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Improvement role of *curcuma longa* against oxidative stress by lithium carbonate in the male rat.

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ABSTRACT

The protective effect of turmeric (*Curcuma longa*) extract against oxidative stress induced by lithium carbonate (Li_2CO_3). The project was carried out on thirty male rats divided into five groups each group has six rats. The first group saved as normal control was administrated normal saline only, the second and third groups were received merely at dose 5, 10 mg/kg for induction of oxidative state on rats, While the fourth group was received lithium carbonate at dose 5 mg/kg with turmeric extract at dose 0.5 g for 500 g of dietand the fifth group wasreceived lithium carbonate at dose 10 mg/kg with turmeric extract at dose 0.5 g for 500 g of diet. The results appeared after administration of the phenolic extract of turmeric in rats has oxidative stress via Li_2CO_3elevated the testes weight, epididymis weight, with raised sperm motility, sperm count, sperm viability, testosterone level, luteinizing hormone level, follicle stimulating hormone level, at the same time, the results demonstrated decreased the sperm abnormality. Conclusively: treatment by turmericextract was produce enhancing role toward oxidative stress induced via Li_2CO_3 in the rats.

Keywords: Oxidative stress, *Curcuma longa*, Li_2CO_3, Male fertility, Rat.

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INTRODUCTION

Lithium is important element its lightest than other elements and chemically similar to sodium and potassium. Lithium used in the pharmaceutical purpose in the form of lithium carbonate or lithium citrate to treat several disorders in the body. Lithium is widely used in the treatment of manic depression also can to reduce suicide risk[1].

The therapeutic used of lithium for long period may result several disorders as neurotoxicity and hyperthyroidism [3].used of lithium and its salts for the purpose of treatment may lead to complications as sexual impotency, renal toxicity, diabetes mellitus [4,5]. but using lithium carbonate at small doses causing side effects on the male reproductive system. The abnormalities occur in the propagation and distinction of germ layer and altered into spermatogenesis. The direct mechanism via differentiation of germ cells while indirect mechanism by reducing the effective of hypothalamic-pituitary-gonadal- axis also, lithium effect on the supporting cells in the seminiferous tubules lead to reduce the process of spermatogenesis[6].used of lithium carbonate in many doses according to the severity of depression [7]. Lithium carbonate used to treat neurodegenerative illnesses [8]. in addition of low doses of lithium have recorded to study this effect in the [9].

Turmeric is a spice that improve the food dishes and used in Indian. Traditionally, *Curcumalonga*is used in treatment of bleeding, leech bites and inflamed joints. As well as, it used to treat the liver, digestive disorders, jaundice and anti-inflammatory agent[10].

In the last years, many studies about the effective compounds from the plants in medicine and their derivatives in healing different ailments related to oxidative stress [11]. One of these plants turmeric (*Curcumalonga*) was used widely in many counteried as spice and coloring factor in food. Ingestion of turmeric has many useful effects in the human health as protection against inflammatory, apoptotic and oxidative processes [12].Curcumin, is the main active component of turmeric. It is a yellow phenolicpigment derived from the rhizome of turmeric which has shown to possess a broad spectrum of biologicaland pharmacological activities. Curcuminhas been claimed to be a potential anti-inflammatory, antineoplastic and antimutagenic agent [13]. It has also shown to be a powerful antioxidant through inhibiting production of ROS inside and outside the body[14] .

Therefore, the present study aimed to investigate whether the use of turmeric (*Curcuma longa*) could alleviate adverse reproductive outcomes, in particular those related to oxidative stress that produced in male rats by prolonged exposure to lithium carbonate.

MATERIALS AND METHODS

Lithium carbonate (Li_2CO_3) was purchased from the Norginecompany, U.K.
Turmeric rhizomes werepurchased from the local market in AL-Najaf city.

Preparation of phenolic extract of turmeric (curcuminoids):The rhizomes were crushed to powder by using a blender, take a bout 100g of powdered were added to 500ml of 80% ethanol and put the mixture in soxhelt system during 24h . After that, resulting extracts were filtered using filter paper and concentrated to dryness in rotary evaporator in the room temperature. Then, the recipient was transferred to a separating funnel, and 2 N (HCl) were added gradually to get pH 2, then, washed with 10 ml chloroform three times. The solution was separated into two levels, the down level contain the phenols (curcuminoids) were residue, weighted and kept in a refrigerated until using it [15].

Experimental Design:

Thirty male albino rats strain (*Rattusnorvegicus*) weighting (225-250g) obtained from the animal house in the science faculty/Kufa university. The rats kept under observation for one week before starting the experiment for acclimatization.fed on standard diet and water *ad libitum* .Then animals were divided into five groups of six rats in each group, group one was fed the standard diet and normal saline keeping as control but the second and third group were received lithium carbonate at doses 5, 10 mg/kg respectively. The fourth and fifth groups were administration lithium carbonate at dose 5, 10 mg/kg plus turmeric extract (curcuminoids) at dose 0.5 g/500g respectively for 50 days.At the end of experiment period (50 days), rats were anaesthetized by

Ketamine andxylazine and blood samples were collected by heart puncture and put into serum tubes in the room temperature for several minutes and were centrifuged for 20 minutes at 3000 rpm. After separate the sera which were kept at-10°C till hormonal analysis. Testes and epididymis of the sacrificed rats were removed for obtained weight and measurement of sperm parameters.

Sperm parameters: sperms content in the epididymis of the experimental rats after cutting epididymis, sperms content were empyting on the slide and stained by eosin nigrosin stain for examination under the microscope to determine the sperm progressive motility[16]and sperm viability, sperm abnormality [17]. Hormones analysis are measurement by ELISA method [18] .

Analysis of data: by using the SPSS program in the computer version (21) with one way ANOVA [19].

RESULTS AND DISCUSSION

The obtained results in table (1) showed that oral administration of $Li_2CO_3$to normal rats were significant decreased the weight of reproductive organs (testes and epididymis) causing degeneration of testes, reduced seminal fluid quality and quantity also decreased testosterone, luteinizing hormone and follicle stimulating hormone. In the present study, Li_2CO_3– induced testicular toxicity was identical to that previously reported in rat [20]. The action of Li_2CO_3on the testes may be ascribed a direct toxic action of Li_2CO_3on the tissues and is likely to impair gonadal response to LH and FSH [21].

Lithium treatment at high doses is contributed with occurrence of toxic side effects as oxidative stress in several tissues such as the male reproductive system. The present study designed to evaluate the toxicity of lithium carbonate in the male rats at dose 5, 10 mg/kg for 50 days. The results appeared a significant reduced in the weight of testes and epididymis.

So that, the reduced spermatogenesis process, the levels of testosterone and raised formation of abnormal sperms accompanied with high doses of lithium carbonate. Other study showed loss the weight of testes, epididymis and other sexual glands these changes resulting from dysfunction of germ cells in the seminiferous tubules [22].

OS is causing degeneration of sex organs, nitrate lead to reduced the weight of testes, epididymis and sperm parameters may indicate increased oxidative stress in response to lithium carbonate toxicity and further strengthen theROS has pivotal role in lithium carbonate toxicity[23]. Nowadays, the used antioxidants in the diets to reduce the ailments in the body. Herbal plants such as turmeric are known to exert their health effects by scavenging free radicals and modulating antioxidant defense system. In the present study, the use of turmeric or its active component, curcumin counteracted nitrate-induced testicular toxicity, however, curcuminwas more effective than turmeric [24].

The present studyturmeric extract with lithium carbonate increased epididymal sperm number, weights of testes, epididymis, male sex hormones, With respectto turmeric extract, several reports have linked its protective action to anti-inflammatory and anti-infectious activities of this plant[25].These effects, taken together, improved fertility and testicular performance, through controlling both lipoperoxidation and NO production, which simultaneously affect sperm motility. The role of turmeric in testicular protectionmay be referred also to its anti-oxidant property [26].Padmaja and Raju[27]showed that treatment of *Curcuma longa* ameliorated selenium induced damage in wister rat lens by reducing lipid peroxidation.

Table (1) Effect of oral administration of lithium carbonate and turmeric extract (*Curcuma longa*) for 50 days on the weight of testes and epididymis of the male rats.

Treatment	Testes %	Epididymis %
Control	866.66 ± 33.33	540.00 ± 20.81
5 mg/kg Li2CO3	366.66 * ± 44.09	417.00 * ± 16.50
10 mg/kg Li2CO3	283.33 * ± 16.66	380.00 * ± 15.27
5 mg/kg of Li2CO3 & turmeric extract	590.00 * ± 5.77	456.66 * ± 6.66
10 mg/kg of Li2CO3 & turmeric extract	480.00 * ± 15.27	386.66 * ± 8.81

Values are mean ± S.E.* significantly different with control group. Six rats in each groups

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Data in table (2) show the oral administration of $Li_2CO_3$to normal rats induced a significant ($p<0.05$) decrease in serum testosterone, gonadotropins (LH,FSH) and oraladministration of turmericextract to toxicity rats caused significant ($p<0.05$) increase in testosterone, gonadotropins (LH,FSH) hormone levels as compared to toxicity rats by lithium carbonate these results agreement with Zarnescu and Zamfirescu[28] after treated with lithium carbonate for 21 days at dose 35 mg/kg when examined under the microscope showed testicular atrophy, loss of spermatogenic connection and formation of large spaces between the germinal epithelium and these changes were developed to become inflamed and enlargement out the nuclear membrane.

Also, showed round spermatid cells with changed and irregular acrosome and decreased sub-acrosomal space, in addition a high number of ruptured mature spermatids distributed in the sections.

Lithium carbonate was effect on the testicular-pituitary-gonadal-axis. This activated production of luteinizing hormone and follicle stimulating hormone via manufacture of gonadotropin releasing hormone which is transferred from the hypothalamus in to the adenohyophysis of the pituitary gland. FSH and LH were stimulated the adenosine monophosphate as secondary messenger act on the testicular somatic cells to synthesis testosterone hormone. Any causes lead to decrease the levels of FSH and LH in the blood which reflect on the sertoli cells and leydigcells[29,30] .

Concerning turmericextract, from the obtained results showed that it is oral administration at 0.5g/500g for 50 days to male toxicity rats increased the weight of testes and epididymis, as well as testosterone, luteinizing hormone and follicle stimulating hormone levels associated with an enhancement of motility and quantity of sperms also increased the degeneration in the tissues of testes of toxicity rats.These results acceptable with Bhagat and Purohit[31]have recorded in their study that turmeric extract can to enhance the sperm parameters. Also improvement in the reproductive functions due to its antioxidant activities, The lesions in the testes showed in toxicity rats can to disappear after administration of *curcumalonga* . in addition, increase in testosterone, gonadotropins (LH,FSH) levels in oral administration of turmeric extract could be attributed to it is direct on the central nervous system and gonadal tissues or their effects on hypothalamus-pituitary-testis axis[32].

Table (2) Effect of oral administration of lithium carbonate and turmeric extract (*Curcuma longa*) for 50 days on serum Testosterone, Luteinizing hormone and Follicle stimulating hormone of the male rats.

Treatment	T (µg/ml)	LH (µg/ml)	FSH (µg/ml)
Control	3.17 ± 0.17	1.77 ± 0.22	2.25 ± 1.33
5 mg/kg Li2CO3	2 * ± 0.57	1.33 ± 0.33	1.16 * ± 0.44
10 mg/kg Li2CO3	1.33 * ± 0.33	1.66 ± 0.33	1.16 * ± 0.44
5 mg/kg of Li2CO3 & turmeric extract	1.66 * ± 0.33	1.66 ± 0.33	1.33 * ± 0.33
10 mg/kg of Li2CO3 & turmeric extract	1.66 * ± 0.33	1.33 ± 0.33	1.66 * ± 0.33

Values are mean ± S.E. * significantly different with normal group. Six rats in each groups

The seminal fluid analysis showed that lithium carbonate reduced the motility of sperms, count of sperms and elevated the abnormality of sperms. also the results appear, oral administration of tumericextract for 50 days to toxicity rats induced significant ($p<0.05$) increases the sperm progressive motility, sperm count and increased the percentage of sperm cell abnormality in the table (3).

According to the result in the following study can to conclude a reduced significantly in the testosterone formation and stopping the normal production of spermatozoa, lithium carbonate causing atrophy in the cells responsible for luteinizing hormone and follicle stimulating hormone manufacture. Reduced spermatogenesis process and elevated production of abnormal sperms after treatment with lithium carbonate accompanied with reduced significantly the levels of testosterone[33].

As well as, lithium causing decrease in the trophic factors and testosterone synthesis have critical role in the development of germ cells and low doses of lithium carbonate identical to high doses when used for long period lead to side effects in the testes and the levels of six hormones [34].

The improvement in fertility parameters that caused by large dose of turmericextract could be attributed to it is previously reported antioxidant activity [35].Naik[36] recorded the natural antioxidants can to elevate the harmful effects on DNA and other components in the cell resulting from the oxidation as well as to improve all sperm parameters.Also,Salama and El-Bahr [37]reported the *curcumalonga* has effective antioxidants against cadmium toxicity in rats. Moreover, Jedlinska[38]demonstrated the uptake of antioxidants in diets as vitamins act as barrier toward the oxidation by the toxic substances and free radicals. Turmeric extract used in the present study has important role to protect the male reproductive system and raised secretion of testosterone hormone and other sex hormones in the present study.

Table (3) Effect of oral administration of lithium carbonate and turmeric extract (*Curcuma longa*) for 50 days on sperms count and semen picture of the male rats.

Treatment	Sperm Count ($\times 10^6$) in testes	Sperm Count ($\times 10^6$) in epididymis	Motility (%)	Viability (%)	Abnormality (%)
control	73.21 ± 1.14	88.86 ± 0.88	83.66 ± 1.85	88.66 ± 0.88	11.00 ± 0.57
5 mg/kg Li2CO3	31.60 * ± 0.89	52.24 * ± 1.52	25.00 * ± 2.88	44.00 * ± 2.08	72.66 * ± 1.45
10 mg/kg Li2CO3	20.40 * ± 0.47	45.90 * ± 2.85	18.33 * ± 1.66	37.66 * ± 0.88	76.00 * ± 1.52
5 mg/kg ofLi2CO3 & turmeric extract	73.51 * ± 1.86	76.21 * ± 0.99	57.00 * ± 1.15	71.00 * ± 0.57	66.66 * ± 4.40
10 mg/kg ofLi2CO3 & turmeric extract	62.50 * ± 1.86	69.84 * ± 0.31	46.00 * ± 1.52	67.33 * ± 0.88	63.33 * ± 1.66

Values are mean ± S.E.* significantly different with normal group. Six rats in each groups

In conclusion, oraladministration of turmeric extract (*curcumalonga*) to toxicity male rats for 50 days increase the weight of testes, epididymis and improve semen quality and quantity also increase testosterone, luteinizing hormone and follicle stimulating hormone levels in serum. Therefore, this study recommended that intake of turmeric in food may be useful for patients who suffer from infertility .

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