Testicular Dysfunction in Malathion Induced Toxicity in Male Rats: Protective Role of NAC and Silymarin.

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KEYWORDS
Malathion;
Testicular toxicity;
NAC;
Silymarin;
Antioxidants

ABSTRACT

This study investigated the biochemical and histopathological testicular dysfunctions following subacute toxic exposure to malathion and the potential counteracting effects of N-acetyl cysteine (NAC) and Silymarin against these induced toxic effects. The study was carried out on 48 Sprague-Dawley male rats. They were divided into six equal groups as follow; control group: received distilled water, the group I: rats were treated with NAC at a dose 200 mg/kg/day; group II: rats were treated with Silymarin at a dose 200 mg/kg/day; group III: rats were given malathion at a dose of 200 mg/kg/day; group IV: rats were given malathion plus NAC; group V: rats were given malathion plus Silymarin. The drugs were given by gastric gavage daily for 30 days. Significant reductions in the testicular weights were observed in malathion exposed rats. Malathion caused significant reductions in follicle – stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, butyryl cholinesterase activity as well, sperm counts and motility with significant increases in abnormal sperm morphology. The histopathological findings showed diffusely necrotic seminiferous tubules and arrested spermatogenesis. Administration of NAC and Silymarin improved the biochemical and histopathological alterations in the testes induced by malathion. However, NAC was much better than Silymarin for inducing these protective effects.

Introduction

Pesticides are one of the chemical classes which have wide different biological activities and thus various potentials to produce deleterious effects in living organisms (Mehri et al., 2016). Organophosphorus (OPs) insecticides are considered the largest and the most diverse group of pesticides that has potentially hazardous impacts on humans, animals, plants and environmental ecosystems (soil, water, air and food). In humans, it produces severe acute and chronic poisoning; due to its many applications that account for its known low toxicity and specific selectivity for insects (Alavinia et al., 2019).

Malathion (MT) is the first synthesized OP and the fourth used agropesticide that is habitually used to eradicate ectoparasites, household insects, disease-inducing arthropods and human miticides, as well to conserve stored grain. It is well declared that it can cause physiological, biochemical, and histological changes due to its lipophilic nature with simple and rapid intestinal assimilation (Mehri et al., 2016). Besides, it inhibited cholinesterase's activity, induced oxidative stress that may be due to generation of reactive oxygen species (ROS) and redox-cycling activity (Selmi et al., 2018).

Oxidative stress is a key factor in the induction of male infertility. At the level of the spermatozoon, ROS causes DNA fragmentation and lipid peroxidation disrupting both its ability to support normal embryonic development and the motility of its cells. Thus at this stage, oxidative stress is capable of disrupting the capacity of the germinal epithelium to differentiate normal spermatozoa (Sharma and Gulati, 2017). The antioxidant system plays an effective role in

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protecting testis below a critical threshold of reactive oxygen species thus preventing testicular affection (Jain and Joshi, 2009).

Growing evidence for sterility in humans and various animals was accounted to various chemicals and pesticides in the environment (Bhardwaj et al., 2018). Natural compounds extracted from plants have been found to have efficient protective effects against some pathological conditions (Ahmadipour et al., 2016). A flavonoid with effective antioxidant properties is silymarin. Its antioxidant activity is due to its capacity to eliminate free radicals produced through the inhibition of the cyclooxygenase cycle and leukotrienes. It allows electron donation to free radicals and ROS to stabilize them and prevents lipid peroxidation by interaction with intracellular glutathione (Zaulet et al., 2017). In recent years, many studies have suggested that antioxidants such as N-acetylcysteine (NAC) can eliminate oxidative damages caused by MT (Ahmadipour et al., 2016; Bhardwaj et al., 2018).

Since many animal studies have linked the toxic effects of malathion to the dysfunction of several organs including testis (Bustos-Obregón and Gonzáles-Hormazabal, 2003; Choudhary et al., 2008; Geng et al., 2015; Zhang, et al., 2016).The main objective of this study is to find out the biochemical and histopathological testicular cchanges following subacute toxic exposure to malathion; and to investigate the potential counteracting effects of NAC, silymarin against these induced toxic effects in male Sprague Dawley rats.

Material and Methods

Chemicals and kits:

Commercial Malathion (57% commercial grade): in the white liquid form. It was purchased from Al Nasr Company for chemical industries, other chemicals (of high purity) and kits were obtained from Sigma-Aldrich Company. Malathion was dissolved in distilled water (solubility in water: 145 mg/L at 25^{o}C) before use. Silymarin powder was dissolved in 0.7% carboxymethyl cellulose.

Animals and experimental design:

Animals

Male Sprague Dawley rats (sexually mature), weighing approximately 250–300g, were obtained from the animal house Faculty of Pharmacy to be used throughout this study. They were housed in plastic cages and maintained at an ambient temperature of 20 ± 2°C and relative humidity (55 ± 5%). The lighting period was maintained at 12 h/12 h light and dark cycle. Food and tap water were provided ad libitum.

Ethical consideration:

In the current study we followed the ethical guidelines in accordance with the ethical norms approved by the Institutional Review Board – Mansoura Faculty of Medicine Mansoura University (R.19.05.520).

Experimental design and treatment

After one week of acclimatization, 48 rats were randomly divided into six groups including five exposure groups and one control group of 8 animals each. In the exposure groups (by oral gavage), group I: rats were treated with NAC at a dose 200 mg/kg/day (Ahmadipour et al., 2016); group II: rats were treated with Silymarin at a dose 200 mg/kg/day (Abdel-Ghany et al., 2016); group III: rats were given malathion at dosages of 200 mg/kg/day (equivalent to 1/5 of LD50) (Geng et al., 2015); group IV: rats were given malathion plus NAC (at same daily doses); group V: rats were given malathion plus silymarin (at same daily doses). The control rats (control group) were received an equivalent volume of distilled water in the same manner (one ml, by oral gavage). All animals were observed daily for clinical signs of toxicity related to malathion exposure.

In groups IV and V, both NAC and silymarin were given two hrs before malathion was administered.

Selected doses were chosen upon previous studies of the NAC and silymarin protective effects against malathion induced cellular dysfunctions in hepatic tissue, as scarce studies that investigate the protective effects on testicular tissues; including one in-vitro study that was conducted to investigate the role of NAC to protect against testicular tissue using NAC, 1 and 10 mM (Bhardwaj et al., 2018).

After 30 days of the exposure, fasted rats were anesthetized with ketamine (50 mg/kg) Five ml of blood will be taken from the retinal vein. After standing for one hr, blood was collected in sample bottles without anticoagulant; centrifuged rapidly for 15 min at 3000 rpm, sera were separated and frozen at -20 °C for later biochemical analysis. After blood sampling, all rats were sacrificed, testis was freshly separated from each rat and cleaned with an ice-cold 0.9% NaCl solution; for preparation to be evaluated by a histopathologist (Moridi et al., 2018).

The body weights of rats were recorded at the beginning and at the end of the study. Absolute testicular weights will be measured, expressed per 100 g body weight to normalize the data for statistical analysis.

Histobiochemical indictors:

1. Biochemical Hormones measurement:

Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T) levels were measured using RIA kits according to the manufacturer's protocols by Cobas e411 analyzer.

2. Butyryl cholinesterase assay:

The Butyryl cholinesterase activity was determined using a Sigma Diagnostic colorimetric kit (Egypt) using UNICO spectrophotometer 1200, WP0803053. UNICO Instruments CO, LTD.

Procedure: colorimetric kinetic method, according to manufacture instruction.

3. Sperm evaluation:

Immediately after dissection, epididymal sperm was collected and testicular tissues from each animal were placed in one ml phosphate buffered saline at 37^{o}C. The tunica albuginea was cut and removed. The remaining seminiferous tubules then were minced using surgical blades in the phosphate buffered saline.

For sperm count, the testicular cell suspension was pipetted and vortexed. One drop of the homogenous suspension placed on a counting chamber, and the testicular sperm count ($\times 10^6$/ml) was calculated; another one drop of the homogenous suspension placed on a slide, covered by a 24×24 mm coverslip, and examined under 200 × magnifications using a phase contrast microscope, and sperm motility was recorded as the ratio between the number of motile sperm and the total number of sperm (Moridi et al., 2018). After assessment of sperm motility, sperm suspension was used for the analysis of sperm morphology. Thus, one drop of the suspension was put onto a glass slide and stained by the commercial ready to use SperMac stain. In total, 2000 sperm on each slide were evaluated and the results were recorded as the percentage of abnormal sperm on each slide. Abnormal heads and tails were assessed by using specific criteria of Okamura et al. (2005) (Uzun et al., 2009).

4. Histopathological examination

The right testis from each rat was fixed in Bouin's solution for 8 h and then was embedded in a 10% neutral bu□ered formalin solution. The paraffin-embedded sections were prepared to be 5 μm thicknesses, and deparaffinized by 100% xylene, followed by rehydration with 100% then 70% ethanol. Transverse sections were stained with hematoxylin and eosin (HE) for light microscopic examination (Geng et al., 2015). Johnson's score was used to evaluate seminiferous tubules for their modified spermatogenesis index in all groups. The scoring method ranged from 1 (tubular section without any cell) to 10 (tubular section with a regular thickness of germinal epithelium with complete spermatogenesis stages) according to Johnsen, (1970) as shown in (Table 1).

Table (1): Modified Johnsen score.

Score	Histological findings
10	Full spermatogenesis.
9	Many late spermatids, disorganized tubular epithelium.
8	Few late spermatids.
7	No late spermatids, many early spermatids.
6	No late spermatids, few early spermatids, arrest of spermatogenesis at the spermatid stage, disturbance of spermatid differentiation.
5	No spermatids, many spermatocytes.
4	No spermatids, few spermatocytes, arrest of spermatogenesis at the primary spermatocyte stage.
3	Spermatogonia only.
2	No germ cells, Sertoli cells only.
1	No seminiferous epithelial cells, tubular sclerosis

Statistical analysis

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 21). The normality of data was first tested with Shapiro test. Continuous variables were presented as mean ± SD (standard deviation) for parametric data and median (min-max) for non-parametric data. ANOVA test was used to compare more than two means and post hoc LSD test was used to compare between each two groups. Kruskal Wallis test (for parametric data) was used to compare more than two medians and Mann Whitney test (for non-parametric data) was used to compare each 2 groups. Pearson correlation was used to correlate continuous parametric data while Spearman correlation was used to correlate non parametric data. The threshold of significance is fixed at 5%, it is considered significant when the probability of error is less than 5% ($p \leq 0.05$).

Results:

No toxic manifestations or mortality among the studied animals reported during the experimental period.

Table (2): Testicular and absolute weights of the studied groups of rats (n=48).

Variables	Control gp (n=8)	Group I (n=8)	Group II (n=8)	Group III (n=8)	Group IV (n=8)	Group V (n=8)	Test of significance	p-value
Testicular wt. (in grams)	1.81±0.09	1.95± 0.08 [a]	1.89± 0.04	1.46± 0.20 [abc]	1.81± 0.05[bde]	1.70± 0.07[abcd]	F=21.93	<0.001*
Absolute weight (in grams) Median (Min-Max)	0.05 (0.04-0.21)	0.031 (0.03-0.04)	0.03 (0.03-0.04)	0.15 (-0.2 -0.75)	0.043 (0.03-0.07`)	0.05 (0.04-0.09)	KW=20.7	0.001*

F: ANOVA test; **KW**: Kruskal Wallis test; ***** significant p <0.05; **wt**.: weight; **min**: minimum; **max**: maximum; **gp**: group; **group 1:** rats treated with NAC; **group II**: rats treated with Silymarin; **group III**: rats exposed to malathion; **group IV**: rats exposed to malathion+NAC; **group V**: rats exposed to malathion+silymarin; **a**: significant with control group; **b:** significant with group I; **c**: significant with group II; **d**:significant with group III; **e**: significant with group V.

Table (3): Histobiochemical indicators (LH, FSH, testosterone, butyryl cholinesterase) and histopathology of the studied rats (n=48).

Variables	Control gp (n=8)	Group I (n=8)	Group II (n=8)	Group III (n=8)	Group IV (n=8)	Group V (n=8)	ANOVA test	p-value
FSH (mIU/ml)	0.172 ± 0.02	0.20 ± 0.01[a]	0.193 ±0.01[a]	0.101 ± 0.01 [abc]	0.17 ± 0.02 [bcde]	0.128 ± 0.02 [abcd]	50.93	<0.001*
LH (mIU/ml)	0.308 ± 0.01	0.35 ± 0.01[a]	0.34 ±0.01[a]	0.159 ± 0.04 [abc]	0.296 ± 0.03 [bcde]	0.24 ± 0.02 [abcd]	83.98	<0.001*
T (ng/dl)	301.6 ± 13.4	320.1 ± 9.1	312.2 ±11.5	115.45 ± 33.5 [abc]	262.8 ± 18.3 [abcde]	232.7 ± 23.6 [abcd]	118.10	<0.001*
BCE (U/L)	125.32 ± 4.4	135.24±6.8[a]	124.02 ± 3.9 [ab]	98.41 ± 8.28 [abc]	113.97±7.37 [abcde]	104.95± 5.93[abcd]	F=38.0	<0.001*
Histopathology	9.37 ± 0.74	9.37 ± 0.74	9.37 ± 0.74	3.25 ± 2.1 [abc]	8.0 ± 2.67 [d]	8.12 ± 2.58 [d]	F=14.8	<0.001*

FSH: follicle stimulating hormone;, **LH**: luteinizing hormone; **T**: testosterone; **BCE**: butyryl cholinesterase; ***** significant p <0.05; **gp**: group; **group I**: rats treated with NAC; **group II**: rats treated with Silymarin; **group III**: rats exposed to malathion; **group IV**: rats exposed to malathion+NAC; **group V**: rats exposed to malathion+silymarin; **a**: significant with control group; **b:** significant with group I; **c**: significant with group II; **d**:significant with group III; **e**: significant with group V.

Table (4): Sperm evaluation (sperm counts, motility and morphology rate) in the studied rats (n=48).

Variables	Control gp (n=8)	Group I (n=8)	Group II (n=8)	Group III (n=8)	Group IV (n=8)	Group V (n=8)	ANOVA test	p-value
Sperm Count (10^6/ml)	162.8±13.33	175.25±8.02	164.7 ± 11.5	81.25±14.2[abc]	121.27±11.64 [abcd]	116.84±17.5 [abcd]	F=62.64	<0.001*
Sperm Motility (%)	85.0 ± 3.77	88.75 ±5.82	85.0 ± 6.54	53.75±9.16[abc]	72.5 ± 9.63 [abcd]	65.62 ±9.04 [abcd]	F=25.67	<0.001*
Abnormal Morphology (%)	5.0 (3-8)	3.5 (2-5)	4.5 (2-8)	18 (10-30)[abc]	7.0 (5-10) [bde]	11 (7-15) [abcd]	KW=20.18	<0.001*

F: ANOVA test;, **KW**: Kruskal Wallis test; ***** significant p <0.05; **gp**: group; **group I**: rats treated with NAC; **group II**: rats treated with Silymarin; **group III**: rats exposed to malathion; **group IV**: rats exposed to malathion+NAC; **group V**: rats exposed to malathion+silymarin; **a**: significant with control group; **b:** significant with group I; **c**: significant with group II; **d**: significant with group III; **e**: significant with group V.

In the present study, tables 3 and 4 show that the all tested parameters (biochemical and histopathological) were significantly reduced in the group of rats treated with malathion (group III), compared to the control groups, as well the groups of rats received combined malathion and NAC (group IV); and combined malathion and silymarin (group V). However, significant improvements were observed in both groups IV and V compared to group III. By comparing the parameters in group IV to that of group V, it was observed that there was significant improvement in group IV treated with NAC to that of group V treated with silymarin.

As shown in figures 1 and 2, the histopathological changes show that group III that was treated with malathion showed diffusely necrotic transversely cut seminiferous tubules with irregular contour, arrested spermatogenesis; when compared to the control group' histological findings. These findings improved in the groups treated with antioxidants NAC and silymarin, wherein both groups IV and V, there were some necrotic and degenerated seminiferous tubules in some sections with normal appearance with the rest of sections.

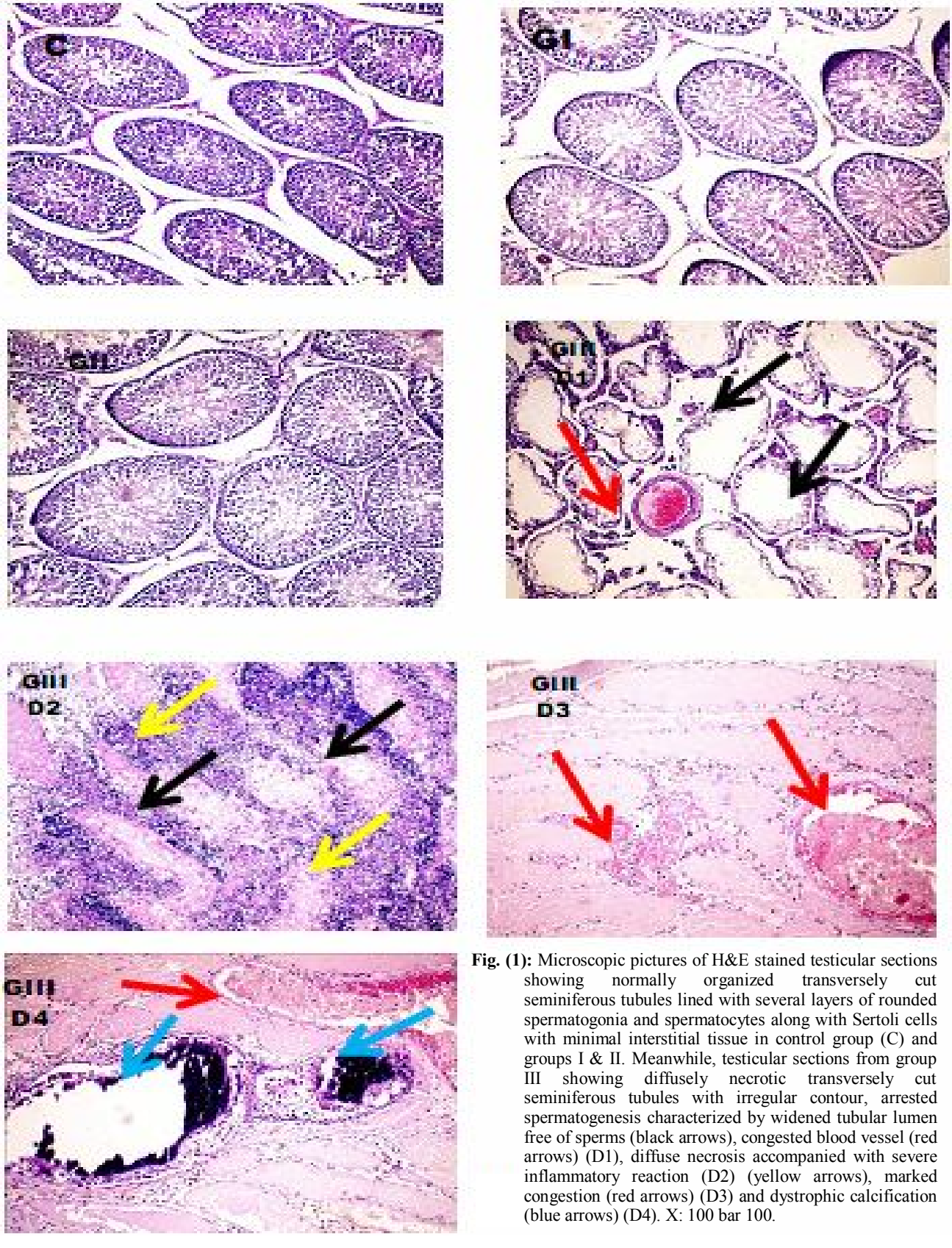

Fig. (1): Microscopic pictures of H&E stained testicular sections showing normally organized transversely cut seminiferous tubules lined with several layers of rounded spermatogonia and spermatocytes along with Sertoli cells with minimal interstitial tissue in control group (C) and groups I & II. Meanwhile, testicular sections from group III showing diffusely necrotic transversely cut seminiferous tubules with irregular contour, arrested spermatogenesis characterized by widened tubular lumen free of sperms (black arrows), congested blood vessel (red arrows) (D1), diffuse necrosis accompanied with severe inflammatory reaction (D2) (yellow arrows), marked congestion (red arrows) (D3) and dystrophic calcification (blue arrows) (D4). X: 100 bar 100.

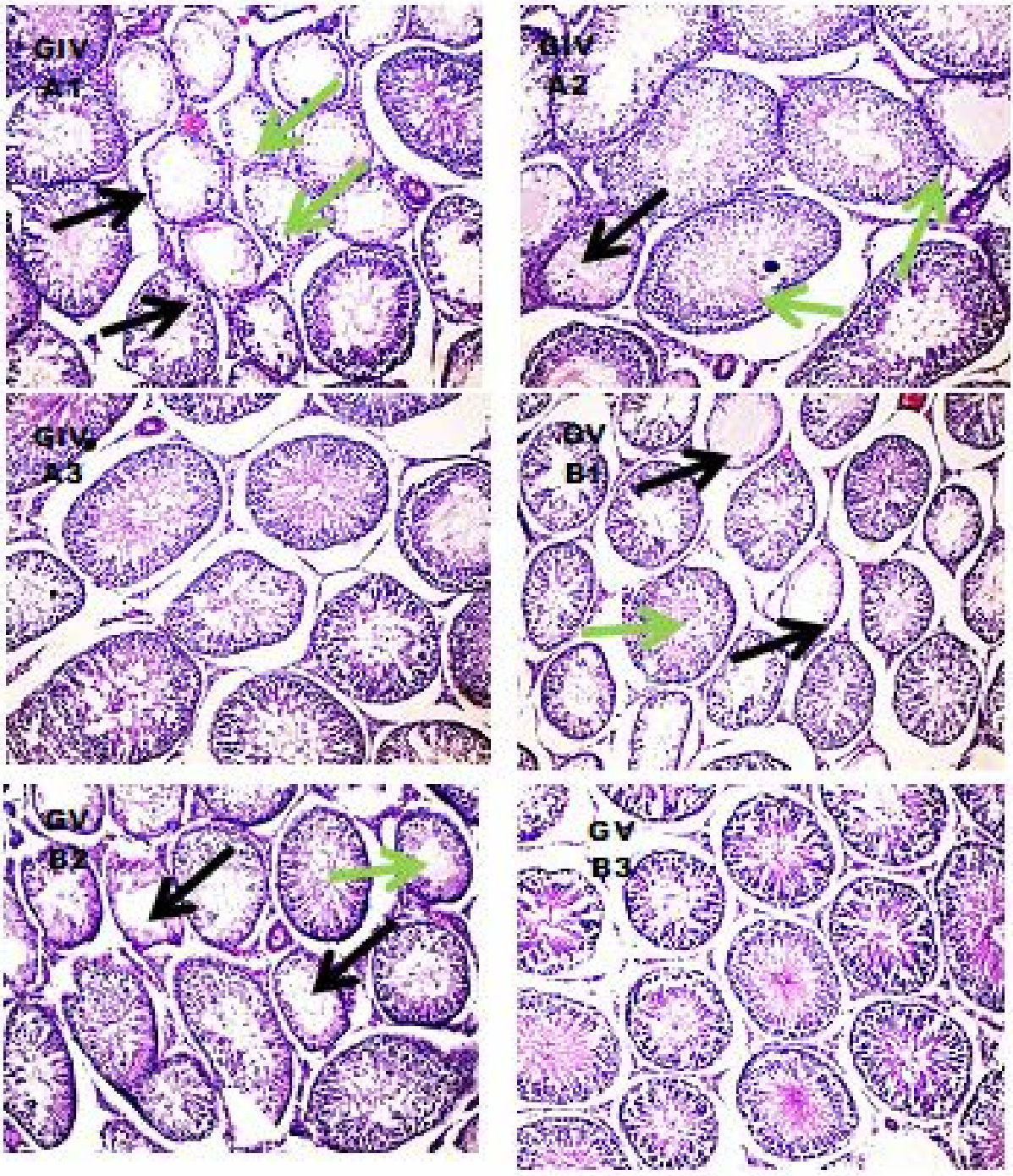

Fig. (2): Microscopic pictures of H&E stained testicular sections from group IV showing some necrotic (black arrows) and degenerated (green arrows) transversely cut seminiferous tubules (A1&A2) in some sections and normal appearance in other sections (A3). X: 100 bar 100.
Microscopic pictures of H&E stained testicular sections from group V showing few necrotic (black arrows) and degenerated (green arrows) transversely cut seminiferous tubules (B1&B2) in some sections and normal appearance in other sections (B3).

Table (5): Correlations between all studied parameters in each studied group of rats (cont.).

Variables		Control gp					Group I					Group II				
		Sperm Count	Sperm Motility	Abnormal Morphology (%)	BCE	Histopathology	Sperm. Count	Sperm Motility	Abnormal Morphology (%)	BCE	Histopathology	Sperm Count	Sperm Motility	Abnormal Morphology (%)	BCE	Histopathology
Testicular weight	r	.795	.729	-.936	.672	-.067	.172	.280	-.238	.894	.702	.685	.808	-.577	.912	-.406
	p	.018*	.040*	.001*	.068	.875	.684	.502	.571	.003*	.052	.061	.015*	.134	.002*	.318
FSH	r	.956	.805	-.955	.696	-.102	.620	.547	-.267	.641	.662	.878	.957	-.502	.759	-.074
	p	<0.001*	.016*	<0.001*	.055	.810	.101	.160	.523	.087	.074	.004*	<0.001*	.205	.029*	.862
LH	r	.870	.941	-.998	.888	.093	.376	.267	.037	.360	.774	.910	.859	-.407	.676	.146
	p	.005*	.001*	<0.001*	.003*	.826	.359	.523	.931	.381	.024*	.002*	.006*	.317	.066	.730
T	r	.516	.375	-.690	.344	-.179	.734	.614	-.548	.577	.344	.936	.872	-.384	.723	.039
	p	.191	.360	.058	.404	.671	.038*	.105	.160	.134	.405	.001*	.005*	.348	.043*	.927

FSH: follicle stimulating hormone; **LH**: luteinizing hormone; **T**: testosterone; **BCE**: butyryl cholinesterase; * significant p <0.05; **gp**: group**; group I**: rats treated with NAC; **group II**: rats treated with Silymarin

Table (5): Correlations between all studied parameters in each studied group of rats.

Variables		Group III					Group IV					Group V				
		Sperm Count	Sperm Motility	Abnormal Morphology (%)	BCE	Histopathology	Sperm. Count	Sperm Motility	Abnormal Morphology (%)	BCE	Histopathology	Sperm Count	Sperm Motility	Abnormal Morphology (%)	BCE	Histopathology
Testicular weight	r	.961	.862	-.916	.973	.615	.861	.867	-.767	.569	-.587	.551	-.596	.804	-.046	.861
	p	<0.001*	.006*	.001*	<0.001*	.105	.006*	.005*	.026*	.141	.126	.157	.119	.016*	.914	.006*
FSH	r	.849	.843	-.916	.809	.849	.942	.919	-.880	.743	-.706	.646	-.739	.836	.302	.942
	p	.008*	.009*	.001*	.015*	.008*	<0.001*	.001*	.004*	.035*	.050	.084	.036*	.010*	.468	<0.001*
LH	r	.978	.955	-.995	.973	.831	.944	.925	-.953	.611	-.507	.803	-.840	.738	-.040	.944
	p	<0.001*	<0.001*	<0.001*	<0.001*	.011*	<0.001*	.001*	<0.001*	.108	.200	.016*	.009*	.036*	.924	<0.001*
T	r	.967	.922	-.975	.931	.945	.930	.911	-.915	.684	-.634	.843	-.907	.763	.164	.930
	p	<0.001*	.001*	<0.001*	.001*	<0.001*	.001*	.002*	.001*	.061	.091	.009*	.002*	.028*	.698	.001*

FSH: follicle stimulating hormone;, **LH**: luteinizing hormone; **T**: testosterone; **BCE**: butyryl cholinesterase; * significant p <0.05; **group III**: rats exposed to malathion; **group IV**: rats exposed to malathion+NAC; **group V**: rats exposed to malathion+silymarin

Table (6): Total correlations between the studied parameters among all the studied groups.

		Total				
		Sperm Count	**Sperm Motility**	**Abnormal Morphology (%)**	**BCE**	**Histopathology**
Testicular weight	**r**	.858	.858	-.932	.879	.687
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
FSH	**r**	.931	.918	-.878	.911	.648
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LH	**r**	.929	.924	-.937	.897	.728
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
T	**r**	.938	.913	-.928	.871	.780
	p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

FSH: follicle stimulating hormone; **LH**: luteinizing hormone; **T**: testosterone; **BCE**: butyryl cholinesterase.

Discussion:

Presently, malathion is one of the environmental pollutants that threatens the ecosystem (Alavinia et al., 2019). Several previous studies have demonstrated the detrimental effects of malathion on male reproduction (Bustos-Obregón and Gonzles-Hormazabal, 2003; Uzun et al., 2009; Geng et al., 2015; Nahid et al., 2016).

Regarding LH, FSH, the study results were in agreement with Uzun et al. (2009) who found reductions in plasma FSH, LH after treatment of rats with malathion. As well, testosterone findings of the present study go in accordance with Simic et al. (1992) who declared that malathion inhibited the formation of 3 alpha-DHT in rat prostatic tissue in a study that was conducted on rat prostatic tissues in-vitro. As well, the study conducted by Bustos-Obregón and Gonzles-Hormazabal (2003) and Uzun et al. (2009), who observed a reduction in plasma testosterone levels after treatment of mice with a single dose of malathion. Geng et al. (2015) declared a reduction in hormonal assay including FSH, LH and testosterone with histopathological alterations in testicular rats as markers for the deleterious effects of malathion on male rat reproductive system.

Regarding the histopathological findings, the results of the present study were similar to those conducted by Uzun et al. (2009) and Nahid et al. (2016). Both researchers found that malathion induced degenerative histopathological changes in testicular tissues. As declared by Uzun et al. (2009), pesticides cause different histopathological and cytopathological changes in the male reproductive system. They accounted for such toxic effects to their ability to cross blood testicular barrier, induction of oxidative stress and lipid peroxidation that damages the testicular membranes, the seminiferous tubules and interstitial tissue, namely, necrosis and edema. This subsequently causes degeneration of the spermatogenic and Leydig cells, a mechanism that explains the reduction of sperm counts due to the disruption of spermatogenesis.

Uzun et al. (2009) reported that oxidative stress affects the sperm activities of mitochondrial enzymes and the structure of the microtubules resulting in the reduction of sperm motility. As well, DNA damage which increases abnormal sperm counts and disrupt spermatogenesis as the sperm morphology is controlled by various autosomal and Y-specific.

Similarly, Nahid et al. (2016) explained their results by oxidative stress after the treatment of some cytotoxic agents such as malathion. Another study that was conducted by Geng et al. (2015); they found that malathion induced dose-dependent effects on spermatogenesis with various histopathological changes in male rats. However, they explained these changes by the ability of malathion to increase the rate of apoptosis in spermatogenic cells; by increased expression of Bax and decreased Bcl-2. Thus, causing delayed spermatogenic cell differentiation and proliferation, and reduced number of sperm cells and spermatozoon.

By studying the sperm parameters, similar results were declared by Uzun et al. (2009); Jain and Joshi (2009) and Moridi et al. (2018), in their study on the toxic effects of malathion on testicular tissue they observed significant reductions in sperm counts and motility with a marked significant increase in abnormal sperm morphology.

In a study that was conducted by Silmen et al. (2014); they observed that malathion lowered testosterone levels, inhibited acetylcholinesterase activity, decreased male reproductive performance with an alteration of semen parameters on exposure to malathion. The lowering effect of malathion on the cholinesterase activity has been proved in this study, as well in the study of Alavinia et al. (2019). They demonstrated significant reversible reductions in cholinesterase activities after rainbow trout treatment with malathion.

This study was the first to investigate and compare the protective antioxidants effects of both NAC and silymarin on testicular tissues. By comparing the group treated with combined malathion and NAC to that treated with combined malathion and silymarin, it was found that NAC has a better improving effect on the toxic action of malathion on testicular tissue. Only one study that was conducted by Bhardwaj et al. (2018) that studied the protective effect of NAC against malathion induced apoptosis in testicular germ cells. They found that NAC mitigated the toxic effects of malathion that is responsible for subsequent infertility.

It has been declared that exposure to OP induces oxidative stress in in-vivo models as the main mechanism of action for OP; the enzymes associated with antioxidant defense are considered the main targets molecular units that are altered due to lipid peroxidation. Depletion of mitochondrial energy (ATP), induction of proteolytic enzymes and DNA fragmentations caused by lipid peroxidation leading to apoptosis in the major biomolecules such as lipids, proteins and nucleic acids that may lead to histopathological changes in the tissues, suggesting a role for oxidative stress mechanism of OPs toxicity (Ojha, et al., 2011).

Supporting the findings that malathion exerts their deleterious effects by promoting destructive oxidation of lipids, proteins and DNA within the testis (Uzun et al., 2009). As well, both NAC and silymarin are well known antioxidant agents (Zaulet et al., 2017 and Bhardwaj et al., 2018). Thus this explains that co-treatment of malathion-exposed rats with NAC and silymarin showed protective effects with a significant reduction in evident damage in testicular tissues may be due to their ability to react with the oxygen metabolites.

Conclusion:

To the best of our knowledge, this is the first study to investigate the protective effects of both NAC and silymarin on malathion induced toxic reproductive dysfunctions in male Albino rats, that was evident and reversible. However, NAC was much better than silymarin for inducing these protective effects. Results of this research can be useful to conduct the bio-magnification studies in the future on a larger sample size; to prove the ability to use these drugs as protective and therapeutic modalities to high risk group males who are exposed to malathion for a long period of time and having risk to develop reproductive dysfunctions.

Conflict of Interest Statement:

Authors have no conflict of interest regarding this research with anyone.

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اضطراب وظائف الخصي التي يسببها الملاثيون في ذكور الجرذان: الدور الوقائي لكل من NAC وسيليمارين

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يهدف هذا البحث الى دراسة الاختلالات البيوكيميائية والهيستوباثولوجية نتيجة التعرض السام للملاثيون. ودور كل من N-acetyl cysteine (NAC) و سيليمارين ضد هذه التأثيرات السامة. أجريت الدراسة على ٤٨ من ذكور Sprague-Dawley ، وتم تقسيم الفئران إلى ست مجموعات متساوية ، وتم إعطاء الأدوية عن طريق المعدة يومياً لمدة ٣٠ يومًا على النحو التالي ؛ المجموعة الضابطة: تلقت ماءً مقطرًا ، المجموعة الأولى: تم علاج الفئران باستخدام NAC بجرعة ٢٠٠ مجم / كجم / يوم ؛ المجموعة الثانية: تم علاج الفئران بسيليمارين بجرعة ٢٠٠ مجم / كجم / يوم ؛ المجموعة الثالثة: أعطيت الفئران ملاثيون بجرعة ٢٠٠ مجم / كجم / يوم ؛ المجموعة الرابعة: أعطيت الفئران الملاثيون بالإضافة إلى NAC ؛ المجموعة الخامسة: أعطيت الفئران ملاثيون بالإضافة إلى سيليمارين. ولوحظ انخفاض كبير في أوزان الخصي في الفئران المعرضة للملاثيون. تسبب الملاثيون في حدوث انخفاضات كبيرة في مستوى هرموني FSHو LH وهرمون التستوستيرون و في نشاط إنزيم الكولين استريز أيضًا ، و نقص عدد الحيوانات المنوية وحركتها مع زيادة كبيرة في أعداد الحيوانات المنوية غير الطبيعية. استخدام كل من NAC و Silymarin يؤدي الى تحسن في التغيرات البيوكيميائية والنسيجية في الخصيتين التي يسببها الملاثيون. و يعتبر NAC أفضل من Silymarin لإحداث هذه التأثيرات الوقائية.