



Equisetum arvense L. Extract Ameliorates Oxidative Stress, Inflammation and Testicular Injury Induced by Methotrexate in Male Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Methotrexate (MTX) is a cytotoxic drug used to treat a wide range of cancers and non-cancerous conditions. However, it can cause unfavorable acute toxic effects in several organs, including the testis. *Equisetum arvense* L. (*E. arvense*) extract is effective in counteracting oxidative stress-related disorders. This study assessed the preventive effect of *E. arvense* extract against MTX-induced testicular toxicity. Gas chromatography-mass spectroscopy (GC-MS) was used to analyze the active constituents of *E. arvense* extract. Testicular toxicity was induced via MTX injection (0.5 mg/kg/ twice a week for 4 weeks). Forty male albino rats were divided into 4 groups: I- control (Cont); II: MTX; III: *E. arvense* (500 mg/kg/daily for 10 weeks); and IV: *E. arvense* + MTX. *E. arvense* main active constituents were squalane (15%), ascorbic acid per methyl (9.55%), phytol (8.69%), 2-pyrroline 1,2-dimethyl (8.63%), and octacosane (8.23%). Treatment of MTX injected rats with *E. arvense* produced a significant rise in body weight, serum testosterone and luteinizing hormone. *E. arvense* significantly increased the sperm counts, viability, and motility relative to the MTX injected rats. The levels of testicular oxidative stress and inflammation significantly reduced in

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the MTX rats treated with *E. arvense*. Furthermore, *E. arvense* markedly improved the testicular tissue and seminiferous tubules' pathologic features in MTX-treated rats. *E. arvense* significantly decreased lipid peroxidation products, interleukin-1 beta, tumor necrosis factor-alpha while increasing superoxide dismutase levels. *E. arvense* prevented MTX-induced testicular damage via anti-inflammatory and antioxidant activities.

Keywords: Methotrexate; *Equisetum arvense* L.; testicular toxicity; antioxidant; anti-inflammatory.

1. INTRODUCTION

In recent years, the epidemiology of cancers in Saudi Arabia has increased threefold [1]. Cancer treatment includes the use of numerous chemotherapeutic agents [2]. MTX is a folic acid reductase inhibitor cytotoxic agent used to treat a large number of malignant tumors (acute lymphoblastic leukemia, osteosarcoma, lung, breast, skin, neck, and head cancers) and non-neoplastic diseases (rheumatoid arthritis) [3,4]. MTX induced severe side effects like nerve, liver, kidney, and lung damage; in addition to diarrhea, mouth sores, hair loss, and reduced blood cell counts [5-7]. Furthermore, several studies have shown that MTX induced damage of testicular seminiferous tubule, apoptosis of spermatocyte, reduction of sperm number, and infertility in males [7,8,9-11]. Numerous studies have linked MTX-induced testicular damage to an imbalance between antioxidant factors and free radical levels [7,12-14].

Medicinal plants and their extracts are good sources of exogenous antioxidants [15-17]. Aside from their excellent effectiveness, they have a low risk of toxicity when used [18]. Several research investigated the effectiveness of antioxidant-rich plants in decreasing and preventing testicular damage caused by MTX [7,8,19].

Equisetum arvense L. (*E. arvense*) called horsetail, is a member of the Equisetaceae family [20]. It's widely utilized in Saudi Arabia because it's suggested for a variety of diseases in folk medicine [21]. *E. arvense* LD50 value reaches 5000 mg/kg, making it a safe plant [22]. Several studies have shown the efficacy of *E. arvense* extract in preventing and treating diseases and disorders caused by oxidative stress [23-26]. *E. arvense* proved to have antioxidant, antitumor, antifungal, analgesic, antibacterial, anti-inflammatory, anticonvulsant, and cytotoxic activities [27-30]. Antidiabetic, renoprotective, and hepatoprotective effects of methanolic *E. arvense* extract have been also demonstrated [31-32]. The plant is used to treat tuberculosis,

gout, rheumatic diseases, ulcers, swelling, and fractures [33-34]. It also proved to cure wounds in animals and humans due to its antioxidants compounds which neutralize, prevent, or hinder ROS [35].

As per our knowledge, no researches assessing the preventive effect of *E. arvense* extract against MTX-induced testicular toxicity in rats. This research was designed to determine the probable protective role of *E. arvense* extract against MTX-induced testicular toxicity and explain its protective mechanism by determining its antioxidants and anti-inflammatory properties.

2. METHODOLOGY

2.1 Drugs, Chemicals, and Kits

MTX "Ebewe, 2.5 mg methotrexate/tablet, Haupt Pharma Amareg GmbH, Regensburg, Germany". All chemicals with high grade were bought from Sigma-Aldrich Chemical Co, USA. Enzyme-linked immunosorbent assay (ELISA) kits to measure the serum testosterone hormone (TH) and luteinizing hormone (LH) purchased from Centronic Chemicals Co, Germany. ELISA kits to measure the testicular antioxidant (thiobarbituric acid reactive substances, TBARS) and superoxide dismutase (SOD), and inflammatory markers (tumor necrosis factor-alpha (TNF- α) and interleukin 1 β (IL-1 β)) were purchased from Glory Science Co., Ltd. Del Rio-TX-USA.

2.2 *E. arvense* Extraction

The aerial parts of the *E. arvense* were purchased from Haraz for Herbs and Medicinal Plant Company Cairo, Egypt. The dried plant was ground using a blender, then 100 g was extracted with ethanol (500 ml, 70 %) in a conical flask, three times in a dark room. The collected extracts were filtered, evaporated to dryness under low pressure at 40 °C using a rotary evaporator, then freeze-dried [36]. The extract was stored at 4 °C for further use.

2.3 Analysis of *E. arvensis* Active Constituents

Analysis of the *E. arvensis* extract was done using a gas chromatography-mass spectroscopy (GC-MS) (Agilent Technologies 7890A) instrument, which connected to a mass-specific detector (MSD, Agilent 7000). Helium gas was used as an eluent and a carrier. Results were represented as a graph from the signal called a chromatogram (the Y-axis measures the intensity of the signal to quantify the component in the extract, and the X-axis shows the retention time (RT)). Each peak is considered a signal created when a compound was eluted from the GC column into the MSD. The extract's constituents were identified by comparing their RT and mass spectra segmentation patterns with those stored in the library of authentic compounds and published literature [37].

2.4 Antioxidant Contents of *E. arvensis*

E. arvensis total phenols were evaluated by the Folin-Ciocalteu method [38], total flavonoids were assessed by Dowd method using aluminum chloride colorimetric method [39], and total antioxidant substances were assessed by the phosphomolybdenum method [40].

2.5 Animal and Experiment Design

Forty male albino rats (170-200 g) were purchased from the animal unit, King Fahd Medical Research Center, KAU. All rats before the experiment were fed a well-balanced diet, had access to unlimited water and kept in standard laboratory conditions for one week as an acclimatization period. Rats were divided into four groups as follows:

Group I: Control (Cont): rats were intraperitoneally (i.p.) injected sodium citrate buffer twice a week for 4 weeks and ingested orally distilled water for 10 weeks.

Group II: MTX: rats were injected i.p. with MTX (0.5 mg/kg body weight/twice a week) for four weeks [41-42].

Group III: *E. arvensis* (ethanolic extract of *E. arvensis*): rats were ingested orally with *E. arvensis* extract for 10 weeks at a dose of 500 mg/kg [43].

Group IV: *E. arvensis* + MTX: MTX-injected rats (as in group II) were ingested orally with *E. arvensis* ethanolic extract for 10 weeks.

The body weight for each rat was measured weekly, and the bodyweight gain percentage (BWG%) was calculated. After 10 weeks, all rats were sacrificed, blood samples were individually collected, and serum samples were separated by centrifugation at 3000 rpm for 10 min, then kept at -80°C for further use in hormones analysis. Testes were collected and preserved either frozen at -80°C (for oxidative stress and inflammation measures) or in 10% buffered formalin solution (for histopathology).

2.6 Sperm Characteristics

To collect semen samples, the cauda epididymides were removed immediately after the rats were sacrificed. The sperm number was counted under the light microscope (LM) in 10 μL of the diluted specimen (1:20 dilution) by hemocytometer. The number of motility sperm was counted under LM. The viability of sperm was measured by mixing 20 μL of sperm suspension with 20 μL of eosin solution (1%), added 5 % nigrosin solution, then calculated the percent of life (discolored) and dead (abnormal head and tails) (appear pink) sperms under LM [44].

2.7 Hormones Assay

Serum levels of TH and LH were measured using ELISA kits.

2.8 Inflammatory Measures Assay

The homogenized testicular tissues were used to measure TNF- α and IL-1 β levels by ELISA kits.

2.9 Oxidative Stress Measures Assay

The homogenized testicular tissues were used to measure the content of TBARS and SOD activity by ELISA kits.

2.10 Histopathological Examination

After the routine procedure for testicular samples and hematoxylin and eosin (H & E) staining, the testicular tissues were examined under LM to detect any pathological changes.

2.11 Morphometric Examination

The dimensional measurements of the thickness of the dividing cells, including the secondary spermatocytes and primary spermatocyte size, and the full thickness of cellular contents of the tubules, which include spermatozoa, were recorded.

2.12 Statistical Calculations

The obtained data were represented as mean \pm SD; means were compared using the LSD test. SPSS version 25 for windows was applied to carry out the statistical calculations of the findings, considering the significance level at $P \leq 0.05$.

3. RESULTS

3.1 Active Constituents of *E. arvensis* (GC-MS)

The GC-MS chromatogram analysis of *E. arvensis* demonstrated the presence of 28 components corresponding to the peaks Fig. (1) and Table (1). Analysis of *E. arvensis* extract proved the presence of major components as follow: squalane (15%) at 18.863 RT; ascorbic acid, per methyl-(9.55%) at 20.356 RT; phytol (8.69%) at 14.553 RT; 2-pyrroline, 1,2-dimethyl-(8.63%) at 8.771 RT; and octacosane (8.23%) at 17.621 RT. Followed by flavone, 5-hydroxy-3,3',4',6,7-pentamethoxy-(6.94%) at 22.636 RT; heptacosane (6.34%) at 18.752 RT; geranyl isovalerate (6.10%) at 21.508 RT and salicylic acid β -D-O-glucuronide (5.93%) at 16.583 RT.

3.2 Total Phenols, Flavonoids, and Antioxidants of *E. arvensis* Extract

The extract of *E. arvensis* contain total phenols amounted 291.10 ± 15.84 (mg/ 100 g gallic acid equivalent); total flavonoids amounted 309.48 ± 6.53 (mg/100g quercetin equivalent); and total antioxidants amounted 2405.5 ± 10.62 (mg/100g ascorbic acid equivalent).

3.3 *E. arvensis* Improved the Sperm Parameters in MTX Injected Rats

When sperm parameters were calculated, it was discovered that the number of cauda epididymis sperms in the MTX group was significantly lower than in the Cont group ($p \leq 0.001$). Furthermore, in the MTX group compared to the Cont group, there were significant decreases in sperm viability and motility percent, as well as a significant increase in sperm abnormalities percent ($p \leq 0.001$). Ingestion of *E. arvensis* extract (500 mg/kg) significantly reverses the impairment of the sperm parameters induced by MTX. The sperm count, motility, and viability percent all increased significantly (61.9 ± 9.48 ; 77.90 ± 5.78 and 79.73 ± 8.02 vs. 35.9 ± 4.82 ; 42.50 ± 8.61 and 50.70 ± 8.78 , for the Cont and the MTX group, respectively), concurrent with a significant decrease in the sperm abnormalities % (9.22 ± 2.04 vs. 39.32 ± 6.63 , for the Cont and the MTX group, respectively). The *E. arvensis* group showed significant increases in sperm count, motility, and viability concurrent with a significant reduction in sperm abnormalities % relative to the Cont group Table (4).

3.4 *E. arvensis* increased TH and LH in MTX Injected Rats

The results revealed that MTX-induced significant decreases in serum TH and LH levels relative to the Cont group ($p \leq 0.001$). The TH and LH levels were elevated significantly in the *E. arvensis* + MTX relative to the MTX group ($p \leq 0.001$). There were no major variations between the *E. arvensis* and the Cont group Fig. (2).

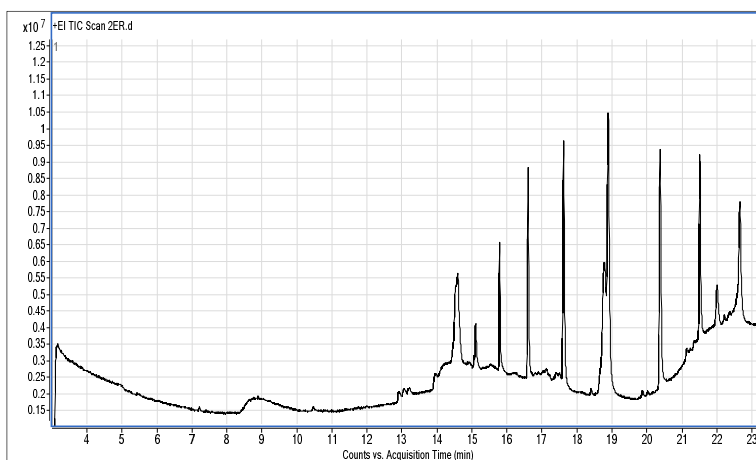


Fig. 1. GC-MS Chromatogram analysis of *E. arvensis* extract major constituents

Table 1. Major phytochemical active constituents in *E. arvense* extract (GC-MS)

Serial No.	Constituents	RT (min)	Concentrations (%)
1	Cubebol	5.511	1.59
2	Gentisic acid	7.221	0.67
3	2-Pyrroline, 1,2-dimethyl-	8.771	8.63
4	γ -Tocotrienol	10.522	0.54
5	3,2',4',5'-Tetramethoxyflavone	10.788	1.04
6	2'-Hydroxy-3,4,5-trimethoxychalcone	12.945	1.11
7	3-(3,4-Dimethoxyphenyl)-4-methylcoumarin	13.089	1.05
8	3,6,3',4'-Tetramethoxyflavone	13.192	0.97
9	6,7,4'-Trimethoxyisoflavone	13.963	1.56
10	Phytol	14.553	8.69
11	Hexa-hydro-farnesol	15.103	1.46
12	Gardenin	15.574	1.71
13	Pentacosane	15.796	3.46
14	3,5-di-tert-Butylcatechol	16.263	1.55
15	Salicylic acid β -D-O-glucuronide	16.583	5.93
16	Probucol	17.079	1.5
17	Vanillic acid	17.424	1.6
18	Octacosane	17.621	8.23
19	Heptacosane	18.752	6.34
20	Squalane	18.863	15.00
21	Flavone, 3,5,7-trimethoxy-	19.868	0.91
22	Coniferyl aldehyde	20.032	0.78
23	Ascorbic acid, permethyl-	20.356	9.55
24	Epicubebol	21.152	0.75
25	Geranyl isovalerate	21.508	6.10
26	Propyl gallate	22.029	1.62
27	2,6-Dihydroxybenzoic acid	22.23	0.71
28	Flavone, 5-hydroxy-3,3',4',6,7-pentamethoxy-	22.636	6.94
	Non-identified compounds	> 22.7	0.01

RT: Retention time

Table 2. Total phenols, flavonoids, and antioxidants of *E. arvense* extract

Antioxidant constituents	Mean \pm SD
Total phenols (mg/ 100 g gallic acid equivalent)	291.10 \pm 15.84
Total flavonoids (mg/100 g quercetin equivalent)	309.48 \pm 6.53
Total antioxidants (mg/ 100 g ascorbic acid equivalent)	2405.50 \pm 10.62

Values were expressed as mean \pm SD for 3 replicates**Table 3. Effect of *E. arvense* on weight gain in MTX injected rats**

Groups	IBW (g)	FBW (g)	BWG%
Cont	186.59 \pm 9.76	274.88 \pm 13.51	32.04 \pm 3.68
<i>E. arvense</i>	187.33 \pm 9.91	271.14 \pm 8.90	30.82 \pm 4.80
MTX	185.26 \pm 9.07	229.03 \pm 8.78*	19.11 \pm 2.72*
<i>E. arvense</i> + MTX	187.73 \pm 8.95	259.32 \pm 10.63#	27.46 \pm 5.07#

IBW: Initial Body Weight; FBW: Final Body Weight; BWG%: Body Weight Gain Percentage
Values were offered as mean \pm SD (n=10). Significant relative to * Cont group and # MTX group**Table 4. Effect of *E. arvense* on sperm parameters (count, motility, viability, and abnormalities) assessed in MTX injected rats**

Groups	Count (x106/ml)	Motility (%)	Viability (%)	Abnormalities (%)
Cont	72.00 \pm 6.89	85.90 \pm 5.97	89.70 \pm 6.67	6.37 \pm 0.58
<i>E. arvense</i>	74.20 \pm 7.32	89.20 \pm 6.25	92.66 \pm 4.09	5.69 \pm 1.12
MTX	35.90 \pm 4.82*	42.50 \pm 8.61*	50.70 \pm 8.78*	39.32 \pm 6.63*
<i>E. arvense</i> + MTX	61.90 \pm 9.48#	77.90 \pm 5.78#	79.73 \pm 8.02#	9.22 \pm 2.04#

Values were offered as mean \pm SD (n=10). Significant relative to * Cont group and # MTX group

3.5 *E. arvensis* Decreased Testicular Levels of Inflammatory Markers (IL-1 β and TNF- α) in MTX Injected Rats

The results demonstrated that MTX induced a significant inflammation as there were significant increases in testicular levels of IL-1 β and TNF- α relative to the Cont group ($p \leq 0.001$). In the *E. arvensis* + MTX group, testicular levels of IL-1 β and TNF- α were significantly lower ($p \leq 0.001$) than in the MTX group. There was no substantial difference between the *E. arvensis* and Cont groups. Fig. (3).

3.6 *E. arvensis* Decreased Oxidative Stress Marker (TBARS) and Increased the Antioxidant Marker (SOD) in MTX Injected Rats

The results revealed that MTX induced significant oxidative stress conditions as there was a significant reduction in testicular content of SOD concurrent with a significant elevation in

testicular content of TBARS in the MTX group relative to the Cont group ($p \leq 0.001$). The testicular level of SOD elevated significantly with a significant reduction in testicular content of TBARS in the *E. arvensis* + MTX group relative to the MTX group ($p \leq 0.001$). Administration of *E. arvensis* (500 mg/kg) only produced a significant antioxidant activity as there was a significant elevation in the testicular content of SOD relative to the Cont group Fig. (4).

3.7 Histopathological Findings

Examined testicular sections of the Cont group revealed normal testicular structures with preserved seminiferous tubules, normal spermatocytes, spermatogonia, Sertoli cells, and spermatids, they contained a variable number of mature spermatozoa in their Lumina. Leydig cells, interstitial tissues, and vascular structures were normal. Neither degenerative nor necrotic or apoptotic changes were observed. Fig. (5A) and Fig. (6A).

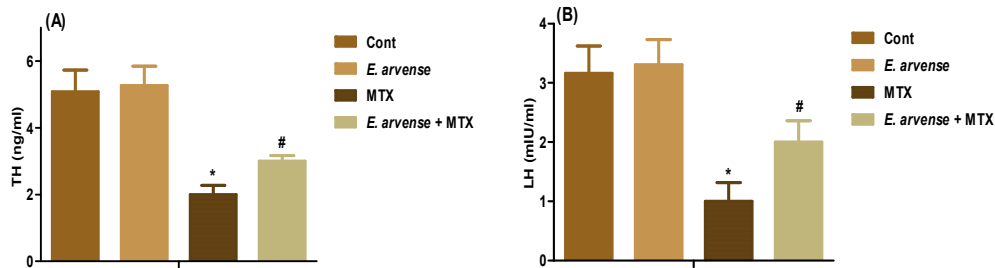


Fig. 2. Effect of *E. arvensis* on serum levels of (A) testosterone hormone (TH) and (B) luteinizing hormone (LH) assessed in MTX injected rats
 Values were offered as mean \pm SD ($n=10$). Significant relative to * Cont group and # MTX group

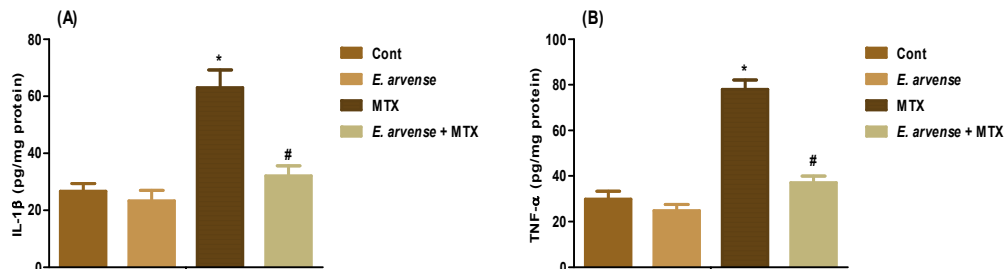


Fig. 3. Effect of *E. arvensis* on testicular levels of (A) interleukin-1beta (IL-1 β) and (B) tumor necrosis factor-alpha (TNF- α) assessed in MTX injected rats
 Values were offered as mean \pm SD ($n=10$). Significant relative to * Cont group and # MTX group

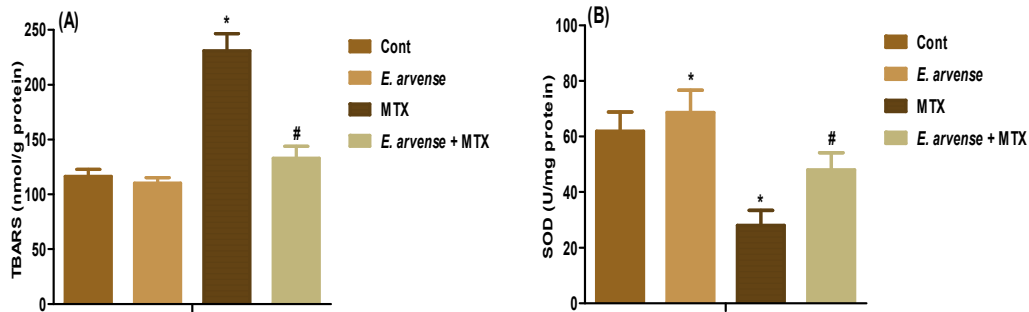


Fig. 4. Effect of *E. arvense* on testicular contents of (A) thiobarbituric acid reactive substances (TBARS) and (B) superoxide dismutase (SOD) assessed in MTX injected rats
Values were offered as mean \pm SD ($n=10$). Significant relative to * Cont group and # MTX group

In *E. arvense* group, testicular sections showed normal histological architectures in most seminiferous tubules with preserved normal spermatocytes, spermatogonia, Sertoli cells, and spermatids, and they contained a variable number of mature spermatozoa in their lumina. Leydig cells, interstitial tissue, and vascular structures were normal. A few sections pointed out mild histomorphological changes represented by interstitial edema with focal thickening of the intertubular septa and increased Leydig cells Fig. (5B) and Fig. (6B).

Examined sections of the MTX group pointed out marked cytotoxic changes in a moderate number of seminiferous tubules with characteristic degenerative, necrotic, and apoptotic morphopathologic changes in almost all the testicular cells, including the spermatogonia, spermatocytes, and spermatids leading to complete testicular aplasia in some parts and partial aplasia in others with almost arrest of spermatogenesis in the affected tubules. Marked interstitial edema with associated multifocal patches of Leydig cells proliferation and compressive atrophic changes in some seminiferous tubules could also be observed in Fig. (5C) and Fig. (6C).

Examined sections of the MTX treated with *E. arvense* demonstrated highly active testicular tissues with active spermatogenesis and spermiogenesis in almost all examined tissues. The seminiferous tubules were lined by normal active spermatogonia, spermatocytes, spermatids, and spermatozoa with a normal thickness of the interstitial tissue and normal

Leydig cells population. Mild focal interstitial edema with moderate Leydig cell proliferation could be observed in a few sections. Neither degenerative nor necrotic or apoptotic changes were seen in Fig. (5D) and Fig. (6D).

3.8 Morphometric Analysis of Testicular Tissue

The collective morphometric analytic was illustrated in Fig. (7) and Table (5). In the *E. arvense* group, there were significant increases in seminiferous tubules size and primary spermatocytes relative to the Cont group ($p \leq 0.001$, and $p \leq 0.05$, respectively). However, there was no significant difference between *E. arvense* group and the Cont group concerning the secondary spermatocytes. MTX group showed significant changes in the morphometric measurements; there were significant decreases in seminiferous tubules size, secondary and primary spermatocytes relative to the Cont group ($p \leq 0.001$). The morphometric measurements were markedly improved in the *E. arvense* + MTX group; there were significant increases in seminiferous tubules size, secondary and primary spermatocytes relative to the MTX group ($p \leq 0.001$).

4. DISCUSSION

MTX is an anti-cancer drug that can also be used to treat rheumatoid arthritis. In human and animal studies, however, it has been shown to cause liver, renal, lung, heart, and testicular abnormalities [9,45-46]. This can heighten the desire to avoid MTX-induced reproductive

toxicity, especially among men of reproductive age [47-49]. *E. arvensis* has been shown to be beneficial in the treatment of diseases and disorders caused by oxidative stress [25-26]. The aim of this study was to see whether *E. arvensis* could protect male rats from MTX-induced testicular oxidative stress, inflammation, and histopathological changes.

In the current research, MTX injection induced a significant reduction in rats' body weight gain, in addition to the significant decrease in sperm counts, viability, and motility relative to the Cont group. These findings are consistent with [42,50-52], who revealed that injected of MTX induced loss in body weight, weakness, and lack of activity. This could be attributed to MTX-

induced mucositis, disturbance in the gastrointestinal, appetite, and nutrient absorption in the rat model [53]. Additionally, the deterioration in sperm characteristics after MTX-treatment may be explained via inhibition of spermatogenesis and reduction in seminiferous tubules, as a result of damaged cell membrane integrity by disturbing proteins and lipids within the sperm membrane and increase lipid peroxidation level [42]. In the current research, MTX induced a marked decline in serum TH and LH levels relative to the Cont group. These findings agree with several previous studies that revealed MTX-induced changes in TH associated with a decrease in steroidogenesis and the number of LH receptors on Leydig cells [54-56].

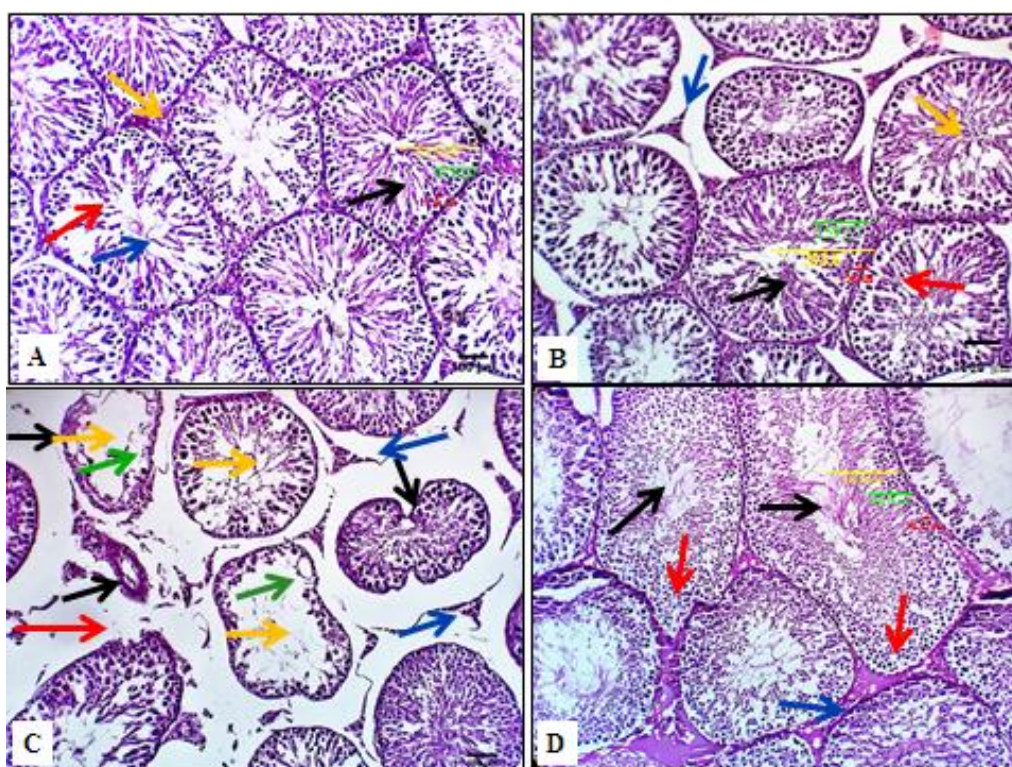


Fig. 5. Effect of *E. arvensis* on testicular histopathology (H & E bar = 100 µm)

Photo (A): Testis of the Cont group showing normal testicular tissue, seminiferous tubules (black arrow), Leydig cells (orange arrow), normal spermatogonia, spermatocytes (red arrow), spermatids, and spermatozoa (blue arrow). Photo B: Testis of the *E. arvensis* group showing normal histological architectures in most of the seminiferous tubules (black arrow) with preserved normal spermatogonia, spermatocytes (orange arrow), spermatids, and Sertoli cells. Mild histomorphological changes are represented by interstitial edema with focal thickening of the inter-tubular septa and an increase in Leydig cells population (blue arrow); Photo C: Testis of the MTX group showing a moderate number of seminiferous tubules, degenerative (green arrows), necrotic and compressive atrophic changes in some seminiferous tubules (black arrow), interstitial edema (red arrow) with associated multifocal patches of Leydig cells proliferation (blue arrows). Photo (D): Testis of the *E. arvensis* + MTX group showing highly active testicular tissue with active spermatogenesis and spermiogenesis in almost all of the examined tissue (black arrows). The seminiferous tubules are lined by normal active spermatogonia, spermatocytes, spermatids, and spermatozoa (red arrows) with a normal thickness of the interstitial tissue and normal population of Leydig cells

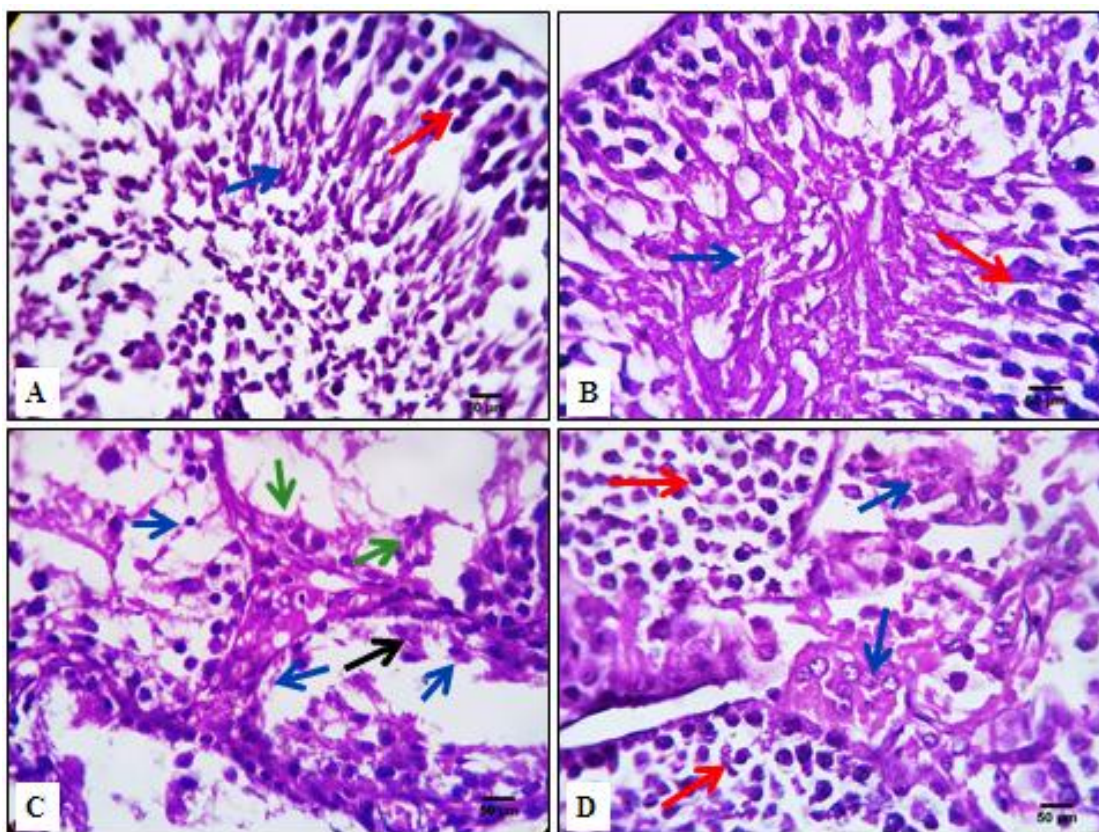


Fig. 6. Effect of *E. arvensis* on testicular histopathology (H & E bar = 50 μ m)

Photo (A): Testis of the Cont group showing normal testicular tissue, seminiferous tubules, normal spermatogonia, spermatocytes (red arrow), spermatids, and spermatozoa (blue arrow). Photo (B): Testis of the *E. arvensis* group showing normal architectures in the seminiferous tubules, spermatogonia, spermatocytes (red arrow), spermatids, and Sertoli cells, they contained a variable number of mature spermatozoa in their lumina (blue arrow); Photo (C): Testis of the MTX group showing marked cytotoxic changes in seminiferous tubules with characteristic degenerative (green arrows), necrotic (black arrow), and apoptotic morpho-pathologic changes (blue arrows) in almost all the testicular cells including the spermatogonia, spermatocytes, and spermatids leading to complete testicular aplasia in some parts and partial aplasia in others with almost arrest of spermatogenesis in the affected tubules; Photo (D): Testis of the *E. arvensis* + MTX group showing mild focal interstitial edema (blue arrows), and moderate Leydig cells proliferation (red arrow)

Table 5. Effect of *E. arvensis* on morphometric analysis of testicular tissues measured in MTX injected rats

Groups	Seminiferous tubules size (μ m)	Secondary spermatocytes size (μ m)	Primary spermatocyte size (μ m)
Cont	270.46 \pm 1.82	152.62 \pm 4.49	20.35 \pm 0.82
<i>E. arvensis</i>	290.36 \pm 3.14 *	153.57 \pm 6.07	23.86 \pm 1.46 *
MTX	106.61 \pm 1.78 *	44.82 \pm 2.03 *	18.05 \pm 1.25 *
<i>E. arvensis</i> + MTX	240.92 \pm 1.72 #	144.27 \pm 7.84 #	23.77 \pm 1.79 #

Values were presented as mean \pm SD (3 measurements). Significant relative to * Cont group and # MTX group

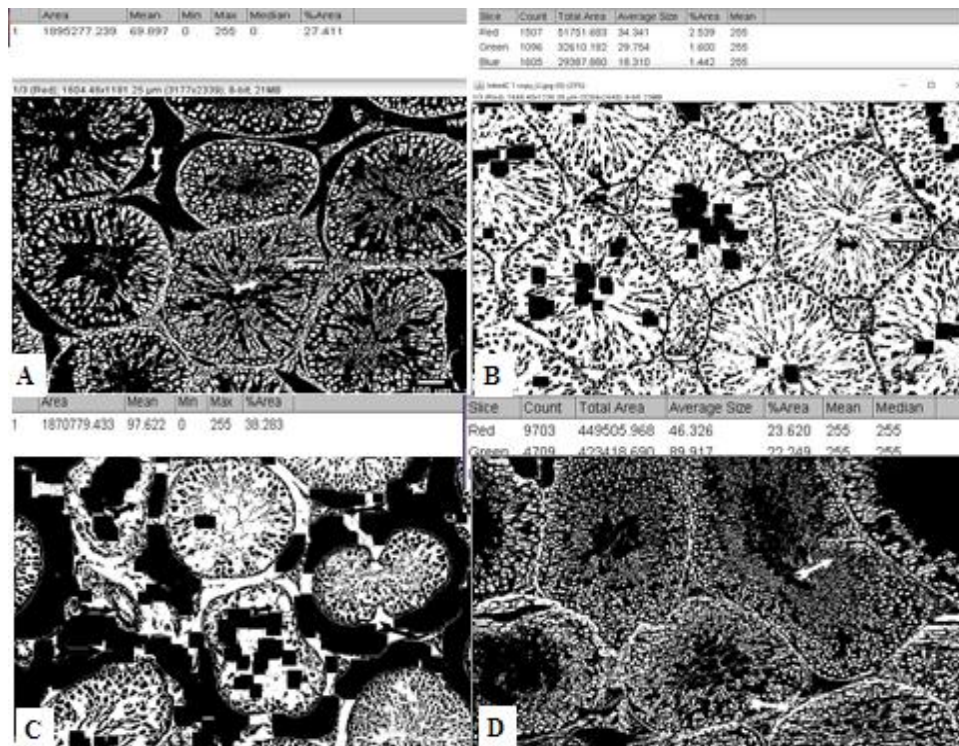


Fig. 7. Effect of *E. arvensis* on testicular morphometric analytic in MTX injected rats
 Photo A: Cont group; Photo B: *E. arvensis* group; Photo C: MTX group; Photo D: *E. arvensis* + MTX group

Oxidative stress proved to have an essential role in testicular damage induced by MTX. Uncontrolled production of ROS resulted in sperm abnormalities and infertility. Our study revealed that MTX induced a significant elevation in TBARS level, simultaneously a significant decrease in SOD content in testicular tissue relative to the Cont group. These findings are consistent with several previous studies [7,57-60]. As a result, SOD is an essential antioxidant enzyme that serves as the first line of defense against ROS production [61]. MTX-induced testicular oxidative stress explained via the sperm membrane, is rich in polyunsaturated fatty acids, thus increase lipid peroxidation, rise in utilization of SOD, and imbalance of resynthesizing machinery [8].

Inflammation has an essential effect on the pathogenesis of MTX- induced testicular toxicity [14-15]. In these findings, MTX induced a noticeable testicular inflammation as evidence by significant increases in testicular tissue content of IL-1 β and TNF- α relative to the Cont group. Several previous studies revealed that MTX induced a marked rise in the inflammatory mediators as in testicular tissues [13-15,56,62]. Our histopathological results confirmed the

biochemical findings. In the seminiferous tubular lumen, the widespread immature germinal cells indicated impaired and not completed spermatogenesis; these changes may be attributed to oxidative and inflammation properties induced by MTX [4,7,59].

This research evaluated unprecedentedly the *E. arvensis* preventive effect as antioxidant and anti-inflammatory versus MTX-induced reproductive damage. *E. arvensis* exhibits significant increases in body weight gain, sperm counts, viability, and motility, as well as the serum levels of TH and LH. Additionally, it attenuated and restored the normal contents of the antioxidant testicular enzyme SOD activity and decreased testicular TBARS content. It reduces the MTX-induced inflammation, as evidenced by significant decreases in the testicular levels of IL-1 β and TNF- α levels as well as marked improved histopathological changes relative to the MTX group. Similarly, several natural plants confirmed their protective role against MTX-induced testicular toxicity [2,13,55]. The antioxidant and anti-inflammatory activities of *E. arvensis* were proved in several studies [28-30,33]. Like the current data. Many previous studies confirmed that *E. arvensis* extract has high amounts of total

phenols, total flavonoids, and total antioxidants [63-66] as well as several previous studies obtained similar active constituents of *E. arvense* using GC/MS [33,67-71].

5. CONCLUSION

Based on the findings of this study, it can be concluded that *E. arvense* reduced the testicular toxicity caused by MTX. Increased reproductive organ activity, reduced oxidative stress, and reduced inflammation-induced semen impairment may explain the positive results.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Handling and procedures of the rats were conducted following regulations of Canadian ethics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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