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RESEARCH ARTICLE

Investigating the Protective Role of L-carnitine and Thymoquinone against Methotrexate-Induced Testicular Damage in Rats

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ABSTRACT

The research aimed to investigate whether the combination of L-carnitine (LC) and thymoquinone (TQ) could lower the adverse effects of methotrexate (MTX), a widely used chemotherapy agent in cancer therapy, on testicular function. A total of seven groups were established (n=7); control group, LC (200 mg/kg PO), TQ (50 mg/kg PO), MTX (single IP dose of 20 mg/kg), LC+MTX, TQ+MTX, and LC+TQ+MTX. The use of MTX resulted in lower levels of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in the blood, accompanied by a significant rise in testicular MDA levels. Additionally, there was a notable decline in GSH, CAT, and SOD, indicating reduced antioxidant activity in the testicles. Furthermore, the histopathological examination of the testicles revealed disruptions caused by MTX, and the expression of caspase-3 in the testicles was documented. The combination of LC and TQ with MTX significantly increased testosterone, FSH, LD levels and restored oxidative damage markers ($p<0.05$) and improved histopathological scores and caspase-3 expression. In conclusion, our results demonstrate that LC and TQ exhibit substantial protective effects against testicular toxicity induced by MTX, primarily attributed to their antioxidant properties.

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INTRODUCTION

Anticancer medications not only target tumor cells but also impact other cells, resulting in comparable adverse effects on both abnormal and healthy cells (Granados-Principal *et al*., 2010). Methotrexate (MTX) is a commonly used cytotoxic chemotherapy drug for treating specific types of cancer (Zhang *et al*., 2022, Behairy *et al*., 2024), but it can cause various side effects when administered over short or prolonged periods (Alahmadi and Abduljawad, 2021). Methotrexate-induced testicular damage through apoptotic cell death has been extensively explored in numerous studies (Maremanda and Jena, 2017). Previous investigations have reported the occurrence of testicular toxicity caused by methotrexate (Abdul-Hamid *et al*., 2023; Hassanein *et al*., 2023).

L-carnitine (LC) is a naturally existing substance crucial for bioenergetic functions, playing a significant role in creating acyl carnitine esters from long-chain fatty acids (Sallam *et al*., 2021). L-carnitine is a potent antioxidant present in elevated concentrations in the epididymis, playing a crucial role in spermatogenesis, sperm maturation, and metabolism (Agarwal and Said, 2004). Several studies have pointed out the positive therapeutic impacts of LC in reducing testicular toxicity (Aktoz *et al*., 2017; Abdel-Emam and Ahmed, 2021; Sallam *et al*., 2021).

Thymoquinone (TQ) is the primary active compound found in *Nigella sativa*. It demonstrates a wide spectrum of biological and therapeutic activities (anti-inflammatory, antioxidant, antiapoptotic, and anticancer properties (Abdo *et al*., 2021; Phua *et al*., 2021). Due to its ability to scavenge free radicals, thymoquinone (TQ) demonstrates significant antioxidant potential (Abdel-Daim *et al*., 2020; Ostadpoor and Gholami-Ahangaran, 2021). Moreover, TQ has been shown to exhibit protective effects on the testes against damage induced by cadmium (Fouad and Jresat, 2015), lead (Hassan *et al*., 2019), valproic acid (Savran *et al*., 2020), and cyclophosphamide (Adana *et al*., 2022).

To mitigate testicular toxicity induced by DOX, it is advised to consider approaches such as employing compounds with antioxidant or anti-inflammatory properties. Hence, combining agents with the drug could potentially offer a more efficient strategy for mitigating tissue damage associated with the medication (Kabir *et al*., 2021; Elsayed *et al*., 2024). In this regard, to enhance treatment efficacy, it is recommended to administer these agents in conjunction with substances capable of reducing drug-induced harm to the intended target and/or other organs (Aboubakr *et al*., 2023b).

This study assessed the effectiveness of LC and/or TQ in ameliorating oxidative damage, biochemical parameters, testicular histopathology, and immunohistochemistry (IHC) induced by MTX.

MATERIALS AND METHODS

Chemicals: Injectable MTX solution (50 mg/5 mL; Mina Pharm Pharmaceuticals, Cairo, Egypt). L-carnitine was obtained from MEPACO Company (Inshas Elraml, Egypt). Thymoquinone, possessing a purity of 98% (Sigma Aldrich – USA).

Animals and experimental design: Forty-nine male Wistar Albino rats, with weights ranging from 185 to 195 grams obtained from the Egyptian Organization for Biological Products and Vaccines. Rats were accommodated at 25±2°C and divided into 7 groups (n=7). 1st group (saline orally), 2nd group (LC orally, 200 mg/kg/day for 30 days (Khedr and Werida, 2022). The third group received thymoquinone (TQ) orally at a dose of 50 mg/kg/day for 30 days (Savran *et al*., 2020). 4th group received 20 mg/kg (single IP dose) on the 23rd day of the study (Aboubakr *et al*., 2023a). 5th group (LC and MTX), 6th group (TQ and MTX), and the 7th group received all three treatments: LC, TQ, and MTX.

Sampling (Blood & tissues): Rats were anesthetized using isoflurane at end of the study. Blood samples were obtained from the retro-orbital plexus; serum was obtained by centrifuging the samples at 1200 g for 15 minutes and stored at -20°C for hormone analysis. The testes were swiftly removed and rinsed with saline. Tissue samples weighing 1 gram were homogenized in a phosphate buffer with a pH of 7.4. Following centrifugation at 1200 x g for 20 minutes at 4°C, the resulting supernatants were stored at -20°C for the measurement of oxidative stress markers in the testicular tissues. A portion of the testicular tissue was immediately fixed in formalin for subsequent histopathological and IHC evaluations.

Hormone level analysis: Testosterone, FSH, and LH hormone levels were quantified using ELISA kits according to the instructions provided by the manufacturers.

Oxidative stress biomarkers: The concentrations of MDA, SOD, CAT, and GSH were determined using diagnostic kits procured from Biodiagnostic Co in Egypt.

Histopathology, and immunohistochemistry (IHC): The testicular tissues were preserved through fixation in 10% formaldehyde and subsequent embedding in paraffin wax. Sections, measuring 5 µm in thickness, were obtained from paraffin blocks using a microtome. These sections were placed on glass slides, deparaffinized, and subjected to staining with H&E stain. To evaluate the severity of damage in seminiferous epithelia, tubular necrosis, congestion, and interstitial edema, testicular injury scoring system was applied. This scoring system ranged from 0 (indicating normal histopathology) to 3 (indicating severe damage), following the methodology previously outlined by Sherif *et al*. (2020). Immunostaining with caspase-3 was conducted following the protocol outlined by Porter and Jänicke, (1999).

Statistical analysis: The findings (mean ± SD) and statistical analysis was conducted using GraphPad Prism 9 software (San Diego, CA, USA), employing one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Statistical significance was established at $P \leq 0.05$.

RESULTS

Effect on serum hormone levels: The findings of the study indicated that exposure to MTX led to decreased levels of testosterone, FSH, and LH in the testes compared to the control group ($p<0.0001$), suggesting the occurrence of testicular toxicity. However, administration of a combination of LC and TQ to rats administered MTX resulted in a notable rise in these hormone levels, albeit they remained below those seen in the control group ($p<0.0001$). So the simultaneous administration of LC and TQ had a more pronounced protective effect against MTX-induced reproductive damage compared to the use of either supplement alone, as illustrated in Fig. 1A-G.

Effect on oxidative damage parameters: Exposure to MTX resulted in a notable elevation in MDA levels, along with a significant reduction in GSH, SOD, and CAT levels in the testicular tissues of rats ($p<0.0001$) compared to control. The findings of the study indicated that when LC or TQ were used separately, they effectively reduced the negative impacts of MTX on MDA, CAT, SOD, and GSH levels in the testes. However, these values still differed significantly from those of the control group. Furthermore, the co-administration of LC +TQ+MTX led to notably decreased oxidative damage in the testes compared to groups that received either LC or TQ alone with MTX ($p<0.0001$), as illustrated in Fig. 1.

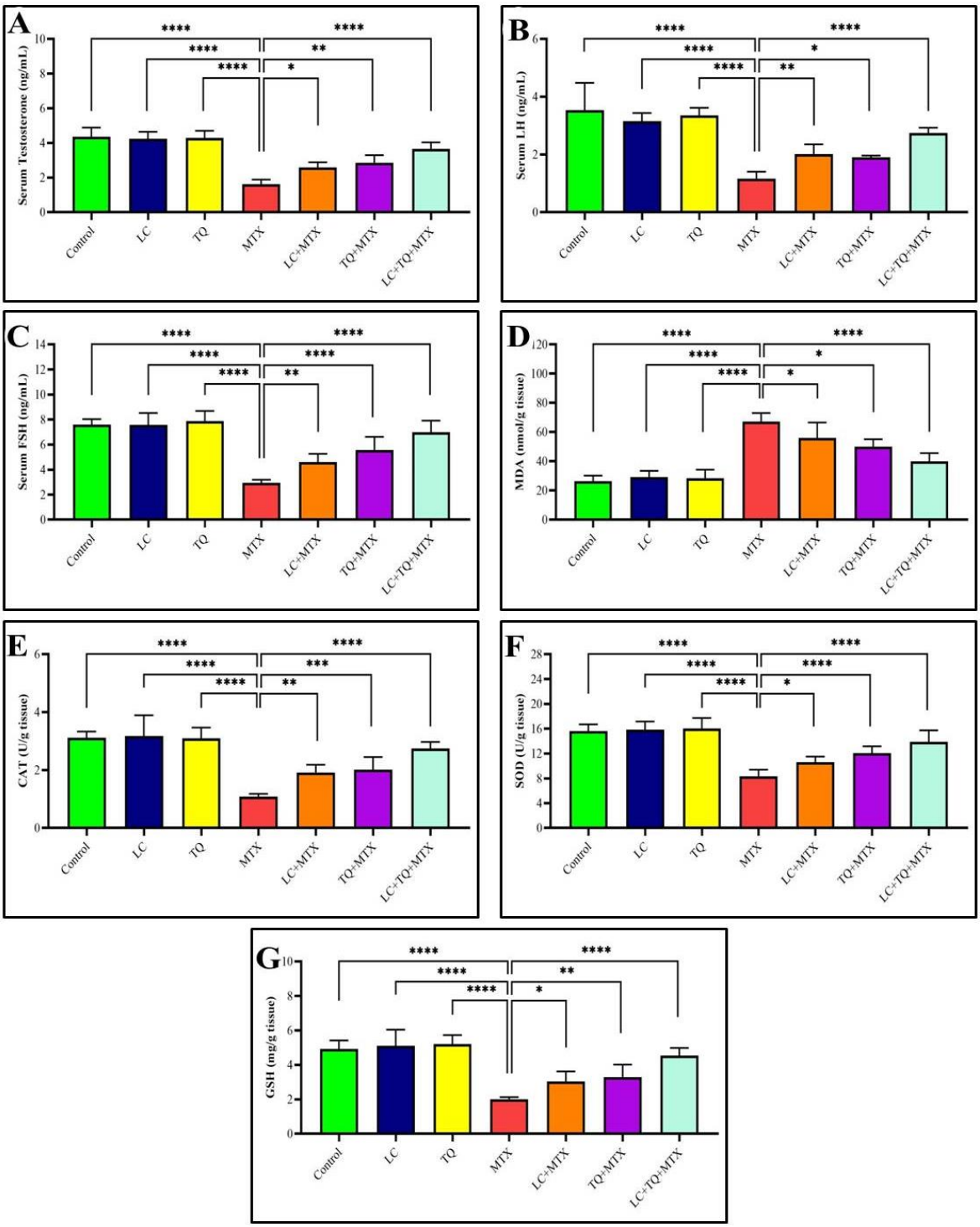


Fig. 1: Effect of L-Carnitine (LC), thymoquinone (TQ) and methoxtrexate (MTX) on serum testosterone, FSH, and LH and antioxidant parameters (MDA, CAT, SOD, and GSH) in testicular tissues in rats. Statistical significance was determined by asterisks, with * indicating $p < 0.05$, ** indicating $p < 0.01$, *** indicating $p < 0.001$, and **** indicating $p < 0.0001$, compared to the MTX-treated groups. Data are expressed as the mean ± SD (n=7).

Histopathological findings: In the light microscopic examination of testicular tissue, notable variations were observed among the study groups. The Control Group and its subgroups (Normal, LC, TQ) displayed typical testicular architecture (Fig. 2A-C), with orderly seminiferous tubules, regular germinal epithelium, and complete spermatogenesis. Spermatogonia were adjacent to a defined basement membrane, primary spermatocytes exhibited large central nuclei, and spermatids with pale cytoplasm were arranged in layers.

The Methotrexate (MTX) group (Fig. 2D-F) presented pronounced pathological changes, including interstitial vessel congestion, oedema, vacuolization in spermatocytes, necrotic spermatids, and impaired spermatogenesis (Fig. 2D). Seminiferous tubules showed severe degeneration, shrinkage, and disrupted basement membranes, with a marked reduction in spermatogenic cells displaying vacuolation and nuclear pyknosis and widened interstitium were clearly noticed (Fig. 2E). The germinal cell lining of the seminiferous tubules was nearly depleted (Fig. 2F).

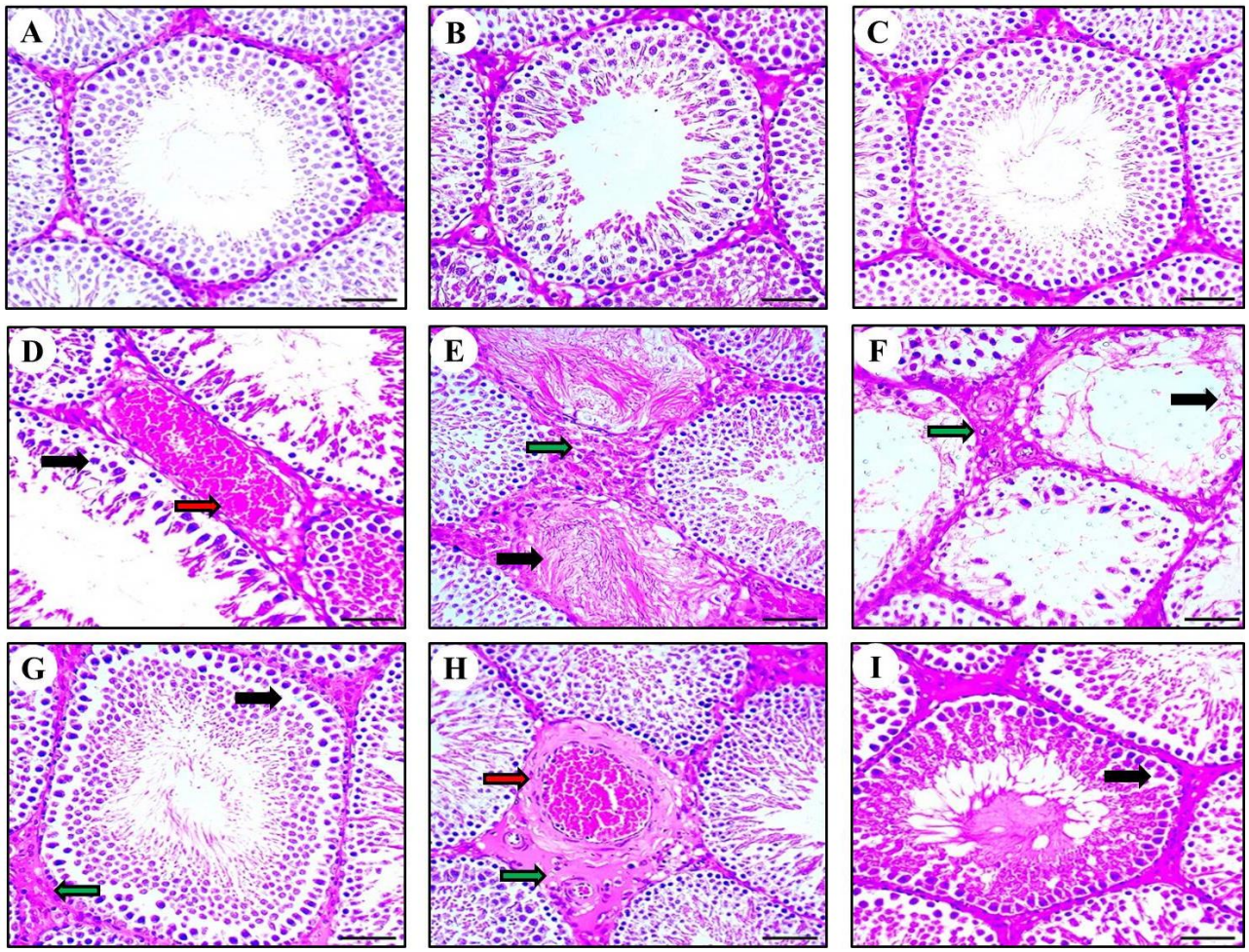
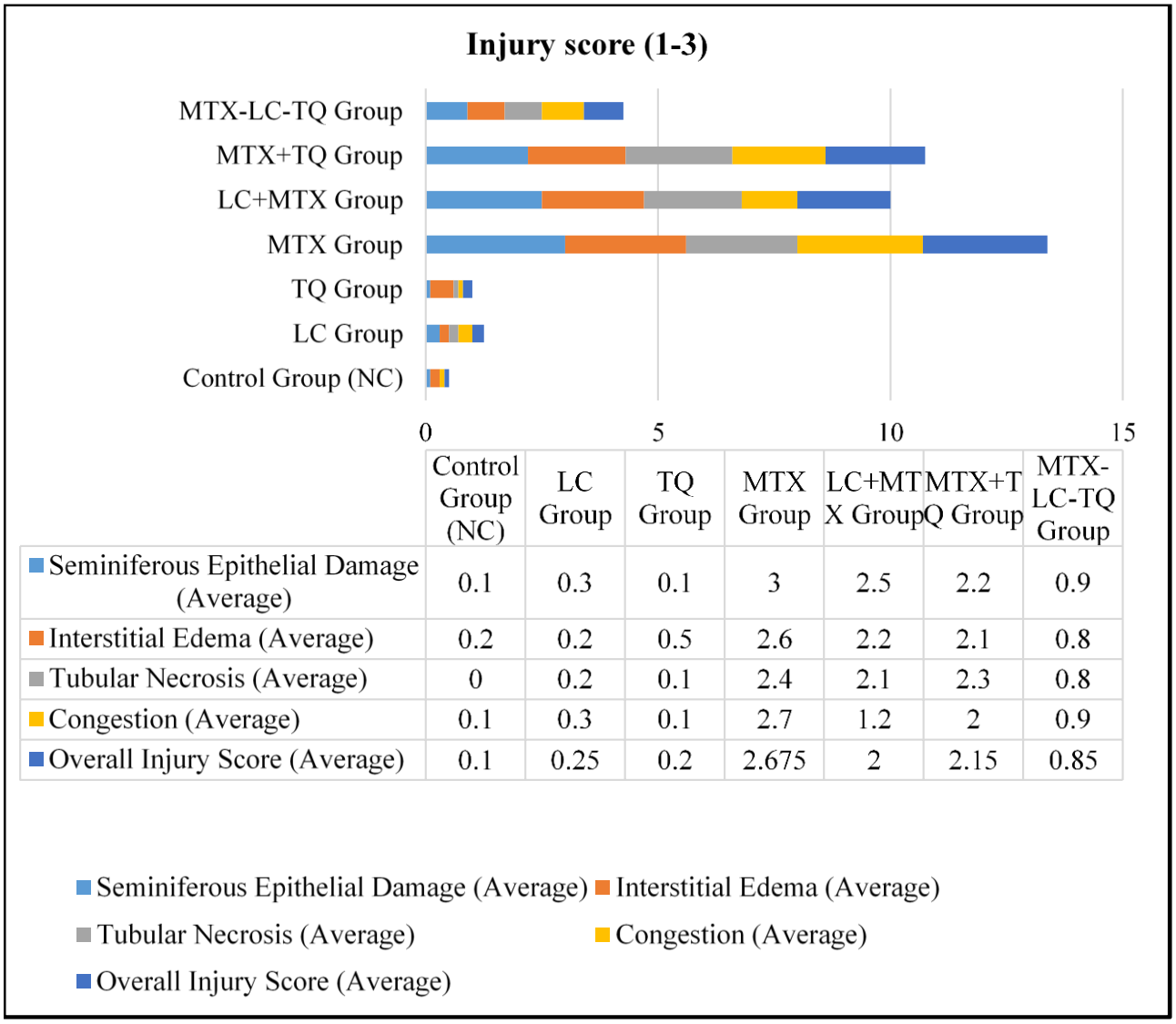


Fig. 2: Histopathological changes; Control (A), LC (B), and TQ (C), displayed characteristic testicular architecture with well-organized seminiferous tubules and comprehensive spermatogenesis, signifying typical physiological conditions. D-F) MTX-treated group exhibited pronounced pathological alterations, as evidenced by marked edema and congestion (D), compromised spermatogenesis (E), and extensive vacuolization and necrosis (F), indicative of significant testicular damage. G-H) The LC+MTX group demonstrated notable histopathological recovery (G), whereas the TQ+MTX group presented a near-normal testicular structure, albeit with some residual vascular congestion (H). The LC+TQ+MTX co-treated group closely paralleled the histology of the control group, suggesting a synergistic ameliorative effect in mitigating MTX-induced testicular impairment (I). Pathological features are delineated with black arrows marking degeneration and necrosis of the germinal epithelium, red arrows highlighting congestion, and green arrows indicating the expansion of the interstitial space.

Conversely, the LC+MTX group (Fig. 2G) demonstrated significant histopathological recovery, albeit with some tubular epithelium detachment. The TQ+MTX group (Fig. 2H) revealed near-normal testicular architecture, with regular seminiferous tubules, stratified germinal epithelium, and mature sperm development despite some vascular congestion and intertubular acidophilic substances.

The LC+TQ+MTX co-treated group (Fig. 2I) closely mirrored the control group's architecture, with well-preserved seminiferous tubules and basement membranes, signifying the most marked recovery among the treated groups, with predominantly normal spermatogenic cells. This highlights a synergistic protective effect in mitigating MTX-induced testicular damage.

The testicular injury scoring system was shown in Fig. 3: control Group (NC) served as the reference or control, demonstrating minimal damage across all categories, with the lowest scores observed for Seminiferous Epithelial Damage (0.1), Interstitial Edema (0.2), Tubular Necrosis (0), and Congestion (0.1), resulting in an Overall Injury Score of 0.1. The LC Group exhibited slightly elevated scores in Seminiferous Epithelial Damage (0.3), Tubular Necrosis (0.2), and Congestion (0.3), leading to an Overall Injury Score of 0.25, while Interstitial Edema remained at 0.2. Within the TQ Group, Interstitial Edema had the highest average score at 0.5, while the scores for other categories remained relatively low, resulting in an Overall Injury Score of 0.2. The MTX Group displayed the most severe testicular injury, with high scores across all categories, including Seminiferous Epithelial Damage (3), Interstitial Edema (2.6), Tubular Necrosis (2.4), and Congestion (2.7), leading to an Overall Injury Score of 2.675, indicating severe damage. Combining LC and MTX in the LC+MTX Group resulted in moderately high scores in all categories, with Seminiferous Epithelial Damage (2.5) and Interstitial Edema (2.2) being particularly elevated, resulting in an Overall Injury Score of 2. Similarly, the MTX+TQ Group also exhibited moderate levels of injury across all categories, with Seminiferous Epithelial Damage (2.2) and Tubular Necrosis (2.3) being notable,



	Control Group (NC)	LC Group	TQ Group	MTX Group	LC+MTX Group	MTX+TQ Group	MTX-LC-TQ Group
Seminiferous Epithelial Damage (Average)	0.1	0.3	0.1	3	2.5	2.2	0.9
Interstitial Edema (Average)	0.2	0.2	0.5	2.6	2.2	2.1	0.8
Tubular Necrosis (Average)	0	0.2	0.1	2.4	2.1	2.3	0.8
Congestion (Average)	0.1	0.3	0.1	2.7	1.2	2	0.9
Overall Injury Score (Average)	0.1	0.25	0.2	2.675	2	2.15	0.85

Fig. 3: The effect of LC and/or TQ on the testicular injury scoring system of MTX treated groups

resulting in an Overall Injury Score of 2.15. Lastly, the MTX-LC-TQ Group demonstrated relatively low levels of testicular injury in all categories, with Seminiferous Epithelial Damage (0.9), Interstitial Edema (0.8), Tubular Necrosis (0.8), and Congestion (0.9) all being minimal. The Overall Injury Score for this group was 0.85.

Immunohistochemistry assessment of testis: As shown in Fig. 4; in the control groups, which included normal, LC, and TQ subgroups, there was a notable absence of cytoplasmic immunostaining for caspase-3 in spermatogenic cells. This lack of staining indicated an absence of apoptosis or cellular stress, corroborating the typical histological architecture observed in these groups and suggesting a healthy testicular environment under these conditions.

MTX-treated group presented strong positive staining for caspase-3 in the cytoplasm of spermatogonia and other spermatogenic cells, particularly in areas showing significant histopathological changes such as vacuolization and necrosis. The pronounced caspase-3 activity indicated a high level of apoptosis, aligning with the severe testicular damage and impaired spermatogenesis induced by MTX, as reflected in the deteriorated testicular architecture.

The LC+MTX group exhibited a mixed pattern of caspase-3 staining, with some areas showing reduced staining intensity compared to the MTX group alone. This reduced caspase-3 activity in certain regions suggested a partial protective effect of LC against MTX-induced damage, aligning with the observed histopathological recovery in this group. Similarly, the TQ+MTX group demonstrated generally lower levels of caspase-3 staining compared to the MTX group, with some areas approaching normal levels. This suggests a protective effect of TQ against testicular damage induced by MTX, as supported by the near-normal testicular architecture and reduced apoptotic activity observed histologically in this group.

Most notably, the LC+TQ+MTX group displayed minimal caspase-3 staining, resembling the control group. This minimal staining signified the most significant recovery among the treated groups, suggesting LC and TQ's strong synergistic protective effect against MTX-induced damage. This observation aligned with the histopathological findings of predominantly normal spermatogenic cells and well-preserved testicular structure in this group.

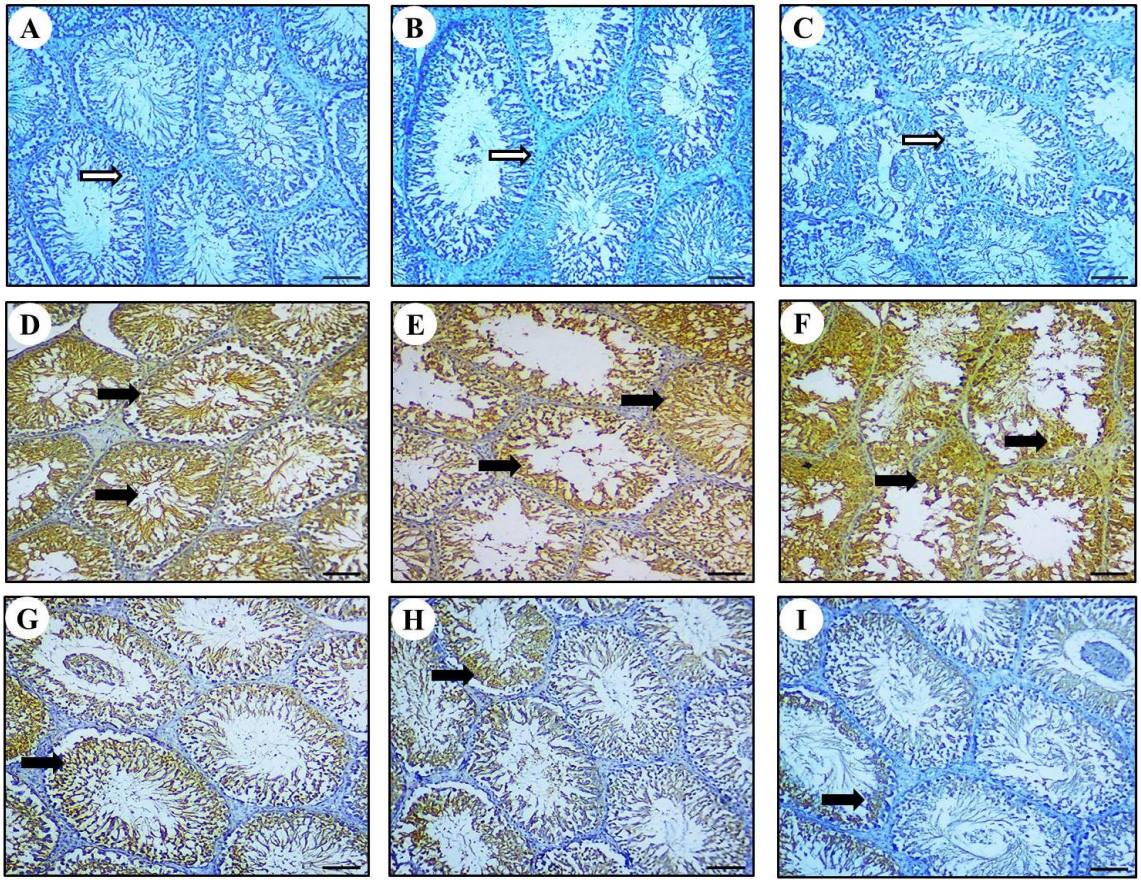


Fig. 4: Caspase-3 immunostaining patterns in testicular tissue among different study groups, highlighting the cellular response to various treatments. In the control groups, encompassing Normal, LC, and TQ subgroups, an absence of caspase-3 staining in spermatogenic cells signifies no apoptosis or cellular stress, indicating a healthy testicular state. Contrasting sharply, the MTX Group shows intense caspase-3 staining in spermatogonia and other cells, particularly in regions with vacuolization and necrosis, revealing a high apoptosis level in response to MTX-induced testicular damage. The LC+MTX and TQ+MTX groups demonstrate intermediate staining patterns, with reduced caspase-3 activity, suggesting the mitigating effects of LC and TQ against MTX-induced damage and partial histological recovery. The LC+TQ+ MTX group exhibits minimal caspase-3 staining, paralleling the control group and indicating significant recovery and a synergistic protective effect against MTX-induced damage.

DISCUSSION

Methotrexate induces testicular toxicity through multiple pathways, including oxidative stress and apoptosis. The study results demonstrated a significant decrease in total testosterone, LH, and FSH levels in the MTX group. These findings suggest that changes in testosterone levels induced by MTX may be associated with a decrease in the quantity of LH receptors present on Leydig cells (Abdul-Hamid *et al*., 2023). A reduction in the production and release of testosterone results from the depletion of germ cells, dysfunction of Sertoli cells, contraction of interstitial cells, and a decline in the anabolic impact of testosterone due to oxidative stress (Ramadan *et al*., 2018). This finding aligns with previous research indicating the testicular damage caused by MTX (Mansour *et al*., 2021; Abdul-Hamid *et al*., 2023; Hassanein *et al*., 2023).

Administering LC or TQ in conjunction with MTX successfully mitigated the adverse impacts of MTX on testicular function. Previous studies have shown that LC restored testosterone, FSH, and LH levels following testicular damage induced by trazadone (Khedr and Werida, 2022), monosodium glutamate (Koohpeyma *et al*., 2022), and busulfan (Hafezi *et al*., 2022). The anti-oxidative characteristics of LC alleviate oxidative stress leading to an improvement in serum levels of sex hormones. LC also has a direct effect on testosterone release by preventing the transformation of testosterone into estrogen, as suggested by Safarinejad, (2008). The decline in testosterone levels in the bloodstream can be attributed to the inhibition of crucial enzymes involved in testosterone production within the testes, as proposed by Wang *et al*. (2013). Significant decline in testosterone secretion could be attributed to reduced LH secretion by the pituitary gland and pathological changes occurring in the Leydig cells within the interstitial tissues of the testes.

Moreover, the role of TQ in enhancing Leydig cell numbers and testosterone levels, thereby improving spermatogenesis, has been documented in several studies (Negi *et al*., 2018). Furthermore, TQ has been identified to exhibit potent antioxidant properties, capable of mitigating the harmful impacts on spermatogenesis resulting from testicular injuries associated with oxidative stress. Earlier research has illustrated the protective effects of TQ on compromised spermatogenesis induced by exposure to cyclophosphamide (Adana *et al*., 2022). Moreover, TQ has been proposed to mitigate the adverse consequences of cadmium chloride by triggering the endocrine and antioxidant systems in the testes (Sayed *et al*., 2014). The

protective function of TQ in testicular tissue is underscored by its antioxidant defense against the generation of ROS (Negi *et al*., 2018; Adana *et al*., 2022). Treatment with TQ significantly counteracted the toxic effect of lead on serum testosterone levels, possibly due to its ability to enhance LH release, which stimulates Leydig cells to produce testosterone, and/or mitigate degenerative changes and apoptosis in Leydig cells (Hassan *et al*., 2019).

The testes are highly vulnerable to oxidative stress, primarily due to their abundant polyunsaturated membrane lipids. A disturbance in the equilibrium between the production and elimination of free radicals can result in pathological states. Oxidative stress has the potential to inflict harm on a range of biological molecules, encompassing lipids, proteins, polysaccharides, and DNA (Yüncü *et al*., 2019). Oxidative stress plays a significant role in the process of tissue damage. It occurs when an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize or repair their harmful effects arises (Elsayed *et al*., 2022; Aboubakr *et al*., 2023b; Elsayed *et al*., 2024). The testicular harm triggered by MTX primarily stems from oxidative stress and disturbances in the body's antioxidant defense mechanisms (Akacha *et al*., 2022; Abdul-Hamid *et al*., 2023; Hassanein *et al*., 2023). In this study, disturbances in testicular oxidation related to MTX were observed, as indicated by increased levels of MDA, decreased production of GSH, and reduced activity of CAT and SOD.

The testes, having limited blood supply, possess low oxygen content, rendering them especially susceptible to the adverse impacts of oxidative stress on spermatogenesis and steroidogenesis within testicular tissue (Abd El-Hakim *et al*., 2018). Likewise, the use of LC or TQ to MTX-intoxicated rats brought back the levels of MDA and the activities of SOD, CAT, and GSH to levels similar to those observed in the control group and consistent with the research examining the protective properties of LC against the harmful effects of substances like cisplatin (Sallam *et al*., 2021).

Thymoquinone (TQ) is recognized for its strong ability to scavenge radicals, making it effective in protecting against tissue damage caused by free radicals. TQ has demonstrated protective effects against various types of testicular injuries, where oxidative stress is the underlying mechanism (Gökçe *et al*., 2011). Notably, when administered orally with lead, TQ significantly reduced the concentration of malondialdehyde (MDA) in testicular tissues, indicating its antioxidant effect against lead-induced testicular toxicity (Hassan *et al*., 2019). The antioxidative properties of TQ can be attributed to its ability to scavenge free radicals and counteract oxidative stress (Mansour *et al*., 2021). Furthermore, there is evidence indicating that TQ can inhibit the lipid peroxidation caused by superoxide anion radicals (Nagi and Mansour, 2000). Previous studies have reported the anti-oxidative effects of TQ in rats against lead and cadmium induced testicular toxicity.

Furthermore, previous studies have reported significant histopathological changes in the testicles of rats treated with MTX compared to control rats (Akacha *et al*., 2022; Abdul-Hamid *et al*., 2023, Hassanein *et al*., 2023). However, the administration of LC demonstrated a significant reduction in the severity of MTX-induced histological changes and their effects on spermatogenesis. Moreover, supplementation with LC has been shown to play a significant role in reducing pathological lesions in the testicles of rats exposed to radiation (Aktoz *et al*., 2017), lead (Abdel-Emam and Ahmed, 2021), cisplatin (Sallam *et al*., 2021). Additionally, it has been discovered that TQ can mitigate the extent of histopathological alterations induced by MTX, similar to its observed effects in cases of testicular toxicity caused by cyclophosphamide (Adana *et al*., 2022), valproic acid (Savran *et al*., 2020), lead (Hassan *et al*., 2019), and cadmium (Fouad and Jresat, 2015).

MTX resulted in an increased expression of caspase-3 as observed in the study by Vardi *et al*., (2009). However, when MTX was co-administered with either LC or TQ, there was a reduction in caspase-3 expression. Moreover, the combined treatment of MTX with both LC and TQ not only decreased caspase-3 expression but also restored normal histological architecture and normalized caspase-3 levels. This suggests that using LC+TQ was more effective than each alone, attributed to their dual antioxidant actions and their capacity to restore enzymatic antioxidant activity.

Conclusions: The present research shows that exposure to MTX results in lipid peroxidation in the testicular tissue, disturbs the antioxidant defense system, and alters the structure and function of the testes. Nevertheless, giving LC or TQ seems to aid in the restoration of testicular tissues in rats harmed by MTX-induced injury. These protective impacts probably stem from inflammation inhibition, along with the reduction of oxidative stress and lipid peroxidation. More research is needed to explore the precise mechanisms behind these protective effects.

Ethics approval: Faculty of Veterinary Medicine's Research Ethical Committee, Cairo University, Egypt (Vet CU 25122023824) approved the study.

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Authors' contribution: Soliman A, Hassan FW, Zakaria M (methodology). Aboubakr M; designed the experimental protocol. Alkafafy M; histopathology and IHC. Abduljabbar MH and Mohamed K; original draft preparation, review and editing. All authors read, revised, and approved the final manuscript.

REFERENCES

Abd El-Hakim YM, Mohamed WA and El-Metwally AE, 2018. Spirulina platensis attenuates furan reprotoxicity by regulating oxidative stress, inflammation and apoptosis in testis of rats. Ecotoxicol Environ Saf 161: 25-33.

Abdel-Daim MM, Abo El-Ela FI, Alshahrani FK, et al., 2020. Protective effects of thymoquinone against acrylamide-induced liver, kidney and brain oxidative damage in rats. Environ Sci Pollut Res Int 27: 37709-37717.

Abdel-Emam RA and Ahmed EA, 2021. Ameliorative effect of L-carnitine on chronic lead-induced reproductive toxicity in male rats. Vet Med Sci 7: 1426-1435.

Abdo W, Elmadawy MA, Abdelhiee EY, *et al*., 2021. Protective effect of thymoquinone against lung intoxication induced by malathion inhalation. Sci Rep 11: 2498.

Abdul-Hamid M, Abdel-Reheim ES, Hegazy W, *et al*., 2023. Impact of gervital against histopathological, ultrastructural, and biochemical alterations caused by methotrexate or azathioprine in albino rat testis. Environ Sci Pollut Res Int 30: 21914-21926.

Aboubakr M, Elbadawy M, Ibrahim SS, *et al*., 2023a. Allicin and lycopene possesses a protective effect against methotrexate-induced testicular toxicity in rats. Pak Vet J 43: 559-566.

Aboubakr M, Elmahdy AM, Taima S, *et al*., 2023b. Protective effects of N acetylcysteine and vitamin E against acrylamide-induced neurotoxicity in rats. Pak Vet J 43: 262-268.

Adana MY, Imam A, Bello AA, *et al*., 2022. Oral thymoquinone modulates cyclophosphamide-induced testicular toxicity in adolescent Wistar rats. Andrologia 54: e14368.

Agarwal A and Said TM, 2004. Carnitines and male infertility. Reprod Biomed Online 8: 376-384.

Akacha A, Badraoui R, Rebai T, *et al*., 2022. Effect of Opuntia ficus indica extract on methotrexate-induced testicular injury: a biochemical, docking and histological study. J Biomol Struct Dyn 40: 4341-4351.

Aktoz T, Caloglu M, Yurut-Caloglu V, *et al*., 2017. Histopathological and biochemical comparisons of the protective effects of amifostine and l-carnitine against radiation-induced acute testicular toxicity in rats. Andrologia 49: 10.1111/and.12754.

Alahmadi AA and Abduljawad EA, 2021. Equisetum arvense L. extract ameliorates oxidative stress, inflammation and testicular injury induced by methotrexate in male rats. J Pharm Res Int 33: 101-114.

Behairy A, Elkomy A, Elsayed F, *et al*., 2024. Spirulina and Thymoquinone Protect Against Methotrexate-Induced Hepatic Injury in Rats. Rev Bras Farmacogn 34: 154-167.

Elsayed A, Aboubakr M, Hassan FW, *et al*., 2024. Testicular injury of acrylamide in rats and potential protection of coenzyme Q10 and rosuvastatin. Pak Vet J http://dx.doi.org/10.29261/pakvetj/2024.146

Elsayed A, Elkomy A, Alkafafy M, *et al*., 2022. Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats. Pak Vet J 42: 107-111.

Fouad AA and Jresat I, 2015. Thymoquinone therapy abrogates toxic effect of cadmium on rat testes. Andrologia 47: 417-26.

Gökçe A, Oktar S, Koc A, *et al*., 2011. Protective effects of thymoquinone against methotrexate-induced testicular injury. Hum Exp Toxicol 30: 897-903.

Granados-Principal S, Quiles JL, Ramirez-Tortosa CL, *et al*., 2010. New advances in molecular mechanisms and the prevention of adriamycin toxicity by antioxidant nutrients. Food Chem Toxicol 48: 1425-1438.

Hafezi H, Vahdati A, Forouzanfar M, *et al*., 2022. Ameliorate effects of resveratrol and l-carnitine on the testicular tissue and sex hormones level in busulfan induced azoospermia rats. Theriogenology 191: 47-53.

Hassan E, El-Neweshy M, Hassan M, *et al*., 2019. Thymoquinone attenuates testicular and spermotoxicity following subchronic lead exposure in male rats: Possible mechanisms are involved. Life Sci 230: 132-140.

Hassanein EHM, Mohamed WR, Hussein RM, *et al*., 2023. Edaravone alleviates methotrexate-induced testicular injury in rats: Implications on inflammation, steroidogenesis and Akt/p53 signaling. Int Immunopharmacol 117: 109969.

Kabir MT, Rahman MH, Akter R, *et al*., 2021. Potential role of curcumin and its nanoformulations to treat various types of cancers. Biomolecules 11: 392.

Khedr NF and Werida RH, 2022. l-carnitine modulates autophagy, oxidative stress and inflammation in trazodone induced testicular toxicity. Life Sci 290: 120025.

Koohpeyma F, Gholizadeh F, Hafezi H, *et al*., 2022. The protective effect of L-carnitine on testosterone synthesis pathway, and spermatogenesis in monosodium glutamate-induced rats. BMC Complement Med Ther 22: 269.

Mansour DF, Saleh DO, Ahmed-Farid OA, *et al*., 2021. Ginkgo biloba extract (EGb 761) mitigates methotrexate-induced testicular insult in rats: Targeting oxidative stress, energy deficit and spermatogenesis. Biomed Pharmacother 143: 112201.

Maremanda KP and Jena GB, 2017. Methotrexate-induced germ cell toxicity and the important role of zinc and SOD1: Investigation of molecular mechanisms. Biochem Biophys Res Commun 483: 596-601.

Nagi MN and Mansour MA, 2000. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol Res 41: 283-289.

Negi P, Rathore C, Sharma G, *et al*., 2018. Thymoquinone a potential therapeutic molecule from the plant *Nigella sativa*: role of colloidal carriers in its effective delivery. Recent Pat Drug Deliv Formul 12: 3-22.

Ostadpoor M and Gholami-Ahangaran M, 2021. A review on hepatoprotective effects of Nigella sativa L. J Med Herb 12: 49-54.

Phua CYH, Teoh ZL, Goh BH, *et al*., 2021. Triangulating the pharmacological properties of thymoquinone in regulating reactive oxygen species, inflammation, and cancer: Therapeutic applications and mechanistic pathways. Life Sci 287: 120120.

Porter AG and Jänicke RU, 1999. Emerging roles of caspase-3 in apoptosis. Cell Death Differ 6: 99-104

Ramadan BK, Schaalan MF and Mahmoud ES, 2018. Protective effect of taurine on thiopurine-induced testicular atrophy in male albino rats. J Steroids Horm Sci 9: 1000192.

Safarinejad MR, 2008. Evaluation of endocrine profile and hypothalamic-pituitary-testis axis in selective serotonin reuptake inhibitor-induced male sexual dysfunction. J Clin Psychopharmacol 28: 418-423.

Sallam AO, Rizk HA, Emam MA, *et al*., 2021. The Ameliorative Effects of L-Carnitine against Cisplatin-Induced Gonadal Toxicity in Rats. Pak Vet J 41: 147-151.

Savran M, Ascı H, Armagan İ, *et al*., 2020. Thymoquinone could be protective against valproic acid-induced testicular toxicity by antioxidant and anti-inflammatory mechanisms. Andrologia 52: e13623.

Sayed MM, Hassanein KMA and Senosy W, 2014. Protective effects of thymoquinone and l-cysteine on cadmium-induced reproductive toxicity in rats. Toxicol Rep 1: 612-620.

Sherif IO, Al-Mutabagani LA and Sarhan OM, 2020. Ginkgo biloba Extract Attenuates Methotrexate-Induced Testicular Injury in Rats: Cross-talk Between Oxidative Stress, Inflammation, Apoptosis, and miRNA-29a *Expression.* Integr Cancer Ther 19: 1534735420969814

Vardi N, Parlakpinar H, Ates B, *et al*., 2009. Antiapoptotic and antioxidant effects of beta-carotene against methotrexate-induced testicular injury. Fertil Steril 92: 2028-2033.

Wang H, Ji YL, Wang Q, *et al*., 2013. Maternal lead exposure during lactation persistently impairs testicular development and steroidogenesis in male offspring. J Appl Toxicol 33: 1384-1394.

Yüncü M, Zengin G, Birinci H, *et al*., 2019. Protective effect of proanthocyanidin against methotrexate-induced testicle damage in rats. Eur J Ther 25: 51-57.

Zhang S, Ye C, Zhao W, *et al*., 2022. Product identification and toxicity change during oxidation of methotrexate by ferrate and permanganate in water. Front Environ Sci Eng 16: 93.