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L- Carnitine mitigates infertility induced by Lithium and Carbamazepine in protein malnourished rats

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Abstract: Male infertility is one of the most important health problems. It can be caused by drugs, especially those used for mental illnesses. Poor nutrition can also affect male fertility as well as mental health. L-carnitine as a natural supplement shows promising effect in preserving male fertility. The purpose of this study was to test whether L-carnitine is protective against lithium and carbamazepine induced male infertility in protein malnourished rats. Forty-eight adult male albino rats were divided in six groups. Lithium and carbamazepine were administered in combination and L-carnitine was given as protective against deteriorating effects on male fertility. This was undergone in both normal feeding and protein malnutrition conditions. Oxidative stress parameter (MDA), testicular testosterone, testicular TNF and gonadosomatic index (GSI) were evaluated. Lithium and carbamazepine administered groups showed significant increase in testicular MDA and marked decrease in testosterone and GSI. Testicular TNF was modestly decreased. L-carnitine administration showed significant reduction in tissue MDA while it showed increase in testosterone and GSI. There was no effect on testicular TNF. Lithium and carbamazepine combination caused decline in male fertility parameters. This decline was more prominent in the protein malnourished animals. L-carnitine caused improvement in male fertility. Its effect was stronger in the normally fed animals.

Keywords: Lithium; Carbamazepine; L-carnitine; Male infertility; Protein malnutrition.

1. INTRODUCTION

Infertility is an important health problem in which 30% is due to male factors, and male infertility alone accounts for approximately 20% of all infertility cases [1]. Male decreased fertility is mainly due to deficits in the semen, and semen quality is used as a measure of male infertility. Diagnosis of infertility is given to a couple who are unable to conceive over the course of one year. When the problem is due to the male partner, it is referred to as male infertility [2].

Free radicals are very reactive molecules derived from oxygen; they are also called reactive oxygen species (ROS). They have unpaired electrons in the outer orbital of valency. They contain radicals with oxygen in the center (hydroxyl, nitric oxide and superoxide anion radicals) and non-radicals (hydrogen peroxide, peroxynitrite, and hypochlorous acid) [3]. ROS are beneficial in signaling of the cells and in homoeostasis. They are generated in sperm cells by small quantities and provide important functions as initiation of capacitation of the sperms, controlling sperm maturation, and modulating cellular signaling pathways [4]. But when levels of ROS increase more than normal, they exert paradoxical effects on sperm function, leading to infertility. These effects are seen in highly damaged DNA and lipid peroxidation in seminal fluid [5]. Excessive ROS amounts or failure of antioxidant activity, causes disruption to the equilibrium between oxidation and reduction, resulting in oxidative stress (OS). Spermatozoa are highly sensitive to OS, being poor in enzymatic

antioxidants, so there is no sufficient protection for the sperm against high ROS levels. Moreover, the sperm cell's plasma membrane contains extremely high amounts of polyunsaturated fatty acids, such as docosahexaenoic acid, attracting ROS-induced oxidation reactants [6].

Malnutrition is one from the several causes of infertility in men. Excessive exercising and severe dietary restrictions have negative effect on sperm count and sperm motility which can lead to reduction or even stopping sperm production. Caloric restriction or ingestion of non-nutritious food can cause deficiencies in many of the hormones and vitamins which are essential to sperm production [7]. It was proved that the quantity of dietary protein impacts the hypothalamic-pituitary-gonadal axis and sub-optimal amount reduces the potential of male fertility in both humans and animals [8].

The most common mental disorders that are prevalent nowadays in numerous countries are bipolar disorder, obsessive-compulsive disorder (OCD), schizophrenia, and depression. The pattern of dietary intake of the general population in some countries reflects that they are often deficient in nutrients, especially proteins, omega-3 fatty acids, essential vitamins and minerals [9]. A closer look at the diet of mentally ill individuals reveals an interesting observation, that their eating pattern is far from healthy and inadequate. They usually select types of foods that may contribute to depression due to their poor food choices [10].

Bipolar disorder is a chronic, recurrent disease of the mood that affects 1–2 % of people [11]. The common treatment for managing its symptoms is medication, usually mood stabilizers. Upon studying lithium, it was proven to be effective in treating both the manic and depressive episodes of bipolar disorder. Furthermore, its mood stabilizing properties can prevent manic episodes in patients taking antidepressants [12]. In moderate to severe manic episodes, adding another medication such as carbamazepine is usually necessary [13].

L-Carnitine is an endogenous substance with an important role in the metabolism of fatty acids. It is biosynthesized from the substrates L-lysine and L-methionine amino acids, inside the human body. L-Carnitine is present in many types of foods, especially red meats, such as beef and lamb. Other good sources for L-carnitine include fish, poultry and milk [14]. An important aspect is the high concentration of L-carnitine found in the male reproductive tract, suggesting its essential role in energy metabolism and in the spermatozoa maturation [15]. Also, a finding of particular interest is the anti-apoptotic effect of L-carnitine, which is explained by the inhibition of programmed cell death mediated by the FAS and FAS ligand and the caspases 3, 7, and 8 [16].

The aim of the present study is to examine whether Lithium and Carbamazepine in combination have detrimental effects on male fertility parameters, especially in protein malnourished male rats.

2. METHODS

2.1. Animals

Forty-eight adult male albino rats were obtained from the animal house of the Egyptian Drug Act (EDA), with an initial weight of 150-200 gm at the beginning of the experiment. All the undergone procedures followed the animal ethical guidelines and were approved by the Committee of Control and Supervision of experiments on Animals, Government of Egypt. (Permit number: NODCAR/I/25/2021).

2.2. Experimental design

Rats were divided at random into two main groups, named Group I and Group II, of 24 animals each. Animals in Group I were normally fed while animals in group II received isocaloric protein-poor (8% casein) diet over 35 days. Each main group was subdivided into three subgroups of 8 rats each, as follows: Group I: Normally fed rats; they were subdivided into three subgroups: Group 1: Control, Group 2: Lithium + Carbamazepine administered, Group 3: Lithium + Carbamazepine + Carnitine administered. Group II: Protein malnourished rats; they were subdivided into three subgroups: Group 4: Protein malnourished, Group 5: Protein malnourished + Lithium+ Carbamazepine administered, Group 6: Protein malnourished + Lithium+ Carbamazepine +Carnitine administered. (The main groups are given the roman numerical while the subgroups are given the Arabic numerical).

2.3. Preparation and doses of drugs

All treatments were administered orally by gavage once daily for a period of 35 days. Lithium Chloride was administered orally in a dose of 1 mmol/kg daily dissolved in distilled water [17].

Carbamazepine was administered orally in a dose of 18 mg/kg daily dissolved in 2% gum acacia [18]. L-carnitine was administered in a dose of 100 mg/kg daily dissolved in distilled water [19].

2.4. Preparation of tissue samples

Rats were sacrificed and a longitudinal incision was made in the skin of scrotum and both testes were exposed and dissected out and weighed, and relative weights of both were calculated. Both testes were homogenized in nine volumes of ice cooled buffered 0.9% saline solution using Teflon motor driven homogenizer (USA). The homogenate was then centrifuged at a speed of 10,000 rpm for 30 minutes at a temperature of 4°C using Sigma 3K30 cooling centrifuge (Germany) and the supernatant was used for tissue parameters assessment (MDA, TNF and Testosterone).

2.5. Preparation of blood samples

Twenty-four hours after the last gavage, blood samples were withdrawn by puncture of retro-orbital plexus of veins in the eye using microcapillary tubes [20]. The samples were kept standing for 15 minutes to clot then centrifuged at 10,000 rpm for 30 minutes at 4°C using Sigma 3K30 cooling centrifuge (Germany) and serum was collected in nonheparinized test tubes. Thereafter, serum was collected from each test tube into clean sample tubes, which kept frozen at −70 °C. Then were subsequently used for the evaluation of serum biochemical parameters (Albumin and Glycogen).

2.6. Protein malnutrition model

The standard diet was given to the rats according to *Bamji and Sharada* (1972) [21] and *Anthony and Edozien* (1975) [22]. The low protein diet was composed of the same constituents of the standard diet except for the casein content reduced to 8g and the sucrose content raised to 82 g / 100 g food.

2.7. Parameters measured

2.7.1. Total thiobarbituric acid reactive substances (TBARS)

The TBARS content known as malondialdehyde (MDA) [23] is determined colorimetrically, by reacting 1 molecule of malondialdehyde (MDA) with 2 molecules of thiobarbituric acid TBA at temperature 95 ° C and PH 2-3 for 45 minutes. The pink pigment produced is extracted by n-butanol, then its optical density is measured at wavelength between 535 nm and 520 nm. The difference in optical density is used to calculate the TBARS value.

2.7.2. Testicular testosterone

The kit used is *Cusabio testosterone* ELISA kit (CSB-E05100r). This assay uses an immunoassay technique based on the enzymatic competitive inhibition. The provided microtiter plate is pre-coated with goat-anti-rabbit antibody. The standards or samples are then put into the corresponding microtiter plate wells with the specific antibody for testosterone and conjugated testosterone with Horseradish Peroxidase (HRP). The competitive inhibition occurs between HRP conjugated testosterone and unconjugated testosterone with the antibody. Then a solution of a substrate is added to the wells and the developed color corresponds to the amount of testosterone in the sample. The color intensity is measured.

2.7.3. Testicular tumor necrosis factor-alpha (TNF)

The kit used is *Rat TNF-alpha ELISA Kit (RK00029)*. This assay applies a quantitative immunoassay technique which uses the sandwich enzyme. A microplate has been coated by a monoclonal antibody which is specific for tumor necrosis factor (TNF)-alpha. Wells are filled by standards and samples and the antibody binds the TNF- alpha present. After incubation a wash step is done to remove unbound samples, and then a specific antibody to the TNF-alpha is added to the wells to detect and bind to the combination of capture antibody-TNF-alpha in the sample. Any unbound combination is then removed by a wash step, followed by adding enzyme conjugate to the wells. After that a substrate is added. The resulting-colored product corresponds to the quantity of TNF-alpha found in the sample. An acid is then added to stop the reaction and absorbance is measured. From the standard dilutions a standard curve is plotted, and TNF-alpha sample concentration determined.

2.7.4. Gonadosomatic index (GSI)

After rats were sacrificed, a longitudinal incision was made in the scrotum and both testes were dissected out and weighed, and relative weights to the total body weight were calculated. The gonadosomatic index (GSI) is calculated by the percentage of total body weight relative to the testicular weight. [GSI = (testicular weight/total body weight) × 100] [40].

2.7.5. Serum albumin

The kit used is Rat serum albumin ELISA kit (SK00383-02) for the quantitative determination of rat albumin concentrations in serum and plasma. The Rat Albumin ELISA kit is based on the binding of rat albumin in samples to two antibodies. One has been pre-coated onto a microplate, and the other is biotinylated. Standards and samples are pipetted into the wells and any albumin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for albumin is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of albumin bound in the initial step. The color development is stopped, and the intensity of the color is measured.

2.7.6. Serum glycogen

The kit used is Cell Biolabs' Glycogen Assay Kit (Colorimetric) MET-5022. Cell Biolabs' Glycogen Assay Kit measures total glycogen within biological samples. Glycogen is broken down into glucose monomers by amyl glucosidase first, glucose is then oxidized by glucose oxidase into D-gluconic acid and hydrogen peroxide. The resulting hydrogen peroxide is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of glycogen standard within the 96-well microtiter plate format. Samples and standards are incubated for 45 minutes and then read with a standard 96-well colorimetric plate reader.

2.8. Statistical analysis of parametric responses

The data obtained were expressed as mean ± SEM of eight animals and were statistically analyzed at $P < 0.05$ (results with $P < 0.05$ are considered statistically significant) using statistical package for social science (SPSS), version 19. The yield of studied tools has been determined by Wilsons's score method. A computer program (*Microsoft Excel 2019*) was used to construct the bar charts and the curves for presentation of all parameters. Groups had been compared by samples (t) test, according to type of data.

3. RESULTS

3.1. Malondialdehyde (MDA) measurement

Lithium and carbamazepine administration caused significant increase in testicular MDA by 429.4 % as compared to control group. L-carnitine administration along with lithium and carbamazepine caused significant decrease in testicular MDA by 78.75% as compared to lithium + carbamazepine, although there is still some increase by 12.5 % as compared to control group. In the protein malnourished model, lithium and carbamazepine administration caused significant increase in testicular MDA by 36.25 % as compared to lithium and carbamazepine administration to the normally fed rats. L-carnitine administration along with lithium and carbamazepine to the protein malnourished rats caused significant decrease in testicular MDA by 64.2 % as compared to lithium + carbamazepine in the protein malnourished rats although there is still marked increase by 129.4 % as compared to L-carnitine administration along with lithium and carbamazepine to the normally fed rats.

Table 1. Effect of L-carnitine on testicular content of TBARS in lithium and carbamazepine administered rats receiving standard and low protein diet.

Group	G1/ Control	G4/ M+ Control	G2 L+Cb	G5 M+ L+Cb	G3 L+Cb+Cn	G6 M+ L+Cb+Cn
MDA (nmol/g)	13.6±2.6	33.1±6.9^{c}	72±7.9^{a}	98.1±18.2^{c}	15.3±2.3^{b}	35.1±6.9^{c}

-Values are mean ±SEM, n = 8.

-a or b: indicate a significant difference from control group (G 1), and Lithium & Carbamazepine group (G 2) respectively at P,0.05 using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at p<0.05 using statistical package for social science (SPSS), version 19.

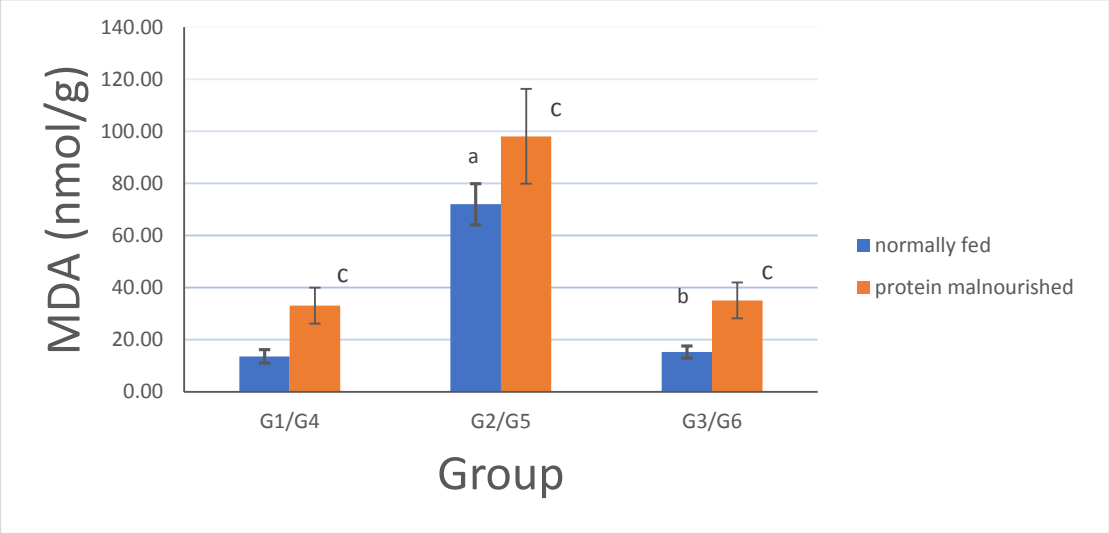

Figure 1. Effect of L-carnitine on testicular content of TBARS in lithium and carbamazepine administered rats receiving standard and low protein diet.

-Values are mean ±SEM, n = 8.

-a or b: indicate a significant difference from control group (G1), and Lithium & Carbamazepine group (G 2) respectively at p<0.05 using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at p<0.05 using statistical package for social science (SPSS), version 19.

3.2. Testosterone measurement

Lithium and carbamazepine administration caused significant decrease in testicular testosterone by 50.2% as compared to control group. L-carnitine administration along with lithium and carbamazepine caused significant increase in testicular testosterone by 84.3% as compared to lithium + carbamazepine, although there is still some decrease by 8.2% as compared to control group (Group 1). In the protein malnourished model, lithium and carbamazepine administration caused significant decrease in testicular testosterone by 17.1% as compared to lithium and carbamazepine administration to the normally fed rats. L-carnitine administration along with lithium and carbamazepine to the protein malnourished rats caused significant increase in testicular testosterone by 98.1% as compared to lithium + carbamazepine in the protein malnourished rats, although there is still decrease by 10.9% as compared to L-carnitine administration along with lithium and carbamazepine to the normally fed rats.

Table 2. Effect of L-carnitine on testicular testosterone in lithium and carbamazepine administered rats receiving standard and low protein diet.

Group	G1/ Control	G4/ M+ Control	G2 L+Cb	G5 M+ L+Cb	G3 L+Cb+Cn	G6 M+ L+Cb+Cn
Testosterone (ng/g)	39.8±3.1	34.6±2.3^{c}	19.8±2.5^{a}	16.4±2.7^{c}	36.5±2.6^{b}	32.5±2.2^{c}

-Values are mean ±SEM, n = 8.

-a or b: indicate a significant difference from control group (G), and Lithium & Carbamazepine group (G 2) respectively at P,0.05 using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at p<0.05 using statistical package for social science (SPSS), version 19.

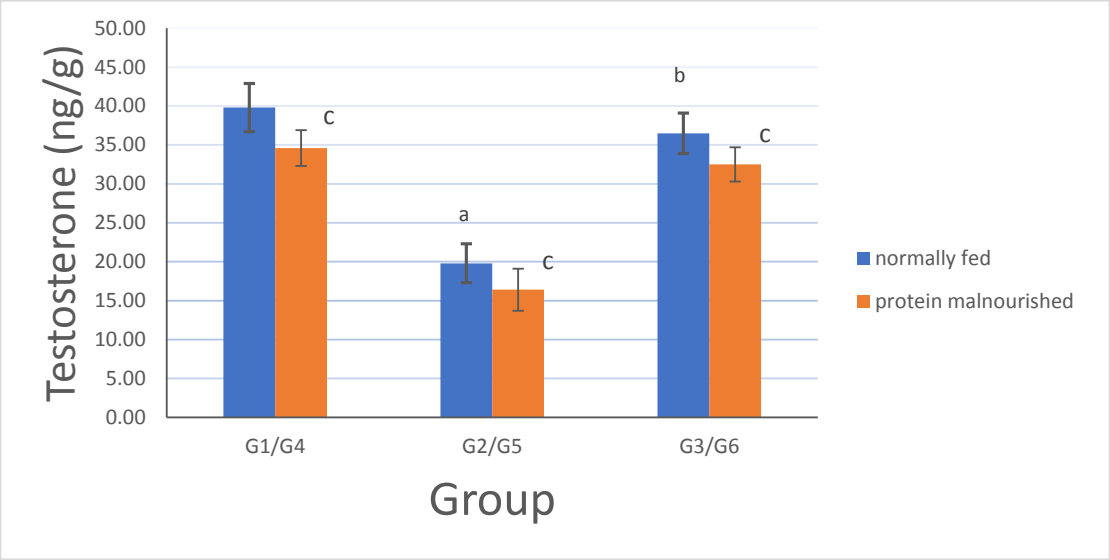

Figure 2. Effect of L-carnitine on testicular testosterone in lithium and carbamazepine administered rats receiving standard and low protein diet.

-Values are mean ±SEM, n = 8.

-a or b: indicate a significant difference from control group (G1), and Lithium & Carbamazepine group (G 2) respectively at $p<0.05$ using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

3.3. TNF measurement

Lithium and carbamazepine administration resulted in slight decrease in testicular TNF by 8.6% as compared to control group. L-carnitine administration didn't cause significant effect on testicular TNF. In the protein malnourished model, lithium and carbamazepine administration resulted in significant increase in testicular TNF by 49.1% as compared to lithium and carbamazepine administration to the normally fed rats. L-carnitine administration along with lithium and carbamazepine to the protein malnourished rats didn't cause significant effect on testicular TNF, although there is still marked increase by 52.8% as compared to L-carnitine administration along with lithium and carbamazepine to the normally fed rats.

Table 3. Effect of L-carnitine on testicular TNF-alpha in lithium and carbamazepine administered rats receiving standard and low protein diet.

Group	G1/ Control	G4/ M+ Control	G2 L+Cb	G5 M+ L+Cb	G3 L+Cb+Cn	G6 M+ L+Cb+Cn
TNF (pg/g)	18.5±2.5	28.8±2.5^{c}	16.9±2.27^{a}	25.2±2.9^{c}	17.4±2.25	26.6±2.34^{c}

-Values are mean ±SEM, n = 8.

-a: indicate a significant difference from control group (G1) at $p<0.05$ using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

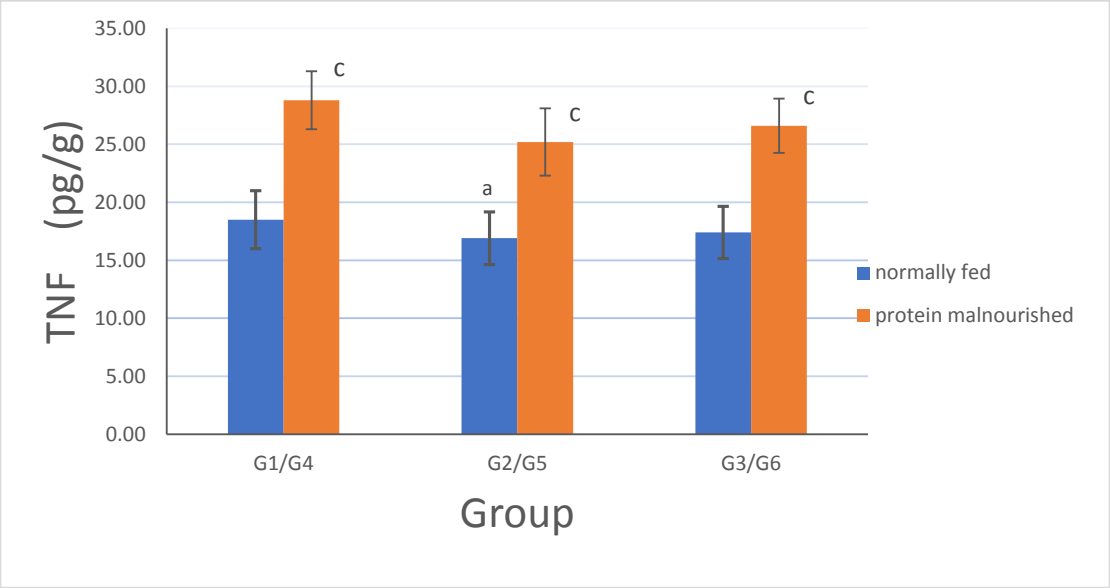

Figure 3. Effect of L-carnitine on testicular TNF in lithium and carbamazepine administered rats receiving standard and low protein diet.
-Values are mean ±SEM, n = 8.
-a: indicate a significant difference from control group (G1) at $p<0.05$ using statistical package for social science (SPSS), version 19.
-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

3.4. GSI measurement

Lithium and carbamazepine administration resulted in significant decrease in GSI by 56.7% as compared to control group. L-carnitine administration along with lithium and carbamazepine resulted in significant increase in GSI by 103.2% as compared to lithium + carbamazepine, although there is still marked decrease by 12% as compared to control group. In the protein malnourished model, lithium and carbamazepine administration resulted in significant decrease in GS by 32.7% as compared to lithium and carbamazepine administration to the normally fed rats. L-carnitine administration along with lithium and carbamazepine to the protein malnourished rats resulted in significant increase in GSI by 134.1% as compared to lithium + carbamazepine in the protein malnourished rats, although there is still marked decrease by 22.5% as compared to L-carnitine administration along with lithium and carbamazepine to the normally fed rats.

Table 4. Effect of L-carnitine on GSI in lithium and carbamazepine administered rats receiving standard and low protein diet.

Group	G1/ Control	G4/ M+ Control	G2 L+Cb	G5 M+ L+Cb	G3 L+Cb+Cn	G6 M+ L+Cb+Cn
GSI (g/kg)	1.41±0.1	1.1±0.1^{c}	0.61±0.11^{a}	0.41±0.08^{c}	1.24±0.11^{b}	0.96±0.11^{c}

-Values are mean ±SEM, n = 8.
-a or b: indicate a significant difference from control group (G1), and Lithium & Carbamazepine group (G 2) respectively at $p<0.05$ using statistical package for social science (SPSS), version 19.
-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

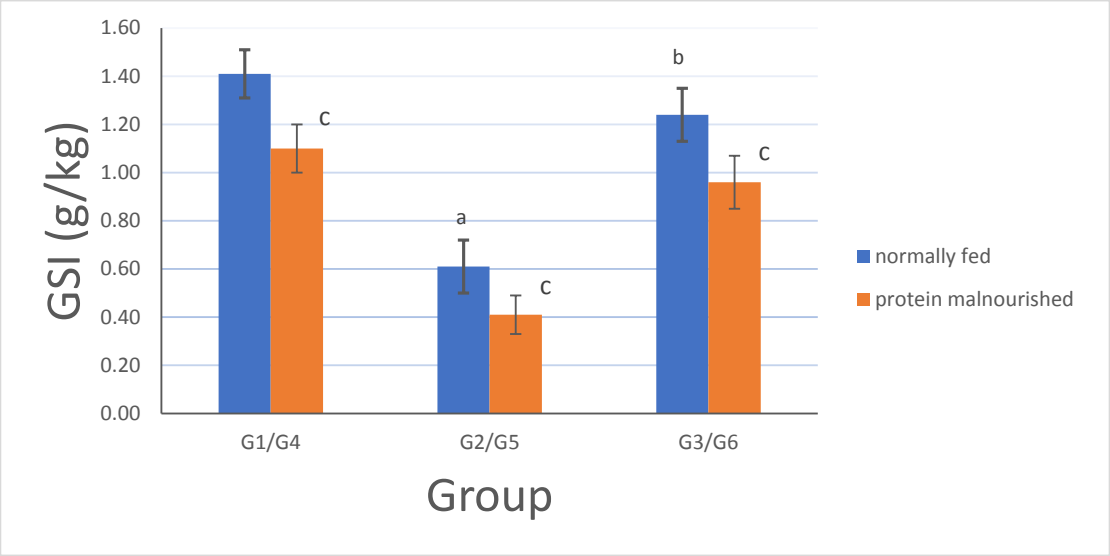

Figure 4. Effect of L-carnitine on GSI in lithium and carbamazepine administered rats receiving standard and low protein diet.

-Values are mean ±SEM, n = 8.

-a or b: indicate a significant difference from control group (G1), and Lithium & Carbamazepine group (G 2) respectively at $p<0.05$ using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

3.5. Serum albumin measurement

Lithium and carbamazepine administration caused significant decrease in serum albumin by 22.8% as compared to control group. L-carnitine administration along with lithium and carbamazepine caused significant increase in serum albumin by 20% as compared to lithium + carbamazepine, although there is still some decrease by 7.3% as compared to control group. In the protein malnourished model, lithium and carbamazepine administration caused significant decrease in serum albumin by 28.7% as compared to lithium and carbamazepine administration to the normally fed rats. L-carnitine administration along with lithium and carbamazepine to the protein malnourished rats caused significant increase in serum albumin by 23.3% as compared to lithium + carbamazepine in the protein malnourished rats, although there is still marked decrease by 26.7 % as compared to L-carnitine administration along with lithium and carbamazepine to the normally fed rats.

Table 5. Effect of L-carnitine on serum albumin in lithium and carbamazepine administered rats receiving standard and low protein diet.

Group	G1/ Control	G4/ M+ Control	G2 L+Cb	G5 M+ L+Cb	G3 L+Cb+Cn	G6 M+ L+Cb+Cn
Albumin (g/dl)	4.2±0.37	2.97±0.18^{c}	3.24±0.14^{a}	2.31±0.28^{c}	3.89±0.27^{b}	2.85±0.16^{c}

-Values are mean ±SEM, n = 8.

-a or b: indicate a significant difference from control group (G1), and Lithium & Carbamazepine group (G 2) respectively at $p<0.05$ using statistical package for social science (SPSS), version 19.

-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

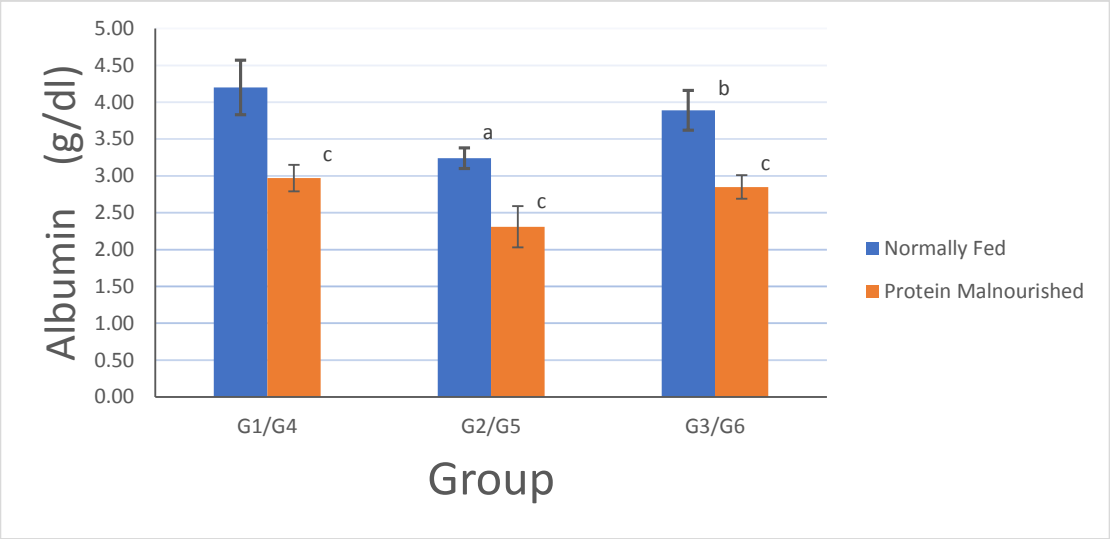

Figure 5. Effect of L-carnitine on serum albumin in lithium and carbamazepine administered rats receiving standard and low protein diet.
- Values are mean ±SEM, n = 8.
-a: indicate a significant difference from control group (G1) at $p<0.05$ using statistical package for social science (SPSS), version 19.
-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

3.6. Serum glycogen measurement

Lithium and carbamazepine administration caused significant decrease in serum albumin by 33.9% as compared to control group. L-carnitine administration along with lithium and carbamazepine caused significant increase in serum glycogen by 39.8% as compared to lithium + carbamazepine, although there is still some decrease by 7.6% as compared to control group. In the protein malnourished model, lithium and carbamazepine administration caused significant decrease in serum glycogen by 35.5% as compared to lithium and carbamazepine administration to the normally fed rats. L-carnitine administration along with lithium and carbamazepine to the protein malnourished rats caused significant increase in serum glycogen by 51.1% as compared to lithium + carbamazepine in the protein malnourished rats, although there is still marked decrease by 30.3% as compared to L-carnitine administration along with lithium and carbamazepine to the normally fed rats.

Table 6. Effect of L-carnitine on serum glycogen in lithium and carbamazepine administered rats receiving standard and low protein diet.

Group	G1/ Control	G4/ M+ Control	G2 L+Cb	G5 M+ L+Cb	G3 L+Cb+Cn	G6 M+ L+Cb+Cn
Glycogen (g/dl)	4.18±0.4	2.99±0.2^{c}	2.76±0.28^{a}	1.78±0.27^{c}	3.86±0.27^{b}	2.69±0.16^{c}

-Values are mean ±SEM, n = 8.
-a or b: indicate a significant difference from control group (G1), and Lithium & Carbamazepine group (G 2) respectively at $p<0.05$ using statistical package for social science (SPSS), version 19.
-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

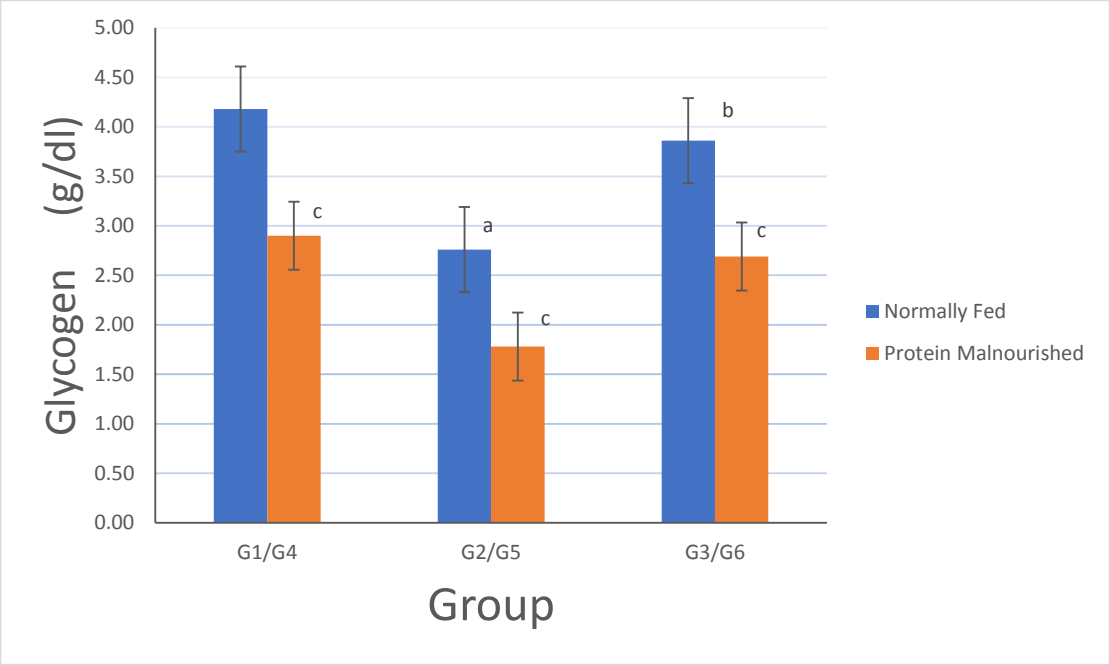

Figure 6. Effect of L-carnitine on serum glycogen in lithium and carbamazepine administered rats receiving standard and low protein diet.
-Values are mean ±SEM, n = 8.
-a: indicate a significant difference from control group (G1) at $p<0.05$ using statistical package for social science (SPSS), version 19.
-c: indicate a significant difference between normally fed group and its corresponding protein malnourished one at $p<0.05$ using statistical package for social science (SPSS), version 19.

4. DISCUSSION

In this study, lithium ion (Li+) and carbamazepine were studied as they are among the drugs affecting sperm parameters negatively and reducing male fertility. The lithium ion is an effective drug known for treating bipolar disorder. Lithium decreases the activity of hypothalamic-pituitary-gonadal axis, and spermatogenesis-stimulating hormones [24]. On another hand, Carbamazepine can cause various hormonal abnormalities; particularly, due to its hepatic enzyme inducing property, which leads to increase in serum testosterone binding globulin concentrations. This increase causes diminished testosterone bioactivity, resulting in diminished potency and thus reduced fertility [25]. It was proved by many studies that the amount of protein in the diet can impact the hypothalamic-pituitary-gonadal axis and lower than average quantity decreases male fertility in both humans and animals [26]. The present study showed that both lithium and carbamazepine have detrimental effects on male fertility parameters, especially in protein malnourished male rats.

Currently, much evidence links oxidative stress to male infertility. This study concluded the lithium and carbamazepine-induced oxidative stress in testicular tissue, expressed by lipid peroxidation through elevated malondialdehyde. This effect was more severe in the protein malnourished rats. These results came in accordance with those of *Saad et al*. (2017) [27] who found that lithium carbonate exposure markedly elevated the level of lipid peroxidation expressed as malondialdehyde and lowered superoxide dismutase, glutathione peroxidase, and catalase activities in testes. *Akorede et al.* (2020) [28] proved the same in their study regarding carbamazepine, mentioning that high amounts of malondialdehyde were found in the testes and the pituitary gland of carbamazepine exposed animals. This high concentration of malondialdehyde might be due to reactive oxygen species activity provoking high oxidative challenge in carbamazepine treated animals.

Also, lithium and carbamazepine administration lowered testicular testosterone markedly, which was more reduced in the protein

malnourished groups. This comes in accordance with *Garacia and Fuentes* (1995) [29] work, which on a 35-day injection of lithium showed destruction of Leydig cells and reduction of plasma testosterone level. In *Joshi et al.* (2007) [30] work, there was a relatively lower testosterone level in the carbamazepine treated groups as compared to the control. This decrease in the concentration of serum testosterone in the carbamazepine treated animals can be linked to the carbamazepine inhibitory effect on the secretion of pituitary gonadotropins (FSH and LH), which have a role in testosterone biosynthesis. Similarly, the decrease in testosterone level seen in the group treated with carbamazepine may be due to direct damage to Leydig cells [31].

On the contrary, lithium and carbamazepine administration showed insignificant effect on tumor necrosis factor-alpha, it showed slight decrease, this may be due to the anti-inflammatory effects of lithium that are responsible of its therapeutic efficacy. The studies of *Nassar and Azab* (2014) [32] agree with this, where they examined lithium effects on pro- and anti-inflammatory mediators. They suggested that lithium has anti-inflammatory properties such as suppressing the expression of the enzyme cyclooxygenase-2, inhibiting the production of tumor necrosis factor-α and interleukin-1β, and enhancing the synthesis of interleukin-2 and interleukin-10. The study performed by *Zhou et al.* (2018) [33], revealed that carbamazepine in combination with vitamin B12 was effective in treatment of epilepsy by reducing serum levels of TNF-α and C-reactive protein.

In this study, lithium and carbamazepine caused significant reduction in reduced glutathione, in both normally fed and protein malnourished rats. This comes in accordance with *Saad et al.* (2017) [27] work, who studied the lithium induced damages in testes of male rats and found it to be negatively affecting the testicular weight than in controls. Similarly, according to *Akorede et al.* (2020) [28] study, which proved that prolonged carbamazepine usage causes decrease in testicular weight and alteration in histology.

This study is concerned about L-carnitine, an amino acid that is biologically active, it is mainly found in tissues that consume high amounts of energy such as skeletal and cardiac muscles. It is also present in organs of reproduction such as the testis and epididymis [34]. The present study showed that the treatment with L-carnitine (100 mg/kg/day) caused marked protection against lithium and carbamazepine induced testicular injury, in both normal feeding and protein malnutrition conditions. This comes in accordance with the study done by *Abd El-Emam and Ahmed* (2021) [35], where they studied the improving effect of L-carnitine on reproductive damage induced by administering lead chronically in male rats and found that L-carnitine co-administration significantly ameliorated sperm parameters, lowered oxidative stress of the testicular tissue, elevated the level of the hormones FSH, LH and testosterone in the serum without affecting the testes weight.

Oxidant status in rat testis after L-carnitine administration was examined through measuring the malondialdehyde content of the testicular tissue. L-carnitine improved it by reducing malondialdehyde after lithium and carbamazepine administration, especially the normally fed group. This obeys *Salem et al.* (2021) [36] who found that L-carnitine effects are mainly through preserving the endocrine functions by its anti- inflammatory and antioxidant properties by a plenty of entangled signal transductions that summarized its beneficial effects on steroidogenesis and spermatogenesis.

Also, this study illustrated that after treatment with the L-carnitine, the level of testosterone was significantly elevated. Researchers who previously worked on this subject confirmed the present result. As seen in the study of *Shi et al.* (2012) [37] who have investigated the effect of L-carnitine on sex hormones in patients with diabetes. Their work indicated that the levels of FSH, LH, and testosterone in the serum were markedly lowered but L-carnitine reversed this. Moreover, *El-Damarawi and Salama* (2014) [38] who studied disorders of infertility in patients with obesity, showed that L-carnitine had positive impact on the serum levels of testosterone, FSH and LH hormones.

The present study showed that L-carnitine administration had a significant positive effect on gonadosomatic index, causing it to increase after lithium and carbamazepine administration, whether in normally fed or protein malnourished groups. Its effect was stronger in normal feeding conditions. This agrees with *Abo-Ghanema et al.* (2012) [39] study which reported that the gonadosomatic index (the testicular average weight in relation to the body weight) showed significant increase upon L-carnitine administration. This may be explained by assuming that the antilipidemic and cholesterol

lowering properties of L-carnitine treatment can be more effective in reducing the body weights in relation to the higher testicular weights in these groups.

According to *Elzanaty et al.* (2007) [41], seminal plasma albumin is directly associated to sperm morphology, which makes it an important marker for male fertility. The present study showed that lithium and carbamazepine administration significantly decreased serum albumin. These results go hand in hand with those obtained by *Małgorzata et al.* (2014) [42] who found in his study that albumin level was decreased in Li-receiving animals. Also, the results of this study obey those obtained by *Mohammad et al.* (2011) [43], who concluded that lithium has the potential of inducing adverse effects in the rat blood (decreased serum albumin concentration and oxidative stress in the rat RBCs also).

Also, in our study there was a marked statistically significant decrease in serum albumin concentration in all subgroups of protein malnourished rats when compared with their identical ones in normally fed group. This means a negative side effect of protein malnutrition. These results coincide with those obtained by *Qureshi and Qureshi* (2001) [44], who concluded that Protein malnutrition is reflected from the decrease in the serum albumin levels of the animals and their weights are also decreased significantly. *Alaverdashvili et al.* (2015) [45] also concluded that protein-energy malnutrition (PEM) results in marked decrease in serum albumin.

Glycogen seems to play a pivotal role within the testicles, particularly during testicular development, where it acts as modulator of germ cell survival, this was proved in *Villarroel-Espíndola et al.* (2013) [46] study. The rate-limiting enzyme in glycogen synthesis is glycogen synthase kinase (GSK). GSK activity and glycogen synthesis in testis could be regulated and a disruption of this process may be responsible for the apoptosis and degeneration of seminiferous tubules and possible cause of infertility [46].

In this study, lithium and carbamazepine caused marked decrease in serum glycogen, in both normally fed and protein malnourished rats, with more decrease in the malnourished ones. This comes in accordance with *Toghyani et al.* (2013) [47] who stated that lithium is considered as a strong ATP production blocker by inhibiting GSK3 enzyme in the glycogen pathway. The strong GSK3 adjusts phosphorylation of glucose metabolism enzymes and its inhibitory effect on severe reduction of flagellar movements is remarkable.

In summary, the findings of this study provide substantial evidence that protein malnutrition has a negative impact on male fertility. L-carnitine, particularly at a dose of 100 mg/kg, decreases the side effects due to lithium and carbamazepine administration. It can decrease the levels of reactive oxygen species through reducing the oxidative stress parameters, such as malondialdehyde, which in consequently modulates the levels of testosterone. Also, it preserved testicular tissue by keeping the gonadosomatic index near normal values. Despite these effects, low protein diet hindered L-carnitine from exerting its full effect as in normal feeding conditions.

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