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Protective Effects of Melatonin and Thymoquinone on Hematological Parameters, Hepatic and Renal Activities against Lithium-Chloride Toxicity in Male Albino Rats

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Abstract: Lithium is a toxic alkaline metal that occur in the environment as industrial pollution and therapeutic use. Lithium is widely used in medicine in the treatment of mania and mood disordered, however its administration causes many side effects. The present study aimed to evaluate the protective effect of melatonin and thymoquinone as antioxidants against lithium toxicity. The study is designed five groups of male albino rats (6 for each group): I (Control)- treated with drinking water and food, II (Li)-treated with lithium chloride (LiCl), III-treated with LiCl and melatonin, IV-treated with LiCl and thymoquinone, and V-treated with LiCl combined with melatonin and thymoquinone. The following parameters were determined: red blood cells, hemoglobin, haematocrit, mean corpuscular volume, mean corpuscular heamoglobin concentration, platelets, white blood cells, neutrophils, eosinophils, basophiles, monocytes and lymphocytes. Also, biochemical analysis such as glucose, total proteins, albumin and activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in plasma as well as urea, uric acid, creatinine, cholesterol and triglycerides were determined. Results showed that oral administration of lithium chloride caused a non-significant decrease in RBCs , whereas it caused a significant decrease in hemoglobin (Hb) concentration, haematocrit(Ht), mean corpuscular volume(MCV), mean corpuscular hemoglobin concentration(MCH), eosinophils ,monocytes and lymphocytes, respectively. But, it caused a significant increase in the WBCs , the platelets and neutrophils, relative to the control group. Lithium significantly decreased serum glucose, total protein, albumin, whereas it significantly increased activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, respectively, relative to the control. On the other hand, it caused a significant increase in the plasma levels of urea, uric acid, creatinine, cholesterol and triglycerides, respectively, relative to that of control.
Administration of lithium chloride combined with either melatonin or thymoquinone, or both of them removed the toxic effects of lithium chloride on the above parameters via decreasing and increasing their values. These findings demonstrate that melatonin and thymoquinone may attenuate the toxic effects of lithium on the blood parameters, hepatic and renal functions in the experimental animals.
Keywords: Lithium chloride, Melatonin, Thymoquinone, Toxicity.

1 Introduction

Lithium (Li) has been widely used in the treatment of bipolar disorder. It has received a great deal of attention in many research literature [1]. It is water soluble, not bound with protein and is initially dispersed in all body fluids including plasma and extracellular fluids, then it is Transported and accumulated in some major organs like liver and kidney [1]. Lithium was readily absorbed after oral administration and its peak level was reached in 2-4 hours [2]. It moves slowly from extracellular fluid to intracellular space, and this movement may require 6-8 days to reach steady blood concentration [3]. Lithium compounds have been also studied as an adjuvant in patients with thyroid diseases undergoing radioiodine therapy and in the cure of neurodegenerative disorders [4]. In spite of that lithium has a beneficial action in the

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treatment of many diseases, but it must be used with accurately determined dose to avoid its side effects which causes disturbances in the nervous system, gastrointestinal tract, hepatic, renal and thyroid dysfunctions [5,6]. It has been stated that prolonged treatment with lithium induced haematological changes [7]. It led to a decrease in red blood corpuscles, hemoglobin concentration, haematocrit, mean corpuscular volume [8,9]. Also, lithium induced an increase in white blood cell neutrophils and lymphocyte counts [10] .

It has been reported that the main target organs for lithium are liver and kidney, however the long-term treatments may be resulted in toxic effects on liver and kidney functions [11,12]. Many studies have been demonstrated that lithium had adverse effects on liver and kidney marker enzymes [13,9,11,14,15,16,17]

Lithium toxicity was found to induce oxidative stress via increasing formation of reactive oxygen species in red blood cells, liver and kidney of rats [17,18,19]. So, oral administration of antioxidants such as melatonin and thymoquinone during lithium supplementation may have a protective role against toxic effect of lithium on hematological parameters and hepatorenal functions in rats.

Melatonin is a tryptophan derivative, an essential amino acid in mammals. It was first isolated from bovine pineal gland [20]. Melatonin was found to be a potent free radical scavenger [21], and it can decrease the toxic effect of many environmental and chemical materials which induce oxidative stress. It is stated that the basic function of melatonin is to protect organisms from oxidative stress [22,23,24]

Thymoquinone is the main constituent of the volatile oil isolated from *Nigella Sativa* seed and exerts various pharmacological actions [25]. It is known that thymoquinone has antioxidant effect. Oral supplementation of thymoquinone protected several organs against oxidative *stress induced by toxic metals and chemicals in liver* [26], kidney [27], brain, erythrocytes and heart in mice [28].

Therefore, the present study aimed to evaluate the ameliorative effect of melatonin and thymoquinone against lithium chloride induced hematoxicity, hepatotoxicity and renal toxicity in rats.

2 Materials and Methods

2.1 Chemical Substances

Lithium chloride (LiCl) was purchased from Loba Cheme (India), melatonin(N-acytyl-5-methoxytryptamine) and thymoquinone (2 isopropyl-5-methyl-1,4-benzoquinone) were purchased from sigma chemical (St. Louis, Mo, USA). All the chemicals used were of highly analytical grade.

2.2 Animals

A total of 30 healthy male albino rats (*Rattus rattus*) were obtained from the animal house of the medicine faculty, Assuit university. The animal's weights ranging from 160-180gm, approximately 8-10 weeks old. The experimental animals were housed six per stainless steel cages at room temperature and acclimated to laboratory conditions two weeks before experimentation and fed commercial rat food and water which were available *add labtum*, lightening cycle of 12 hours (light/dark) was also in consideration.

2.3 Experimental Design

After an acclimation period, the animals were randomly divided in to five groups (six/group) and treated with treatments used in this study for 12 weeks. The control group was given drinking water and food. The second group received 20 mg/kg b. wt. of lithium chloride dissolved in water. The third group received 20 mg/kg b. wt. of lithium chloride combined with 10 mg/kg b.wt. of melatonin. The fourth group received 20mg/kg of lithium chloride combined with 10mg/kg of thymoquinone. The fifth group received 20mg/kg of lithium chloride combined with10mg/kg of melatonin and 10 mg/kg b. wt. of thymoquinone. All groups were administrated the treatments orally daily morning, except melatonin was administrated between 09.00-10.00pm. At the end of the treatment period, the animals were anaesthetized with light ether. Blood samples were collected by cardiac puncture in tubes containing anticoagulant (EDTA), and then divided into two parts. The first part was used immediately analyzed for hematological parameters , while the second part was centrifugated at 3000 rpm for 20 minutes to obtain plasma. The obtained plasma was stored at -20^{o}c until use.

2.4 Evaluation of Hematological Parameters

After 12 weeks of treatment, the animals were anaesthetized and the blood samples were collected by cardiac puncture into tubes containing anticoagulant. They were immediately analyzed for determination of following hematological parameters: red blood cells (RBCs), white blood cells (WBCs), hemoglobin (HB), haematocrit (HCT), mean corpuscles volume (MCV), mean corpuscles hemoglobin (MCH), platelets (PLT), neutrophils, eosinophils, monocytes and lymphocytes by using spectrophotometer (Cline check plus, Italy)

2.5 Evaluation of Enzymes Activities

At the end of the experiment (12 weeks), biochemical parameters representing liver function (glucose, total proteins, albumin, triglycerides and activities of blood enzymes: alkaline phosphatase (ALP), aspartate aminotransferase (ALT) was measured in serum. Also, serum levels of urea, uric acid, creatinine and cholesterol (indicator of kidney function) were measured colorimetrically using UV 2300 spectrophotometer (USA).

2.6 Statistical Analysis

The mean ± standard deviation was determined for each studied group. The data was analyzed by one-way

ANOVA. The values were considered significant with $P < 0.05$.

3 Results

At the end of the experimental period, the ameliorative role of melatonin and thymoquinone against lithium toxicity on hematological parameters and the activities of hepatorenal enzymes were evaluated. All the results from various groups have been compared with the normal control group, and also with lithium treated group. All the animal survived till the entire course of the study.

3.1 Hematological Parameters

The results of the current study showed a non-significant decrease in RBCs count, whereas a significant ($P < 0.05$) decrease in hemoglobin, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, white blood cells count, neutrophils, eosinophils, monocytes and lymphocytes volumes after oral administration of lithium chloride for twelve weeks in relative to the normal control group. On other hand, a significant ($P < 0.05$) increase in the platelets and white blood cell counts were observed(Table 1).

Oral administration of melatonin only, thymoquinone and the combination of melatonin with thymoquinone removed the negative effect of lithium chloride on hematological parameters examined. It caused a significant ($P < 0.05$) increase in the lithium decreased hematological parameters and a significant ($P < 0.05$) decrease in the lithium increased parameters (Table 1). It should be noted that a pronounced effect on the hematological parameters was observed after administration of the combination of melatonin and thymoquinone than that of either melatonin or thymoquinone alone, also. Moreover, administration of thymoquinone had a positive effect on most hematological parameters than that of melatonin.

3.2 Hepatic Parameters

Oral administration of lithium chloride caused a significant ($P < 0.05$) decrease in the plasma levels of glucose, total proteins and albumin, while it caused a significant ($P < 0.05$) increase of triglycerides and the activities of ALP, ALT and AST enzymes relative to the normal control group. Administration of melatonin, thymoquinone and melatonin combined with thymoquinone had an opposite effect of lithium chloride. They led to a significant ($P < 0.05$) increase in plasma levels of glucose, total proteins and albumin. But they led to a significant ($P < 0.05$) decrease in plasma level of triglycerides and the activities of ALP, ALT and AST enzymes (Table 2).

3.3 Renal Parameters

The results of the current study showed a significant ($P < 0.05$) increase in the plasma levels of urea, uric acid, creatinine and cholesterol after administration of lithium chloride for 12 weeks, in comparison with the normal control group. Melatonin, thymoquinone and melatonin combined with thymoquinone treatments removed the negative effect of lithium chloride on the renal parameters examined. They caused a significant ($P < 0.05$) decrease in the above parameters (Table 3).

4 Discussions

Lithium salts have been used as a medicine in psychiatry diseases since so long time ago [29, 30]. In spite of its beneficial effects, it may be accompanied by disturbances in nervous system, gastrointestinal tract, disorders of liver, kidney functions [31,32] and changes in the haematological parameters in the experimental animals [8].

There are scarce studies on the correlation between lithium and either melatonin and /or thymoquinone. So, the current study was aimed to evaluate ameliorative effect of melatonin and thymoquinone against lithium toxicity on the haematological parameters and plasma biochemical parameters representing hepatorenal functions.

Lithium chloride administration caused a non-significant decrease in RBCs count which is consistent with that reported by [8]. However, in lithium-treated rats, it was found that lithium caused a marked increase in RBCs [9]. These results disagree with the results of the present study and also with that of [9] who stated that lithium caused a significant decrease in RBCs in rats-oral administration of melatonin and melatonin combined with thymoquinone resulted in a significant increase in the RBCs count.

The present study revealed significant haematological changes as a result of administration of lithium chloride which induced a marked reduction in Hb, Ht, MCV and MCH relative to the control. These results are in agreement with that obtained by [8] and [9] who suggested that the reduction in haematological parameters is due to occurrence of anaemia. This may be due to diminished erythropoietin, reduction in haemoglobin synthesis and elevation in erythrocyte destruction rate of hematopoietic tissues. So, it can be concluded that lithium chloride caused a production of oxidative radicals which reach the cell membrane causing membrane lipid peroxidation [33] and promote damage in erythrocytes [9]. However, administration of thymoquinone and thymoquinone combined with melatonin were able to reactive erythropoiesis mechanism and stimulate the production of erythropoietin. Melatonin and thymoquinone was found to have antioxidant properties able to inhibit li-induced haematological changes.

Table 1: The influence of different treatment of the hematological parameters in rats.

Parameter	control	Lithium Chloride	Lithium Chloride + Melatonin	Lithium Chloride+ Thymoquinone	Lithium Chloride +Melatonin +Thymoquinone
RBCs [10^6/mm^3]	4.52±0.29	4.22±0.25 •	4.39±0.23•	4.43±0.27•	4.81±0.13•
HB [g/dl]	12.8±0.39	11.55±0.216 **	12.56±0.25 •	13.6±0.236 **	13.06±0.45•
HCT [%]	37.4±2.44	35.8±2.08*	37.266±0.84•	38.43±1.011•	39.516±1.799•
MCV [µm^3]	85.84±0.26	85.31±0.37 *	86.24±0.27 •	86.208±0.35 •	86.71±0.289 *
MCH [pg]	28.61±0.087	28.27±0.311*	28.54±0.277**	28.64±0.087 •	28.82±0.168 **
WBCs [10^3/mm^3]	11.95±0.98	13.4±0.31*	12.26±0.44•	13.29±0.77*	13.06±0.762•
PLT [10^3/mm^3]	172.33±17.44	254.67±26.64**	232.33±9.09**	217.5±5.822**	209.67±8.914**
Neutrophils [10^3/mm^3]	18.5±1.76	32.83±7.55*	31.66±4.23**	26.33±3.56**	27.33±2.88**
Eosinophils [10^3/mm^3]	3±0	1.67±0.52 **	2.9±0.17 •	1.66±0.51 **	2.78±0.27 •
Monocytes [10^3/mm^3]	2.33±0.52	1.5±0.55 *	2.95±0.08 •	2.167±0.41•	2.62±0.39•
Lymphocytes [10^3/mm^3]	73.83±2.71	66.33±5.16 *	65.33±3.20 **	72.66±2.16 •	76.66±3.61 *

Table 2: The influence of different treatment on the blood hepatic parameters in rats.

Parameter	control	Lithium Chloride	Lithium Chloride + Melatonin	Lithium Chloride+ Thymoquinone	Lithium Chloride +Melatonin +Thymoquinone
Glucose [mg/dl]	87.33±2.06	74.66±2.58**	75±2.19**	91.16±2.78*	92±3.34*
Albumin [mg/dl]	2.87±0.052	2.42±0.26**	2.9±0.154•	2.73±0.488•	2.66±0.186•
Total proteins [g/dl]	6.67±0.39	5.55±0.30**	6.26±0.34•	6.73±0.78•	6.43±0.33•
Triglyceride [mg/dl]	40±3.34	86.8±1.47**	40.33±3.01•	40±4.604•	39.33±5.046•
Cholesterol [mg/dl]	71.33±7.2	93.83±1.47**	68.83±5.6•	67.66±2.338•	68.16±5.7•
ALP [U/L]	168.66±4.7	182±5.06*	164.83±3.86•	162.16±4.26*	166.17±2.40•
AST [U/L]	26.5±2.88	30.33±1.37*	24.167±4.21•	24.167±1.72•	25.5±2.07•
ALT [U/L]	75.5±6.025	154.83±13.5**	73.67±2.34•	76.17±11.1•	74.83±4.83•

Table 3: The influence of different treatment on the blood renal parameters in rats.

Parameter	control	Lithium Chloride	Lithium Chloride + Melatonin	Lithium Chloride+ Thymoquinone	Lithium Chloride +Melatonin +Thymoquinone
Urea [mg/dl]	34±1.55	74±2.97**	35.33±1.03•	33.83±3.54•	33±1.41•
Uric Acid [mg/dl]	2.85±0.25	4±0.18**	2.65±0.33*	2.916±0.133•	2.816±0.16•
Creatinine [mg/dl]	0.63±0.05	1.56±0.103**	0.566±0.1•	0.616±0.08•	0.63±0.05•

• P 0.05>(Non-significant).
* P <0.05(Significant difference with respect to control group).
** P <0.009(Highly Significant difference with respect to control group).

The present study showed a significant increase in the total count of WBCs, neutrophils and platelets. But it showed a significant decrease in lymphocytes, monocytes and eosinophils in lithium-treated rats. These results are in agreement with that stated in literatures [9,34,35,8,13]. It has been stated that the increases WBCs count, neutrophils and the decreased lymphocytes, monocytes and eosinophils in lithium-treated rats may be due to the phagocytic activity. All of these activities are caused by the stimulation of the immune system of the body [36,37,35]. Also, it is known that lithium stimulates bone marrow and lead to an elevation in leucocytosis [38,39].

Co-administration of melatonin, Thymoquinone , and melatonin plus thymoquinone removed the toxicity of lithium and restored the total count of WBCs, neutrophils, platelets, lymphocytes, monocytes and eosinophils which may confirm the usefulness of antioxidants for ameliorative lithium toxicity. Thus, the co-treatment of melatonin and thymoquinone may be act on immune system and restore the function of bone marrow through removing the toxicity of lithium chloride on blood parameters.

It is known that glucose, albumin, total protein and hepatic markers enzymes such as ALP, ALT and AST, total cholesterol and triglycerides, when decreased or increased can be considered as a sign of liver injury in the experimental animals. In the current study, reduction of glucose, albumin and total proteins in the plasma of lithium-treated rats are indicative of liver damage. Glucose is considered as parameter more vulnerable to the presence of lithium influencing carbohydrate metabolism [8]. Studies also, demonstrated that albumin and total proteins were significantly decreased in lithium-treated rats [8]. So, these results indicate that lithium treatment could affect the protein metabolism of liver via inducing free radicals which is the cause of the changes in glucose and protein metabolism. Additionally, the present study revealed that lithium increased the plasma levels of total cholesterol and triglycerides which could be an indicative of lipid metabolism damage of liver.

Similar results indicated that these changes may be cause a change in the serum lipid profile since lithium is known to cause oxidative stress via producing free radicals in the hepatic cells of the experimental animals [40]. In the present study, treatment with thymoquinone and thymoquinone combined with melatonin normalized the plasma levels of glucose, albumin, total protein, total cholesterol and triglycerides. So, melatonin and thymoquinone may have a protective effect against hepatotoxicity and oxidative stress induces by lithium in rats. Moreover, lithium seemed to have other biochemical defects in liver indicated by elevation in serum levels of hepatic marker enzymes such as, ALP, AST and ALT [4,14,35,8,19,13]. These results are in agreement with the results obtained in the present study. It has been reported that AST and ALT can be used to evaluate the function and integrity of hepatic cells inducing a rise in serum levels [17,8]. Moreover, the elevated of these enzymes indicated the cytotoxic effect induced by lithium in liver which produce free radicals in their metabolic pathways which in turn result in oxidative stress [41]. Co-administration of melatonin, thymoquinone and melatonin combined with thymoquinone restored theplasma levels of ALP, AST and ALT. Again, the treatment with melatonin and thymoquinone could be act as a protective antioxidant against lithium intoxication in the liver of rats.

It is known that urea, uric acid and creatinine measurement in the serum used as indicators of renal functions which may provide an additional information of renal function [42]. The present results revealed a significant rise in the plasma levels of urea, uric acid and creatinine in lithium-treated rats. It has been reported that the elevation of serum urea, uric acid and creatinine resulted from decreased glomerular filtration due to increased tubular reapsorption [43]. The elevation in these parameters is a clear indicator of toxic effect of lithium on the renal function. Several studies performed on rats administrated with lithium carbonate showed an elevation in the plasma urea and creatinine levels, but urinary urea was significantly reduced revealing to an alternation in the kidney function [32,12]. Moreover, altered levels of glucose and urea in lithium-treated rats are indicator of hepatic and renal injuries [17]. Studies revealed that among other things, lithium toxicity can be connected with oxidative stress [44,9]. Treatment with melatonin and thymoquinone as antioxidants

ameliorates the changes in kidney parameters induced by exposure of rat to lithium in the present study. Finally, it should be noted that there are not so much studies regarding the relationships between lithium and both melatonin and thymoquinone.

5 Conclusions

Haematological parameters, plasma glucose level, albumin, total protein, total cholesterol, triglycerides and hepatic markers enzymes (ALP, AST and ALT] in rats exposed to lithium chloride for twelve weeks showed alteration in their levels. Also, plasmurea, uric acid and creatinine which are indicators of kidney functions showed a significant change in lithium-treated rats. Co-administration of melatonin and thymoquinone ameliorate and improve the pathological changes in the haematological parameters and the biochemical parameters representing the hepatorenal functions with the priority for combination. Further studies are needed to clarify the mechanism behind the activity of melatonin and thymoquinone as antioxidants against lithium toxicity.

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