy control group showed the normal structure of the testis (Figure 3A). Histopathological investigation of testis tissue sections of rats treated with 220 mg/kg/day extract of rosemary extract groups showed no significance abnormalities in semniferous tubules (Figure 3B). On the other side, the histopathology of testis tissue sections of rats that treated with (Li_2CO_3) group showed irregular tubular wall and massive loss of spermatonic cells. Massive necrosis of spermatocytes and spermatides (Figure 3C), while, microscopic examination of kidney tissue sections of rats in the (Li_2CO_3)group treated with rosemary extract showed normal arrangement of seminefrous tubules (Figure 3D).

Groups / Parameters	Group I (Control)	Group II (220mg/Kg/day extraction treated)	Group III (100 mg/Kg/day) (Li_2CO_3) treated)	Group IV (extraction/(Li_2CO_3) treated)
MDA (nmol/ml)	14.75 ± 1.8	15.82 ± 1.4 7.25%	21.6 ± 1.2[a] 46.44%	17.85 ± 1.63[a,b] -17.36%
SOD (U/ml)	120.8 ± 20.17	122.17 ± 18.9 1.13%	40.61 ± 8.97[a] -66.4%	107.20 ± 13.41[a,b] 16.39%
GSH (mg/ml)	20.84 ± 3.2	19.01 ± 2.68 -8.78%	5.45 ± 2.01[a] -73.85%	15.5 ± 3.01[a,b] 18.44%

Table 1: Effect of treatment with *Rosmarinus officinalis* extract on plasma MDA, SOD and GSH levels of lithium toxicity rat model. MDA: Malondialdehyde; SOD: Superoxide Dismutase; GSH: Glutathione; a: Significant change at P<0.05 in comparison with the healthy control group. b: Significant change at P<0.05 in comparison with (Li_2CO_3) group. (%): percent difference with respect to the corresponding control value.

Groups / Parameters	Group I (Control)	Group II (220mg/Kg/day extraction treated)	Group III (100 mg/Kg/day) (Li_2CO_3) treated)	Group IV (extraction/(Li_2CO_3) treated)
Creatinine (mg/dL)	0.98 ± 0.25	1.06 ± 0.29 8.2%	4.83 ± 0.68[a] 392.9%	2.9 ± 0.53[a,b] -39.96%
Urea (mg/dL)	35.16 ± 2.95	33.7 ± 5.73 -4.15%	44.13 ± 4.8[a] 25.51%	38.6 ± 2.62[a,b] -12.53%

Table 2: Effect of treatment with *Rosmarinus officinalis* extract on plasma creatinine and urea levels of lithium toxicity rat model. a: Significant change at P<0.05 in comparison with the healthy control group. b: Significant change at P<0.05 in comparison with (Li_2CO_3) group. (%): percent difference with respect to the corresponding control value.

Parameters \ Groups	Group I (Control)	Group II (220mg/Kg/day extraction treated)	Group III (100 mg/Kg/day) (Li_2CO_3) treated)	Group IV (extraction/(Li_2CO_3) treated)
Total testosterone (ng/ml)	3.74 ± 0.83	4.27 ± 0.74 14.2%	1.12 ± 0.46[a] -70.1%	2.61 ± 0.89[a,b] 13.3%
Free testosterone (pg/ml)	30.11 ± 2.93	27.87 ± 3.1 -7.44%	11.98 ± 2.9[a] -60.21%	19.75 ± 1.61[a,b] 64.85%

Table 3: Effect of treatment with *Rosmarinus officinalis* extract on plasma total and free testosterone levels of lithium toxicity rat model. a: Significant change at P<0.05 in comparison with the healthy control group. b: Significant change at P<0.05 in comparison with (Li_2CO_3) group. (%): percent difference with respect to the corresponding control value.

Parameters \ Groups	Group I (Control)	Group II (220 mg/Kg/day extraction treated)	Group III (100 mg/Kg/day) (Li_2CO_3) treated)	Group IV (extraction/(Li_2CO_3) treated)
HCG (mU/ml)	1.36 ± 0.58	1.61 ± 0.52[a] 18.4%	7.85 ± 1.44[a] 477.2%	5.47 ± 1.78[a,b] -30.32%
NSE (ng/ml)	6.73 ± 1.27	6.9 ± 0.87	22.03 ± 3.8[a] 227.3%	12.85 ± 2.77[a,b] -41.67%

Table 4: Effect of treatment with *Rosmarinus officinalis* extract on plasma HCG and NSE levels of lithium toxicity rat model. HCG: Human chorionic gonadotropin; NSE: Neuron Specific Enolase. a: Significant change at P<0.05 in comparison with the healthy control group. b: Significant change at P<0.05 in comparison with (Li_2CO_3)group. (%): Percent difference with respect to the corresponding control value.

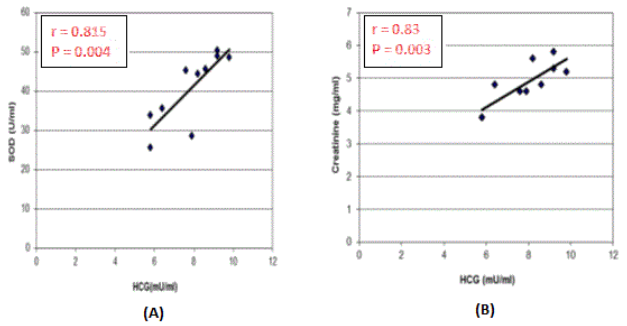

Figure 1: (A) Positive significant correlation between HCG and SOD. (B) Positive significant correlation between HCG and creatinine both in (Li_2CO_3) treated group.

Discussion

The results of the actual study indicated significant increase in plasma Malondialdehyde levels (MDA) in (Li_2CO_3)-induced group against the healthy control group. In rats (Li_2CO_3) administration, the increase in renal MDA levels could be associated with damage of proximal tubules, the primary site of (Li_2CO_3) accumulation [3,22]. Propose that high doses of lithium 200 mg/kg lead to increased lipid peroxidation and destruction to the erythrocyte membrane, MDA is one of the end products of lipid peroxidation, could produce modulation of the structure, and permeability of the erythrocyte membrane, leading to its harm, and increased sensitivity of the cells toward to hypotonic conditions [11]. When lithium is used in its carbonate form, it is found that carbonate reason selective increase of lithium and sodium permeability which is due to ability of carbonate to compose ion pairs with them. In our studies it has been shown that lithium is over view an electrochemical gradient through a counter transport mechanism which depends on the presence of a contrary directed gradient for sodium ion [1].

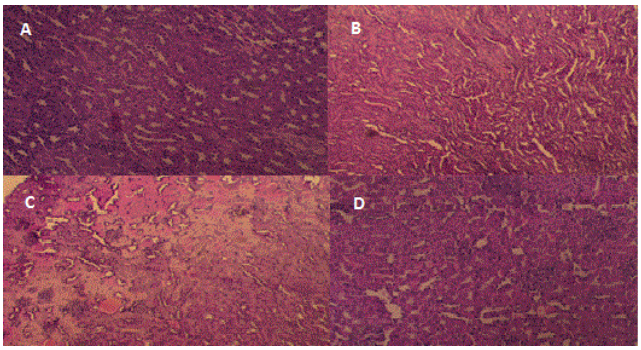

Figure 2: Photomicrographs of kidney tissue section of (A) Healthy control group showing normal structure of the kidney, (B) rats treated with 220 mg/kg/day extract of rosemary showing no change in the glomeruli, proximal tubules or descending loops of Henle, (C) rats treated with 100 mg/kg/day of Li_2CO_3 showing necrotizing cell in damaged tubules with hemorrhagic glomerulitis, (D) rats treated with rosemary extraction and Li_2CO_3 showing normal structure in the glomeruli, proximal tubules (H&E ×100).

Over view of the present results, significant decrease in plasma SOD and GSH levels has been detected in (Li_2CO_3)-induced group when compared with the healthy control group. These results coincide with other study published 3. The activities of SOD and GSH in renal tissue were specified to evaluate the changes of antioxidant status. Kidney

tissue contains antioxidants that prevent destruction from excessive oxygen metabolites. They action either by decomposing peroxide or trapping the free radicals.

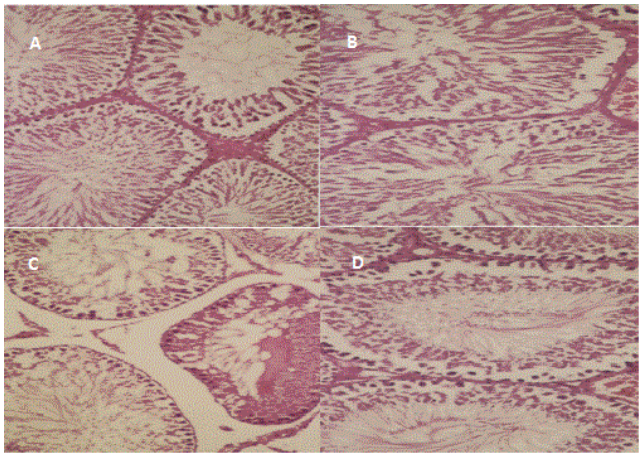

Figure 3: Photomicrographs of testis tissue section of: (A) Healthy control group showing normal structure of the testis; (B) Rats treated with 220 mg/kg/day extract of rosemary showing no significance abnormalities in semniferous tubules; (C) Rats treated with 100 mg/kg/day of Li_2CO_3, showing irregular tubular wall and massive loss of spermatonic cells. Massive necrosis of spermatocytes and spermatids; (D) Rat treated with rosemary extraction and Li_2CO_3, showing normal arrangement of seminefrous tubules (H&E ×100).

SOD is a specific antioxidant enzyme that dismutates O-2, forming H_2O_2, which is scavenged by GSH. It protects the cell against toxic effect of superoxide radicals. The extracellular SOD of renal tissue is distributed in the vessel walls for detoxifying superoxide anions produced in sera of oxidative stress exposed animals. It is possible that Lithium take will affect the mitochondrial membranes to make large amounts of oxygen radicals that caused extreme use of SOD in rat kidneys in this study. The highly rapid interconversion of ROS (O^{-2}, H_2O_2) within the cell can produce it difficult to describe the originating types. The mitochondrial respiratory chain is the major site for the generation of superoxide radicals (O^{-2}) [23]. Reduced Glutathione (GSH) is central to the cellular antioxidant defenses and acts as an important cofactor for antioxidant enzymes. Under oxidative stress, glutathione is used by the glutathione-related enzymes to detoxify peroxides created because of increased Lipid Peroxidation (LPO) and elevation in LPO is a consequence of consumed GSH stores, which are otherwise able to moderating the amount of LPO [24].

According to our results, significant increases both plasma creatinine and urea levels in group, has been detected in the (Li_2CO_3) group as compared to the healthy control group. These results agreement with research published [25], who notify that lithium stimulate hepatic and renal toxicity in rats at physiological and histopathological grades. It has the chance of inducing dangerous effects in the rat blood and oxidative stress in the rat RBCs also. High elevation in plasma urea and creatinine appear related to the observed kidney damage [26].

The present data detect a significant decrease in plasma total and free testosterone levels in the (Li_2CO_3) group compared to the healthy control group. This data agreement with other research [27]. Lithium spends its effect directly at the level of the Leydig cells rather than by the pituitary-gonadal axis. Since the known lithium-induced reduction of testosterone was detected when the plasma lithium levels were within (or around) the therapeutic range [28].

Plasma Human Chorionic Gonadotropin (HCG) level exhibited significant increase in (Li_2CO_3) group compared with the healthy control group as shown in the present study. HCG is a **hormone** produced by the **syncytiotrophoblast**, a portion of the **placenta** after fertilization [29].

Significant increase in plasma NSE level has been detected in (Li_2CO_3) group as contrast to the healthy control group. In general, NSE is a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate. NSE is also repeatedly overexpressed by neural crest-derived tumors [30]. In this study there was great increase in NSE level in (Li_2CO_3) treated group and improve its level in group treated with extraction and (Li_2CO_3). This elevation of NSE was accompanied by neuronal damage in specific brain areas such as cortex, thalamus, amygdala, septum, and hippocampus [31].

Histopathological investigation of kidney tissue sections of rats that treated with (Li_2CO_3) showed necrotizing cell in damaged tubules with hemorrhagic glomerulitis. Oxidative stress is considered to perform a central role in the pathogenesis of (Li_2CO_3) because the increased production of ROS is known to cause lipid peroxidation, followed by kidney damage.

Also, the histopathology of testis showing irregular tubular wall and huge loss of spermatonic cells (Li_2CO_3) treated group, this agreement with other study [32]. Who indicate that lithium carbonate treatment lead to ultrastructure change in testicular epithelium of semniferous tubules contracted and surrounded by irregular and wavy basal lamina.

Rosemary contains many of biologically active compounds; include antioxidants substances carnosic acid, rosmarinic acid, ursolic acid, betulinic acid, rosmaridiphenol and rosmanol. So rosemary extract appear as a cytoprotective agent when exposed to a free radical-scavenging activity that cause widespread damage to cell component such as membrane lipids and highly increased the normal cells viability and the antioxidant enzymes activity SOD and GSH and significantly decrease MDA contents [33]. Rosemary extract are able to donate electrons to reactive radicals, changing them to more stable and on reactive types, therefore preventing them from incoming biomolecules for example: lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological systems [34].

Plasma creatinine and urea levels had been depletion by *Rosmarinus officinalis* extract. These results are agreement with other research [9] who reported that rosemary aqueous extract improve the renal toxicity. This was manifested by normal appearance of kidneys tissues and decreased levels of creatinine and urea. In addition, an investigation conducted by other study [35], concluded that rosmarinic acid, at a dose of 200 mg/Kg body weight/day, for 8 weeks in diabetic rats, could significantly decrease glomerular hypertrophy, glomerulosclerosis and attenuated urea and creatinine. Similarly, another study [36] reported that rosemary prevented histopathological lesions and oxidative stress induced by doxorubicin in liver, kidney and heart of mice.

The present data revealed an important increase in plasma total and free testosterone levels in the *Rosmarinus officinalis* extract treated group. These results agreement with other research [37], who found that the total or free testosterone concentration was increase in viability of Leydig cells something like: (interstitial cells found testicle and they produce testosterone), so increased the testosterone level which was increase spermatogenesis in the rat testis.

Plasma HCG and NSE levels exhibited significant decrease in *Rosmarinus officinalis* extract treated group as shown in the present study flavonoids have been shown to generated antiandrogenic activity and affect fertility effect on the male reproductive system, by interfering with sexual maturation, the output, and transport of spermatozoa, the spermatogenic cycle, sexual activity and fertility. The action of a toxic agent in the testis may cause a reduction in the sperm concentration and the produce of abnormal gametes [38]. *Rosmarinus officinalis* prevented histopathological lesions and oxidative stress induced by (Li_2CO_3) in kidneys and testis tissues. Rosemary was also found to have a therapeutic potential in treatment or prevention of inflammatory nephrotoxicity. Many studies reported that the preventive effects of rosemary and its extracts are attributed to its antioxidant activity [39].

In conclusion, the present results provide a clear experimental evidence for the promising role of *Rosmarinus officinalis* extract leaves in protective effect against lithium-Induced renal and testis toxicity. The active constituents of *Rosmarinus officinalis* namely flavonoids, polyphenols, such as (rosmarinic acid, carnasol, and rosmaridiphenol) this effect through their antioxidant capacity and anti-inflammatory property improves the nephrotoxicity and testis toxicity.

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