Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2023.057

RESEARCH ARTICLE

Allicin and Lycopene Possesses a Protective Effect against Methotrexate-induced Testicular Toxicity in Rats

Mohamed Aboubakr[1*], Mohamed Elbadawy[1], Samar Saber Ibrahim[2], Eman Khalil[3], Marwa Darweish[4], Ahmed Farag[5], Ahmed Elfadadny[6], Mohamed Alkafafy[7], Ahmed Soliman[8] and Asmaa Elsayed[1]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qaliobiya, Egypt; [2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qaliobiya, Egypt; [3]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qaliobiya, Egypt; [4]Department of Pathology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Qaliobiya, Egypt; [5]Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt; [6]Department of Animal Internal Medicine, Faculty of Veterinary Medicine, Damanhur University, Damanhur, Egypt; [7]Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; [8]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt
*Corresponding author: mohamed.aboubakr@fvtm.bu.edu.eg

ARTICLE HISTORY (23-223)

Received:	June 03, 2023
Revised:	July 01, 2023
Accepted:	July 07, 2023
Published online:	July 28, 2023

Key words:
Testicular damage
Oxidative stress
Apoptosis
Histopathology
Antioxidant

A B S T R A C T

Methotrexate (MTX) is a chemotherapeutic medicine frequently used to treat various forms of cancer. The objective of our research was to examine whether allicin (ALC) and lycopene (LP) could alleviate the harmful effects of MTX-induced testicular toxicity in rats. A total of 49 male white albino rats were divided into 7 groups (7 rats in each group). The rats of group 1 was kept as a control group and received normal saline orally; while rats of group 2 and group 3 administered ALC (20mg/kg/BW) LP (10mg/kg/BW) orally. On day 15, the rats of group 4 received a single dose of MTX (20mg/kg, IP). In group 5, rats received ALC and MTX; in group 6, rats received LP and MTX, and in group 7, rats received ALC, LP, and MTX. Results showed that MTX induced significant increase in values of testicular malondialdehyde (MDA) levels with a substantial reduction in glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and lowered the serum testosterone, follicle-stimulating hormone (FSH), and lutenizing hormone (LH) levels. MTX induced a marked decline in seminiferous tubule diameter and epithelium height. MTX showed a widening of the interstitial space of seminiferous tubules, atrophy, vacuolar degeneration of the germinal epithelium and Leydig's interstitial cells had scanty cytoplasm and vesicular nuclei. Also, testicular proliferating cell nuclear antigen (PCNA) expression was recorded. In MTX-intoxicated rats, ALC and LP increased antioxidant biomarkers, decreased MDA, and attenuated changes in serum hormonal parameters. It is concluded that ALC and LP have significant protective effects on MTX- induced testicular toxicity due to their antioxidant properties.

To Cite This Article: Aboubakr M, Elbadawy M, Ibrahim SS, Khalil E, Darweish M, Farag A, Elfadadny A, Alkafafy M, Soliman A, Elsayed A, 2023. Allicin and lycopene possesses a protective effect against methotrexate-induced testicular toxicity in rats. Pak Vet J, 43(3): 559-566. http://dx.doi.org/10.29261/pakvetj/2023.057

INTRODUCTION

Methotrexate (MTX), is a substance that hinders the function of folic acid, is being utilized utilized to handle a variety of health conditions, including psoriasis, rheumatoid arthritis, and cancer (Owumi *et al*., 2019). Prior investigations have documented that MTX has detrimental impacts on different organs of the body and can also impair the fertility by adversely affecting the development of spermatogenesis (Belhan *et al*., 2019). Various antioxidant substances can diminish the toxic effects of MTX on the testicles (Ipek *et al*., 2022, Hassanein *et al*., 2023).

Allicin (ALC) is formed enzymatically when alliin, along with alliinase, undergoes cell rupture in raw garlic cloves. Allicin is a type of thiosulfinate compound that exhibits a range of biological and pharmacological activities (Abdel-Daim *et al*., 2019). ALC can offer

significant advantages in terms of decreasing oxidative stress, mitigating inflammation, and enhancing the integrity of mitochondria (Lv *et al*., 2015).

Lycopene (LP) is a member of the carotenoid family, which is a primary source of vitamin A. It is a natural antioxidant found in fruits and vegetables that inhibits the growth of various human cancer cells, protects against DNA damage, and is more potent than both alpha- and beta-carotenes (Iftikhar *et al*., 2022). Sources such as carrots, tomatoes, watermelons, and other foods contain lycopene. Lycopene has various advantageous characteristics, including antioxidant, anti-lipidemic, and anti-inflammatory (James *et al*., 2023). Furthermore, LP has chemo-preventive properties against certain types of cancer (Elsayed *et al*., 2021; Yin *et al*., 2023). Lycopene exerts its antioxidant properties due to its long and non-polar structure, as well as its conjugated double bonds. Its ability to dissolve free radicals is similar to that of other compounds such as retinol, alpha-tocopherol, and carotenoids (Kaya *et al*., 2019). Lycopene has protective properties against testicular toxicity induced by gentamicin (Aly, 2019), diethylnitrosamine (Kaya *et al*., 2019), cisplatin (Elsayed *et al*., 2022b), and cadmium (Iftikhar *et al*., 2022).

The current study aimed to investigate whether the administration of ALC and LP (single or in combination) could improve the biochemical profile, and reduce the oxidative stress, and cellular (testicular) damage in rats against MTX toxicity.

MATERIALS AND METHODS

Chemicals: The Methotrexate injectable solution (50mg/5mL; Mina Pharm Pharmaceuticals, Egypt), Allicin (35% powder; Delta Vet Center, Cairo, Egypt) and Lycopene (LP) was obtained as a powder from Sigma Aldrich Company in Saint Louis, MO, USA. Both LP and ALC were dissolved in ethanol.

Animals and experimental design: A total of forty-nine male Wister Albino rats (190-210 grams) were obtained from the Egyptian Organization for Biological Products and Vaccines. The rats were kept in a controlled environment at a temperature of 25±2°C with a 12-hour light/dark cycle. They were fed standard pellet diet and provided free access to water. The rats were allowed to acclimate for one week before the trial started. Seven groups of rats were formed, each with seven rats. The first group was given only saline and was used as a control, while the second group (ALC group) received ALC at a dosage of 20 mg/kg (orally) (Abdel-Daim *et al*., 2017). LP was given to the third group (LP group) at a dosage of 10 mg/kg (orally) (Elsayed *et al*., 2022a). The fourth group (MTX-treated group) was given saline (orally) and a single dose of MTX (20 mg/kg, IP) on the 15th day of the study (Mahmoud *et al*., 2021). The fifth group (ALC+MTX group) was given ALC and MTX, the sixth group (LP+MTX group) was given LP and MTX, and the seventh group (ALC+LP+MTX group) was given ALC, LP, and MTX. The protective agents (ALC and LP) were administered once daily throughout the 20-day experiment.

Sampling (Blood & tissues): At the end of the experiment, these rats were anesthetized with isoflurane. Blood samples were taken from the retro-orbital plexus followed by serum separation at 1200g centrifugation for 15 minutes and stored at -20°C for further analysis. The testes were quickly removed, homogenized in a phosphate buffer with pH 7.4. After centrifugation at 1200g for 20 minutes at 4°C, the supernatants were stored at -20°C for measuring oxidative stress markers in the testicular tissues. A part of the testicular tissue was fixed in formalin immediately to perform histopathological and immunohistochemical (IHC) assessments.

Testicular oxidative stress analysis and serum hormone level and: Diagnostic kits from Biodiagnostic Co, Egypt were utilized to measure the concentrations of MDA, SOD, CAT and GSH. The serum testosterone, FSH, and LH hormones were measured using ELISA kits based on the manufacturer's protocol and were obtained from MyBioSource Co, San Diego, USA.

Morphometry, histopathology, and immunohisto-chemistry (IHC): The testes were fixed in 10% formaldehyde and embedded in paraffin wax. Sections with a thickness of 5µm were cut via microtome and mounted on glass slides. The sections were then deparaffinized followed by H&E staining. The diameter of the seminiferous tubules and epithelium height were measured using Image J software.

Immunostaining for PCNA was carried out following a previously described protocol (Hall *et al*., 1990).

Statistical analysis: The obtained results were expressed as mean±SD. Statistical comparisons were conducted using GraphPad Prism 9 software (San Diego, CA, USA), which included one-way ANOVA and Tukey's post hoc test for multiple comparisons. A significance level of $P \leq 0.05$ was employed.

RESULTS

Effect on oxidative damage parameters: No significant changes in oxidative damage parameters were observed between control, ALC, and LP treated groups. The level of MDA was significantly increased while GSH, SOD, and CAT levels were dramatically decreased in the testicular tissues of rats exposed to MTX. Additionally, the group that received ALC and LP together along with MTX had a considerably higher level of oxidative damage induced by MTX in the testes compared to the groups that received ALC or LP alone along with MTX, as presented in Fig. 1.

Effect on serum hormone levels: As indicated in Fig. 1, the study revealed no significant changes in hormonal levels were observed between control, ALC and LP treated groups. While, exposure to MTX resulted in lower levels of testosterone, FSH, and LH in the testes compared to the control group, indicating testicular toxicity. However, when rats exposed to MTX were given a combination of ALC and LP, there was a significant increase in these hormones. These results suggest that the combined use of ALC and LP was more effective in protecting against MTX-induced reproductive damage than using either supplement alone.

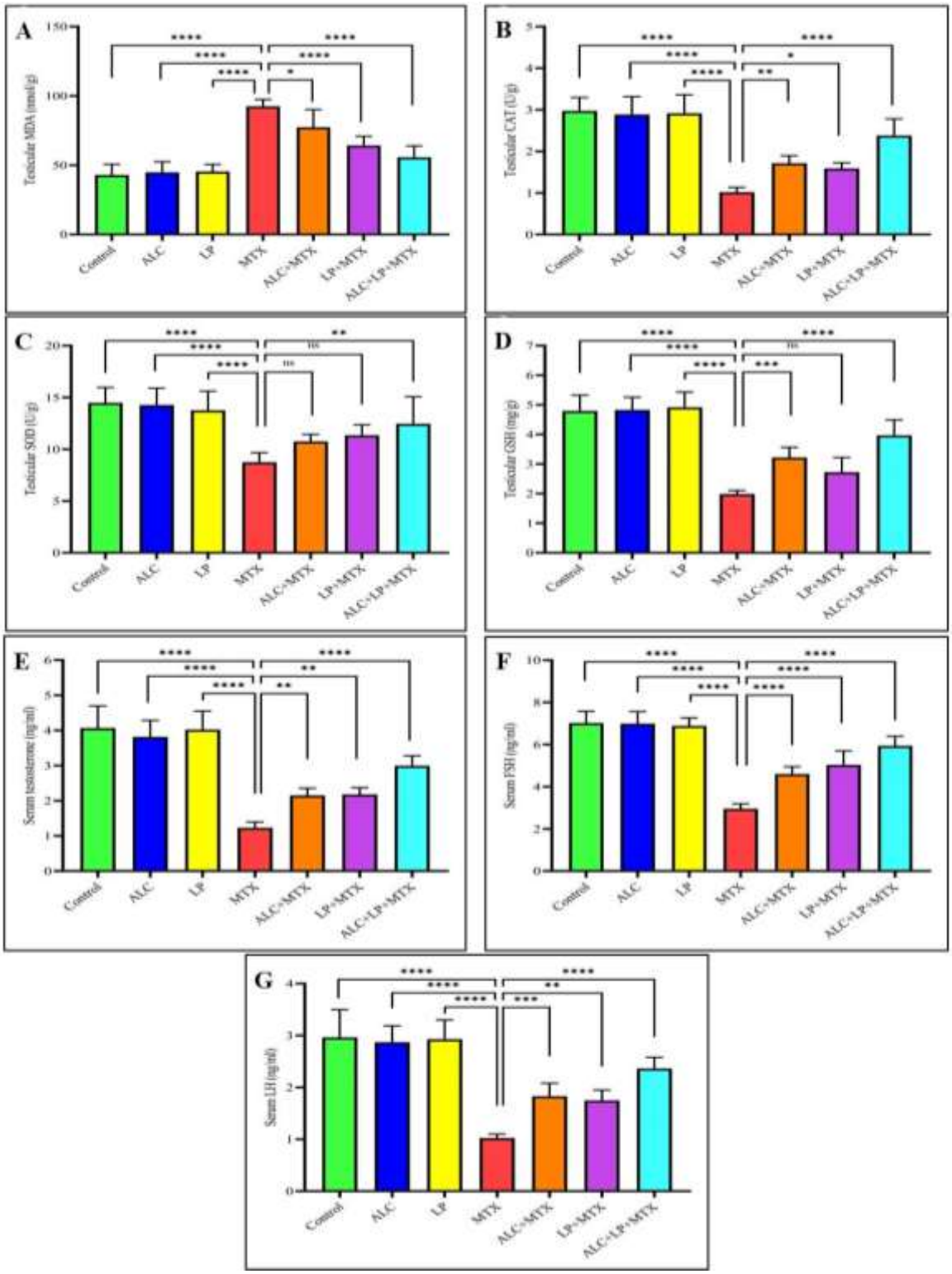

Fig. 1: Effect of ALC, LP, and/or MTX on testicular antioxidant parameters; malondialdehyde (MDA); catalase (CAT); superoxide dismutase (SOD); reduced glutathione (GSH) and serum testosterone, follicle-stimulating hormone (FSH), and lutenizing hormone (LH) levels. Data are expressed as the mean±SD (n=7).

Histopathological findings: Control, ALC and LP treated groups displayed mature, active seminiferous tubules with complete spermatogenesis and fully defined basement membranes in their testes (Fig. 2, 3 A-C). However, rats exposed to MTX, most seminiferous tubules decreased in size with widening of the interstitial space (Fig. 2D), atrophy, and shedding of normal mucosa (Fig. 2E). There were also degenerative changes, such as incomplete spermatogenesis in which the seminiferous tubules were almost emptied of spermatozoa and spermatogonia as well as vacuolar degeneration of the germinal epithelium (Fig. 2F). Many seminiferous tubules were filled with sloughed germinal epithelial cells along with giant cell formations (Fig. 3D). Spermatogenesis was halted in most seminiferous tubules due to loss of germinal epithelium cells (Fig. 3F). There was thickening and irregularity in

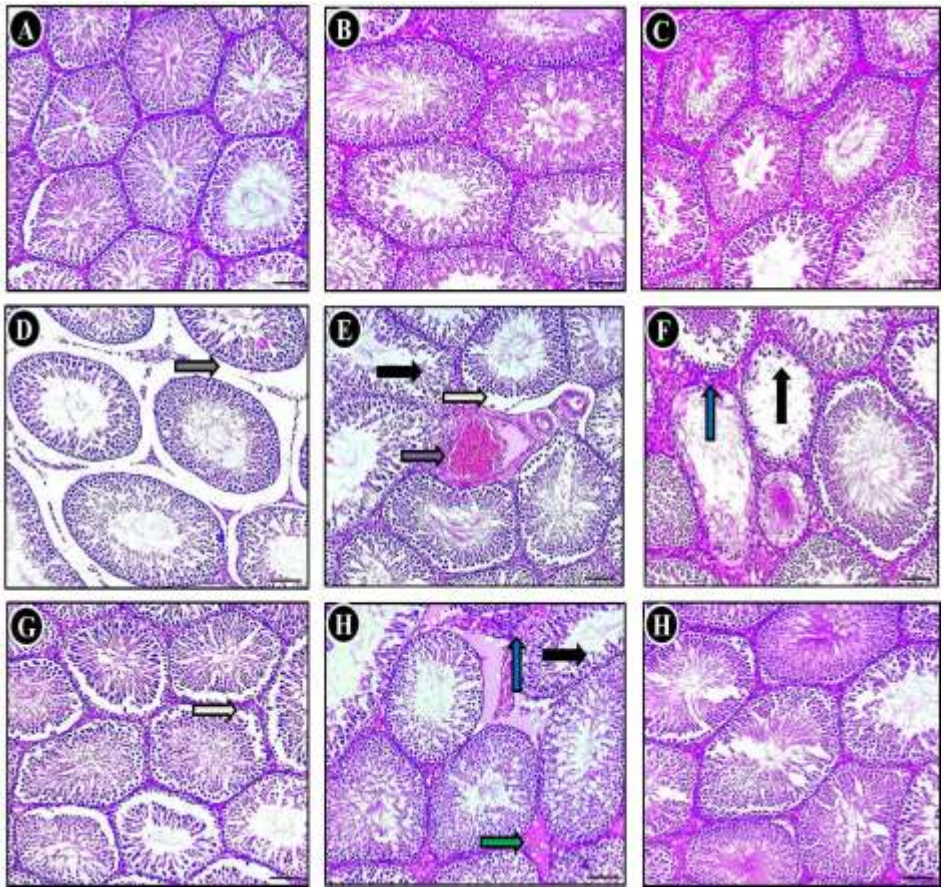

Fig. 2: Testicular histopathological findings are depicted, including representative sections from different experimental groups. The normal control group (Fig. 2A), ALC group (Fig. 2B), and LP-treated group (Fig. 2C) exhibited intact seminiferous tubules with a well-defined basement membrane, along with normal interstitial connective tissue. In contrast, rats treated with MTX (Fig. 2D-F) displayed distinct testicular alterations. These included shrinkage of certain seminiferous tubules and an expansion of the interstitial space (Fig. 2D). Moreover, interstitial blood vessels exhibited congestion (Fig. 2E). At the same time, other tubules demonstrated coagulative necrosis, germinal epithelium depletion, germ cell dissociation from the basal membrane, and accompanying inflammatory cell infiltration within the interstitium (Fig. 2F). However, a noticeable amelioration of testicular lesions and spermatogenesis improvement was observed in rats treated with either ALC+MTX (Fig. 2G) or LP+MTX (Fig. 2H). Notably, the ALC+LP+MTX group showed nearly normal seminiferous tubules, accompanied by the restoration of the typical spermatogenic series (Fig. 2I). Green arrow; edema, blue arrow; inflammatory cell infiltration, black arrow; degenerated and sloughed epithelium, purple arrow; congested blood vessel, and gray arrow; widening of the interstitial space.

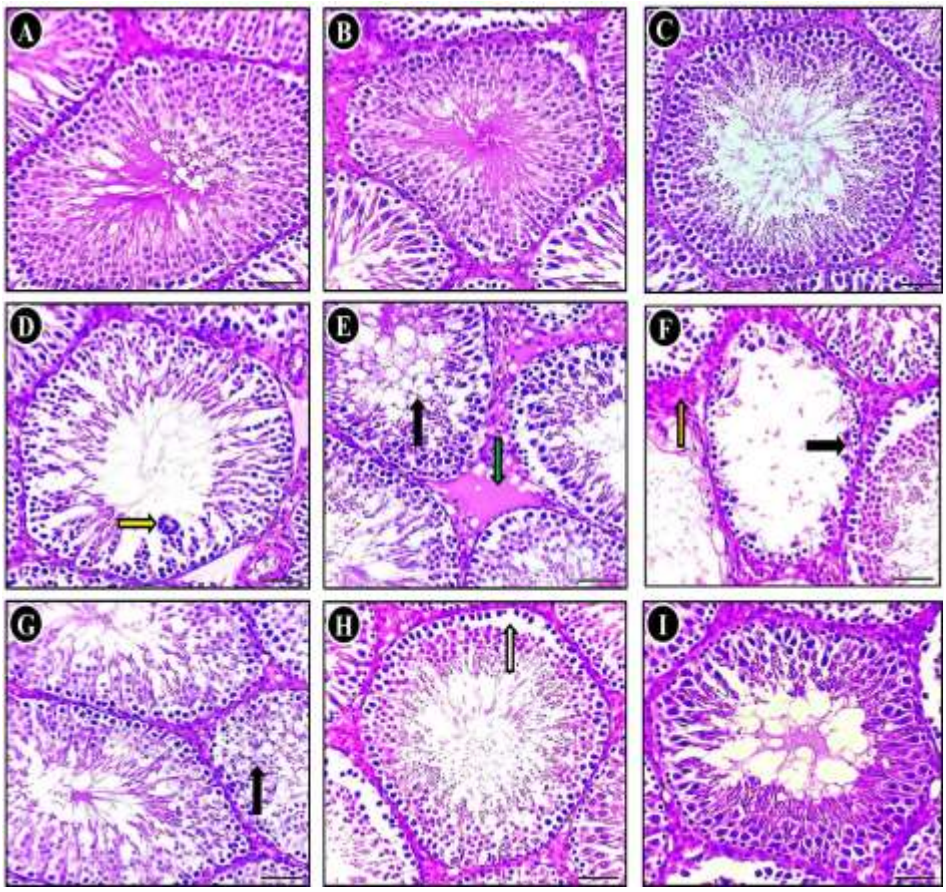

Fig. 3: Testicular histopathological findings, including sections from different experimental groups, are presented. The normal control group (Fig. 3A), ALC group (Fig. 3B) and LP-treated group (Fig. 3C) exhibited normal histological architecture of mature active seminiferous tubules. In contrast, rats treated with MTX (Fig. 3D-F) displayed distinct histological changes. These included the formation of giant cells (Fig. 3D) and vacuolar degeneration of the germinal epithelium. Additionally, the interstitium exhibited oedema, appearing as faint eosinophilic material (Fig. 3E). Sloughing of the germinal epithelium into the lumen of seminiferous tubules was observed, along with the loss of germinal epithelium cells in certain seminiferous tubules (Fig. 3F). However, a moderate improvement of testicular lesions and spermatogenesis was evident in rats treated with either ALC+MTX (Fig. 3G) or LP+MTX (Fig. 3H). Notably, the ALC+LP+MTX group showed nearly normal seminiferous tubules, accompanied by restoring the normal spermatogenic series (Fig. 3I). Yellow arrow; giant cell formations, green arrow; edema, orange arrow; thickening basement membrane, black arrow; degenerated and sloughed epithelium and gray arrow; widening of the interstitial space.

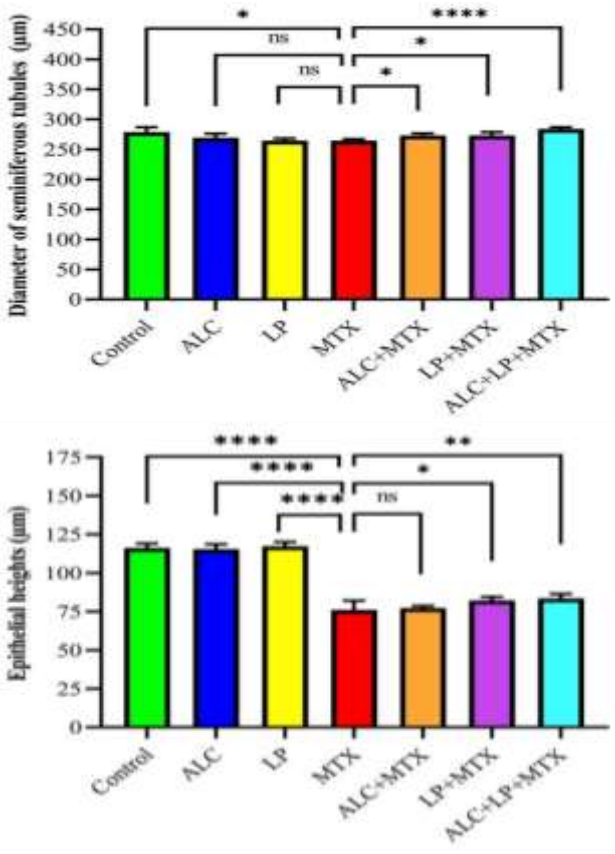

Fig. 4: Determination of the histometric measures of the seminiferous tubules from testicles of different experimental groups.

the basement membrane. Leydig's interstitial cells had scanty cytoplasm and vesicular nuclei (Fig. 3E). ALC+MTX showed significant improvement in testicular lesions characterized by re-establishing normal spermatogenic series (Fig. 2, 3 G). Whereas LP+MTX, testicular lesions showed mild degeneration in some seminiferous tubules with interstitial edema (Fig. 2, 3 H). Upon administering ALC+LP+MTX to rats, the outline and architecture of most seminiferous tubules were highly preserved, with enhanced spermatogenesis and normal appearance of spermatogenic cells (Fig. 2, 3 I).

Analyzing images and performing morphometric evaluations: Using Image J measurement software, images of histological slides of testicles were analyzed in order to determine the diameter (Fig. 4A) and germinal epithelial height of seminiferous tubules (Fig. 4B). The tubular diameter was significantly decreased in the seminiferous tubules of the MTX group relative to the control group and slightly increased in the ALC+MTX and LP+MTX relative to the MTX group, while the ALC+LP+MTX group showed marked significant increase. The epithelial height of the seminiferous tubules in the groups of control, ALC, and LP groups was significantly different from the MTX group. There was no difference in the ALC+MTX group. The tubular luminal area was significantly increased in the LP+MTX group, but much more pronounced in the ALC+LP+MTX group.

The immunostaining results for PCNA: In the seminiferous tubules of rats in the normal control group, most basal spermatogenic cells showed a normal immunoreactive response (Fig. 5A). Moreover, it can be seen from Fig. 5 B, C that rats administered with ALC or LP only showed an immune response comparable to that of the normal group in the nuclei of the basal spermatogenic cells of the seminiferous tubules. Among the testicular abnormalities caused by MTX only, nuclei of the basal spermatogenic cells in the seminiferous tubules showed an obvious reduction in immunoreactivity (Fig. 5D). Following ALC+MTX and LP+MTX showed a considerable improvement in immunoreactivity in basal spermatogenic cells (Fig. 5E, F). Combination of ALC+LP improved rats' resistance to MTX, restoring their spermatogenic cells immunoreactivity and seminiferous tubules architecture (Fig. 5G). Comparing MTX-administered rats with controls, the area percentage of PCNA immune-positive cells was significantly decreased. As a result of co-administration of ALC with LP, the area percentage of PCNA-positive cells was significantly increased (Fig. 5H).

DISCUSSION

Chemotherapeutic drugs have been found to be highly harmful to testicular tissue and MTX, in particular, has demonstrated its gonadal toxicity (Waly *et al*., 2023). The study findings showed that there was a significant reduction in serum levels of total testosterone, LH, and FSH in the MTX group compared to the control group. The observed decline in serum testosterone levels in rats treated with MTX may be due to Leydig cell dysfunction. Similar results indicating that MTX-induced alterations in testosterone levels are related to a reduction in the number of LH receptors on Leydig cells (Felemban *et al*., 2020). The injection of MTX resulted in a decrease in testosterone levels, which can be attributed to a reduction in steroidogenesis (Badri *et al*., 2000). The administration of ALC and LP can restore the hormonal balance and biomarkers of testicular function to alleviate the toxic effects of MTX on the testes (Aly, 2019; Ekeleme-Egedigwe *et al*., 2019).

Oxidative stress is considered the primary contributor to anticancer drugs-induced testicular injury as MTX (Waly *et al*., 2023) and cisplatin (Sallam *et al*., 2021). The study suggested that the high content of polyunsaturated fatty acids in the testis wall can accelerate the process of lipid peroxidation caused by oxygen radicals. Our study found that administering MTX caused a noteworthy elevation in testicular MDA level and a significant reduction in the activity of SOD, CAT, and GSH enzymes. These findings are consistent with the results of earlier studies that have been published (Belhan *et al*., 2019; Abdelzaher *et al*., 2020).

Allicin and lycopene have the ability to remove free radicals, increase the antioxidant ability of cells, slow down the rate of cellular aging and death, and thus safeguard cellular functions (Chan *et al*., 2014, Aboubakr *et al*., 2021; Elsayed *et al*., 2022b). Administration of ALC or LP resulted in a reduction of previously elevated MDA levels, and an increase in GSH levels and the enzyme activities of CAT and SOD compared to the other three groups, particularly the MTX group. The addition of garlic oil to the diet of rats treated with cyclophosphamide was shown to protect the testicular antioxidant defenses,

as demonstrated by a significant increase in SOD and CAT enzyme activities, as well as the level of GSH, which were comparable to those of the normal control group. Additionally, the garlic oil supplementation inhibited lipid peroxidation, resulting in a significant decrease in MDA levels (Ekeleme-Egedigwe *et al*., 2019). Garlic oil contains allin, allicin, and other diallyl trisulfides (Liu *et al*., 2006). Additionally, comparable outcomes were observed in rats treated with LP to counteract testicular toxicity induced by gentamicin (Aly, 2019). Lycopene was found to have a protective effect against testicular toxicity caused by cyclosporine A (Türk *et al*., 2007), gentamicin (Aly, 2019), and diethylnitrosamine (Kaya *et al*., 2019).

The histopathological analysis of the testes confirmed that the decrease in antioxidant status caused significant oxidative damage and changes in morphology in the animals treated with MTX. However, the preservation of the testicular morphology observed in the animals treated with ALC or LP was similar to that seen in the control animals. This suggests that the chemoprotective effect of ALC or LP may be due to their anti-apoptotic and antioxidant properties. The administration of garlic oil in rats treated with cyclophosphamide improved the histopathological changes (Ekeleme-Egedigwe *et al*., 2019). Lycopene reduced histopathological changes in testes after cyclosporine A (Türk *et al*., 2007), gentamicin (Aly, 2019), diethylnitrosamine (Kaya *et al*., 2019) and cadmium administration (Iftikhar *et al*., 2022). Türk *et al*. (2007) found that cisplatin treatment resulted in reductions in the seminiferous tubules' diameter and germinative cell layer thickness, which led to abnormalities in spermatozoa and seminiferous tubule atrophy. However, the study also indicated that lycopene was able to protect against all of these harmful effects.

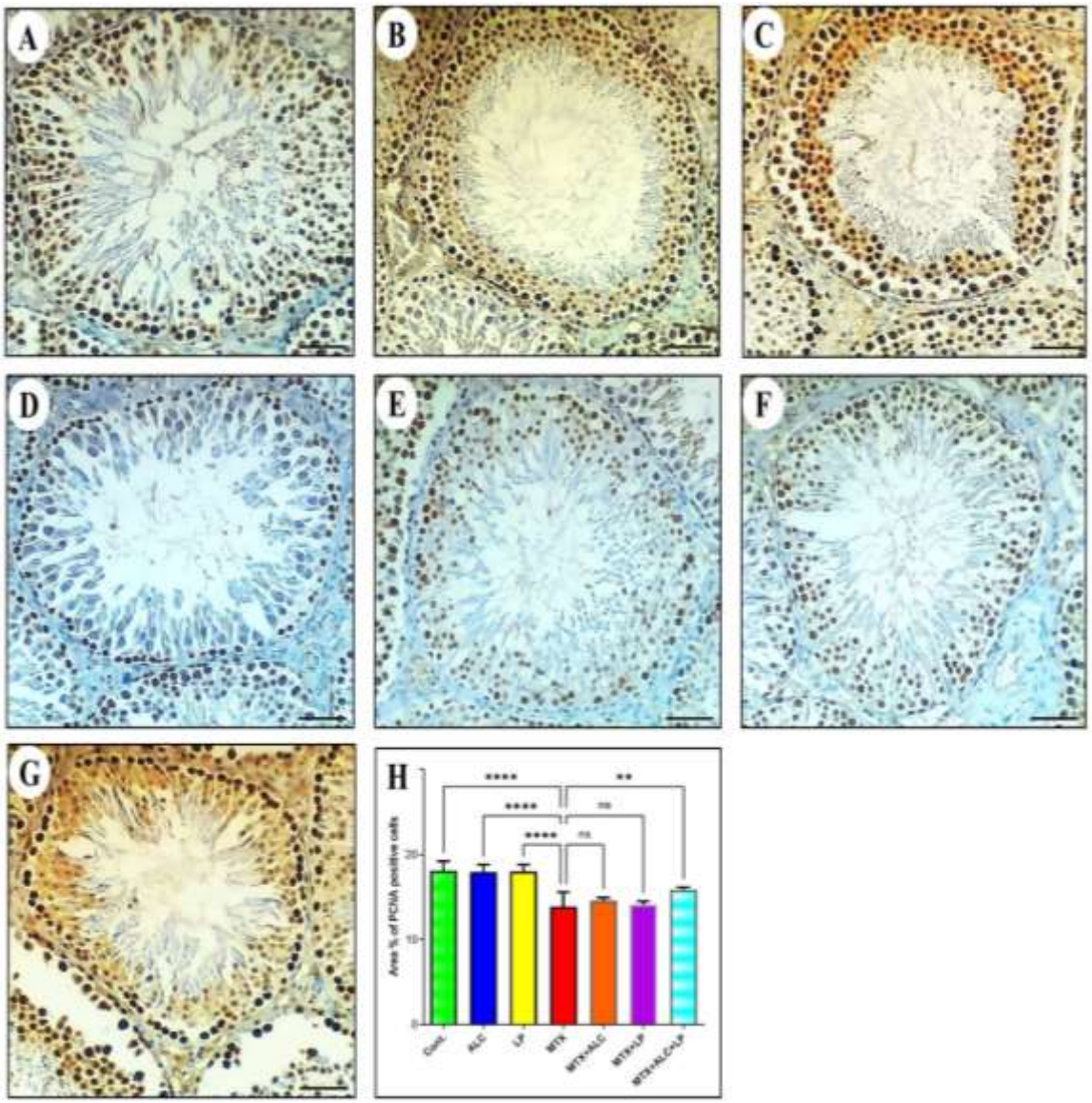

Fig. 5: PCNA activity in the nuclei of spermatogenic cells of control rats, PCNA immunoreactivity was positive (Figure 5A). Rats receiving either ALC (Figure 5B) or LP (Figure 5B) expressing intense immune positive reaction in nuclei (Brown nuclear reaction) of most of the basal spermatogenic cells in the seminiferous tubules comparable to control group. Rats intoxicated with MTX showing negative immunostaining in the nuclei of most seminiferous tubules (Figure 5D). Expressing a reduced immunoreactivity in nuclei of the basal spermatogenic cells in the seminiferous tubules in ALC+MTX (Figure 5E) and LP+MTX (Figure 5F) treated groups. Seminiferous tubules with multiple positive PCNA immunostaining of the nuclei of the germ cell in ALC+LP+MTX treated group (Figure 5G). Quantitative image analysis for PCNA-positive, brown-stained cells as a percentage of the whole area (Figure 5H).

The process of apoptosis is essential in the development of testicular damage caused by MTX (Sarihan *et al*., 2020). MTX causes a reduction in PCNA expression (Felemban *et al*., 2020), but treatment with ALC or LP can counteract this effect and increase PCNA expression. When ALC or LP was given along with MTX, it improved sexual toxicity, reduced oxidative stress, and prevented apoptosis caused by MTX. This suggests that ALC or LP may be effective in reducing the harmful effects and oxidative damage caused by MTX.

Lycopene, a type of carotenoid, is known to be the most effective at scavenging free radicals, as noted by Rao and Balachandran (2002). Its high lipid solubility allows it to cross the blood-testis barrier, which suggests that it may be an effective treatment for testicular toxicity caused by oxidative stress, according to Khachik *et al*., (2002). The protective effects of lycopene have been attributed to a reduction in ROS production and inhibition of the mitochondrial apoptotic pathway, as demonstrated (Aly, 2019; Iftikhar *et al*., 2022).

Conclusions: Allicin and lycopene was found to protect against MTX-induced reproductive dysfunction by reducing oxidative stress and apoptotic activity. The combined effect of these mechanisms may improve spermatogenesis, endocrine function, and cellular redox status in rats. As a result, ALC or LP may be a promising supplement during MTX therapy in order to enhance the reproductive health. However, further studies are needed to confirm the safety and efficacy of these agents in clinical settings. Other mechanistic studies would be required to provide a more complete understanding of how these compounds act to mitigate testicular injury.

Ethics approval: Faculty of Veterinary Medicine's Research Ethical Committee, Cairo University, Egypt (Vet CU 01122022620) approved the study.

Acknowledgments: The researchers would like to acknowledge Deanship of Scientific Research, Taif University for funding this work.

Authors contribution: Experiment design: MA (Mohamed Aboubakr), and AS. Data collection and statistical analysis: SI and ME. Histopathology & IHC: EK, MD, and AE (Ahmed Elfadadny). Writing and drafting: AE (Asmaa Elsayed), AF, and MA (Mohamed Alkafafy). All authors reviewed and edited the final version.

REFERENCES

Abdel-Daim MM, Abushouk AI, Donia T, *et al*., 2019. The nephroprotective effects of allicin and ascorbic acid against cisplatin-induced toxicity in rats. Environ Sci Pollut Res Int 26:13502-9.

Abdel-Daim MM, Kilany OE, Khalifa HA, *et al*., 2017. Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Cancer Chemother Pharmacol 80:745-53.

Abdelzaher W, Khalaf H, El-Hussieny M, *et al*., 2020. Role of nitric oxide donor in methotrexate-induced testicular injury via modulation of pro-inflammatory mediators, eNOS and P-glycoprotein. Hum Exp Toxicol 39:1700-9.

Aboubakr M, Elshafae SM, Abdelhiee EY, *et al*., 2021. Antioxidant and anti-Inflammatory potential of Thymoquinone and Lycopene mitigate the Chlorpyrifos-Induced toxic neuropathy. Pharmaceuticals 14:940. doi: 10.3390/ph14090940.

Aly H, 2019. Testicular toxicity of gentamicin in adult rats: Ameliorative effect of lycopene. Hum Exp Toxicol 38:1302-13.

Badri S, Vanithakumari G and Malini T, 2000. Studies on methotrexate effects on testicular steroidogenesis in rats. Endocr Res 26:247-62.

Belhan S, Çomaklı S, Küçükler S, *et al*., 2019. Effect of chrysin on methotrexate-induced testicular damage in rats. Andrologia 51:e13145. doi: 10.1111/and.13145.

Chan JY, Tsui HT, Chung IY, *et al*., 2014. Allicin protects rat cardiomyoblasts (H9c2 cells) from hydrogen peroxide-induced oxidative injury through inhibiting the generation of intracellular reactive oxygen species. Int J Food Sci Nutr 65:868-73.

Ekeleme-Egedigwe CA, Famurewa AC, David EE, *et al*., 2019. Antioxidant potential of garlic oil supplementation prevents cyclophosphamide-induced oxidative testicular damage and endocrine depletion in rats. J Nutr Intermed Metab 18:100109. https://doi.org/10.1016/j.jnim.2020.100109

Elsayed A, Elkomy A, Alkafafy M, *et al*., 2022a. Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats. Pak Vet J 42:107-11.

Elsayed A, Elkomy A, Alkafafy M, *et al*., 2022b. Testicular toxicity of cisplatin in rats: ameliorative effect of lycopene and N-acetylcysteine. Environ Sci Pollut Res Int 29:24077-84.

Elsayed A, Elkomy A, Elkammar R, *et al*., 2021. Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats. Sci Rep 11:13979. doi: 10.1038/s41598-021-93196-7.

Felemban SG, Aldubayan MA, Alhowail AH, *et al*., 2020. Vitamin B17 ameliorates methotrexate-induced reproductive toxicity, oxidative stress and testicular injury in male rats. Oxid Med Cell Longev 2020:4372719. doi: 10.1155/2020/4372719.

Hall P, Levison D, Woods A, *et al*., 1990. Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: An index of cell proliferation with evidence of deregulated expression in some, neoplasms. J Pathol 162:285-94.

Hassanein EH, 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. doi: 10.1016/j.intimp.2023.109969.

Iftikhar A, Akhtar MF, Saleem A, *et al*., 2022. Comparative potential of Zinc Sulfate, L-Carnitine, Lycopene, and Coenzyme Q10 on Cadmium-Induced male infertility. Int J Endocrinol 2022:6266613. doi: 10.1155/2022/6266613.

Ipek V, Kaya K, Cebi C, *et al*., 2022. Effects of fish oil on methotrexate-induced reproductive damage in rats. Andrologia 54:e14638. doi: 10.1111/and.14638.

James AS, Ugbaja RN, Ugwor EI, *et al*., 2023. Lycopene abolishes palmitate-mediated myocardial inflammation in female Wistar rats via modulation of lipid metabolism, NF-κB signalling pathway, and augmenting the antioxidant systems. Nutr Metab Cardiovasc Dis 33:671-81.

Kaya E, Ozer Kaya S, *et al*., 2019. Evaluation of ameliorating effect of lycopene against testicular toxicity due to diethylnitrosamine using biochemical, spermatological and histopathological data. Andrologia 51:e13274. doi: 10.1111/and.13274.

Khachik F, Carvalho L, Bernstein PS, *et al*., 2002. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. Exp Biol Med 227:845-51.

Liu C-T, Wong P-L, Lii C-K, *et al*., 2006. Antidiabetic effect of garlic oil but not diallyl disulfide in rats with streptozotocin-induced diabetes. Food Chem Toxicol 44:1377-84.

Lv R, Mao N, Wu J, *et al*., 2015. Neuroprotective effect of allicin in a rat model of acute spinal cord injury. Life Sci 143:114-23.

Mahmoud RH, Mohammed MA, Said ES, *et al*., 2021. Assessment of the cardioprotective effect of liraglutide on methotrexate induced cardiac dysfunction through suppression of inflammation and enhancement of angiogenesis in rats. Eur Rev Med Pharmacol Sci 25:6013-24.

Owumi SE, Ochaoga SE, Odunola OA, *et al*., 2019. Protocatechuic acid inhibits testicular and epididymal toxicity associated with methotrexate in rats. Andrologia 51:e13350. doi: 10.1111/and.13350.

Rao A and Balachandran B, 2002. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutr Neurosci 5:291-309.

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 41:147-51.

Sarihan KK, Yilmaz MY, Eraldemir FC, *et al*., 2020. Protective effects of apocynin on damaged testes of rats exposed to methotrexate. Turk J Med Sci 50:1409-20.

Türk G, Ateşşahin A, Sönmez M, *et al*., 2007. Lycopene protects against cyclosporine A-induced testicular toxicity in rats. Theriogenology 67:778-85.

Waly OM, El-Mahdy NA, El-Shitany NAE-A, *et al*., 2023. Protective role of naftidrofuryl against methotrexate-induced testicular damage via the amelioration of the p53/miRNA-29a/CDC42 apoptotic pathway, inflammation and oxidative stress. Environ Toxicol Pharmacol 98:104067. doi: 10.1016/j.etap.2023.104067.

Yin Z, Wang Q and Cheng H, 2023. Synergistic Protective Effect of Interactions of Quercetin with Lycopene Against Ochratoxin A-Induced Ulcerative Colitis. Appl Biochem Biotechnol doi: 10.1007/s12010-022-04287-8