Original Article

Effect of Methotrexate and Matricaria Chamomile on testis tissue and some antioxidants in Albino rats

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Abstract

Objective: Depending on the antioxidant as well as anti-inflammatory properties of Matricaria Chamomile (MC), the current study aimed to investigate if MC could protect testicles against methotrexate (MTX)-induced testicular damage. Methods: 24 male Wistar albino rats were randomly separated into four groups (n=6/each) as follows. G1, the control group was given a single oral daily dose of distilled water (D.W) for 30 days; G2, (MTX group) rats were administered a single oral daily dose of D.W. and in day 28 the animals have received a single IP dosage of MTX; G3 six rats were given an aqueous extract of chamomile flowers at a dose of 200 mg/kg dissolved in 1 ml D.W. and on day 28 the rats have received a single intraperitoneal dose of MTX, and the last group was six rats received a methanolic extract of chamomile flowers at a dose of 200 mg/kg dissolved in 1 ml D.W. and on the day 28 the animals were received a one dosage intraperitoneal of MTX. At the end of the experiment, all animals were anesthetized and sacrificed, blood was also collected for the biochemical test as well as the testicular tissues were removed for histology examination. Results: According to biochemical assessments, notably there was no significant difference between the MTX and the other studied groups. In terms of histological examinations of animals treated with MTX, several pathological changes including vacuolization of seminiferous tubules with a reduced population of germinal epithelium were seen as compared to the control. Animals of G3 and G4 (pre-treatment with equates and alcoholic extract of MC before MTX administration) also showed some notable histopathological changes including disorganization of seminiferous tubules and tubular vacuolation. Conclusion: When comparing the control group, examination of testes with H&E staining showed that pre-treatment with Matricaria Chamomile did not affect MTX-induced testicular damage.

INTRODUCTION

Methotrexate (MTX), a folic acid antagonist with anti-neoplastic properties, is used to treat distinct types of cancer. Since the 1950s, it has been used to treat rheumatic disorders [1]. Methotrexate inhibits enzymes required for DNA and RNA synthesis, as well as lowers the cellular NADPH levels, by blocking the pentose phosphate cycle. In usual conditions, NADPH is utilized to sustain the lowered glutathione storage, which also protects against ROS as a key anti-oxidant [2, 3]. The kidneys, liver, and intestines are among the organs affected by MTX at higher doses or long-term treatment [4-6]. The most serious potential side effect of MTX is testicular injury, which might result in infertility [7]. Methotrexate induced testicular damage, involving reduction of germ cells and loos of spermatozoa [1]. Furthermore, previous research has linked MTX to damage to the testes' seminiferous tubules, a reduction in sperm count, and sperm DNA damage [8, 9] as well as to changes in testicular functional proteins in adult rats [10].

Because most synthetic pharmaceuticals have negative side effects, discovering low-cost natural remedies with protective properties is desirable. The Matricaria Chamomilla (MC) has been used in traditional medicine for treating a variety of human illnesses such as inflammation, rheumatism, and gastrointestinal problems. Chamomile has a short hairy stem with narrow irregular incisions and a green-white stem. Flavonoids, which are excellent antioxidants in scavenging free radicals, are abundant in the chamomile plant [11, 12]. The popular pharmacological activities of Matricaria Chamomilla are reliant on its antioxidant activity, which decreases free radicals, according to several clinical and experimental research [13, 14]. As a result, we chose to explore the impacts of MC extract on oxidative stress and histological changes in testes of Rats induced by Methotrexate.

Methodology

Animals

Twenty-four male albino rats (Rattus rattus) were used in the current study, animals weight range from 200 to 300gm. All animals were housed under controlled environmental condition and placed in the animal house of the college of Science, University of Babylon. Experimental animals were supplied with the commercial pellets and had given a free access to food and water [15].

Experimental animals were separated into four groups as follows:

Group I (G1): six rats given a single dosage orally of distilled water every day for 30 days.

Group II(G2): six rats given a single dosage orally of distilled water for 30 days and on day twenty-eight the animals were received a single intraperitoneal dose of MTX (0 .5 ml for rat weight 200 gram).

Group III (G3): six rats were given an aqueous extract of chamomile flowers at a dose of 200 mg/kg by dissolving in 1 ml D.W. and on day 28 the rats have received a single intraperitoneal dose of MTX (0.5 ml for rat weight 200 gram)

Group VI (G4): six rats were given a methanolic extract of chamomile flowers at a dose of 200 mg/kg by dissolving in 1 ml D.W. and on day 28 the rats have received a single intraperitoneal dose of MTX (0.5 ml for rat weight 200 gram).

On day thirty-one, all of these animals were anesthetized using chloroform and sacrificed, then the blood samples and testicular tissues were collected for biochemical and histological examination in all groups.

Evaluation of Biochemical Parameters

After the centrifugation for 10 minutes at 3000 rpm, the serum was then put into 3 microtubes (500 mL) and stored at -20°C until the time of the experiment. Malondialdehyde, catalase, and superoxide dismutase levels were measured in blood samples following the procedure described in [16, 17].

Histological Study

Tissue sections from the rats' testis were taken and preserved in neutral formalin 10%. After paraffin embedding, 5-micron slices of the tissues were produced and stained with hematoxylin and eosin. Following that, they were examined under a microscope and tissue damage was assessed. [18].

Matricaria chamomilla Extract

Preparation of Methanolic Extract

Eighty grams of crushed powder dry chamomiles were placed in 1 L flasks, subsequently 800 mL of 80% was added to the samples, and the samples were shaken by a shaker for 8 hours at 37 °C. Then the suspension were filtrated using filter paper, and the extract was evaporated in a 45°C by oven before being kept at -4°C until use[19].

Aqueous Extract Preparation

The aqueous extract was prepared by adding 80g of crushed powder dry chamomiles to boiling water. The extract solution then suspended in a 1L flask and placed on a shaker for 4 hours at 37 °C. The suspension then filtered through filter paper then the extract was evaporated using an oven afterward stored at -4°C until it was used [19, 20].

Statistical Analysis

SPSS 20 was used to conduct the statistical analysis (IBM, USA). All the data was given as a mean with standard deviation (SE). To compare the biochemical factors, a one-way analysis of variance (ANOVA) was used, following by a Tukey post hoc test. $P<0.05$ was used to assess statistical significance levels.

Results

Biochemical results are given in Table 1. In this study, serum activity of CAT, SOD, as well as MDA showed no significant alteration in the studied groups.

In the control group, histological investigations revealed normal testis histology with normal seminiferous tubules pattern (Figure 1). However, deterioration and depletion of germinal cells, disruption in layers of the germ cell, and also vacuolization in the seminiferous tubules were found in the Methotrexate (MTX) treated group when compared with the control (Figure 2).

Comparing with the results of control, the pre-treatment with aqueous extract resulted in disordered of germ cell organization, vacuolation, and degenerative changes in seminiferous tubules (Figure 3). Furthermore, MC methanolic extract-pre-treated group light microscopic image showed a deterioration of the germinal epithelium and cytoplasmic vacuolization, as well as seminiferous tubules with a few spermatids, were obvious compared to the control group (Figure 4).

Discussion

Methotrexate is indeed a cytotoxic chemotherapeutic medication that is used to treat cancer and autoimmune illnesses [21]. Testicular toxicity is a prominent adverse effect of MTX[22, 23]. The purpose of this research was to see if Matricaria chamomilla might protect rats against testicular toxicity caused by MTX.

Biochemical results showed that rats administered MTX only did not cause any notable change in the levels of MDA, CAT, and SOD when compared with the control group. In contrast with our findings were the results of previous studies by Yuluğ et al., and Vardi et al., who revealed that methotrexate caused an increase in the level of MDA and decrease the activities of CAT and SOD [9, 24]. The inconsistency in results may well be compared to the findings of Papandreou et al., who stated that sometimes stress antioxidant response varies depending on the intensity, duration, and the type of the stress as well as the history record of the animal to stress situation [25].

Matricaria chamomilla extract has been shown in several studies to reduce lipid peroxidation (as measured by MDA levels) and raise serum levels of SOD, catalase, and glutathione [14, 26, 27]. However, in the present study, the administration of MC along with MTX exhibited no significant difference in the serum levels of MDA, CAT, and SOD in contrast to other studied groups. The variations in the levels of oxidative stress biomarkers may be linked to the kind and severity of stressors that seem to be the principal factor in determining oxidative stress measurement in rats as recorded in a previous study[28].

In our study, light microscopic images for the MTX group displayed remarkable testicular damage in comparison with the control group. Several studies in the literature show such effects of MTX [9, 29, 30]. While the specific mechanism by which MTX causes testicular injury is unknown, it has been established that oxidative stress, inflammatory cytokines, and resulting apoptotic are involved [31].

Accumulating data has pointed to the protective role of Matricaria chamomilla [6, 14] Yet, in the existing study, the pre-treatment by MC extracts induced a status of histological changes in the testes of adult rats including disorganization of the seminiferous tubules, the appearance of cytoplasmic vacuolization, and increased interstitial space.

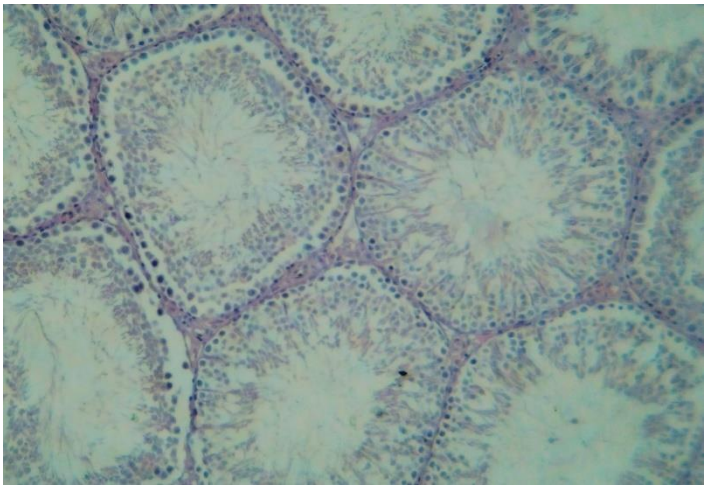
The histological changes in the testicular tissue could be the result of a changes in the microenvironment of the testes due to the influence of MTX and to the involvement of a toxic microenvironment presence in Matricaria chamomilla as some previous studies showed that ethanolic extract of Matricaria recutita possesses antispermatogenic properties [32]. Additionally, another

study indicates that the treatment of Matricaria recutita extract (100 and 150 mg/kg/day) for 56 days dramatically lowered the counts of sperm and motility in experimental animals compared to control animals [33].

Matricaria chamomilla has a great history of herbal usage, yet in the present study, the pre-treatment with Matricaria chamomilla before MTX administration did not show a notable enhancement in testes architecture compared with control group.

Table 1. Serum levels of malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) contents in the studied groups. Data are expressed as mean + S.D. Group1= control; Group 2= methotrexate (MTX) treated group; Group 3= received aqueous extract of Matricaria chamomilla before methotrexate administration; and Group 4= received methanolic extract of Matricaria chamomilla before methotrexate administration. NS: means not significant.

Parameters	Group 1	Group 2	Group 3	Group 4	p values
MDA	0.95±0.26	0.96±0.11	0.93±0.04	1.01±0.25	NS
CTA	0.61±0.33	0.76±0.79	1.01±0.55	0.75±0.26	NS
SOD	0.86±0.09	0.85±0.1	0.79±0.09	0.87±0.06	NS



Figure(1) Cross histological section of control rats group with normal testis histology and normal seminiferous tubules pattern (10x).

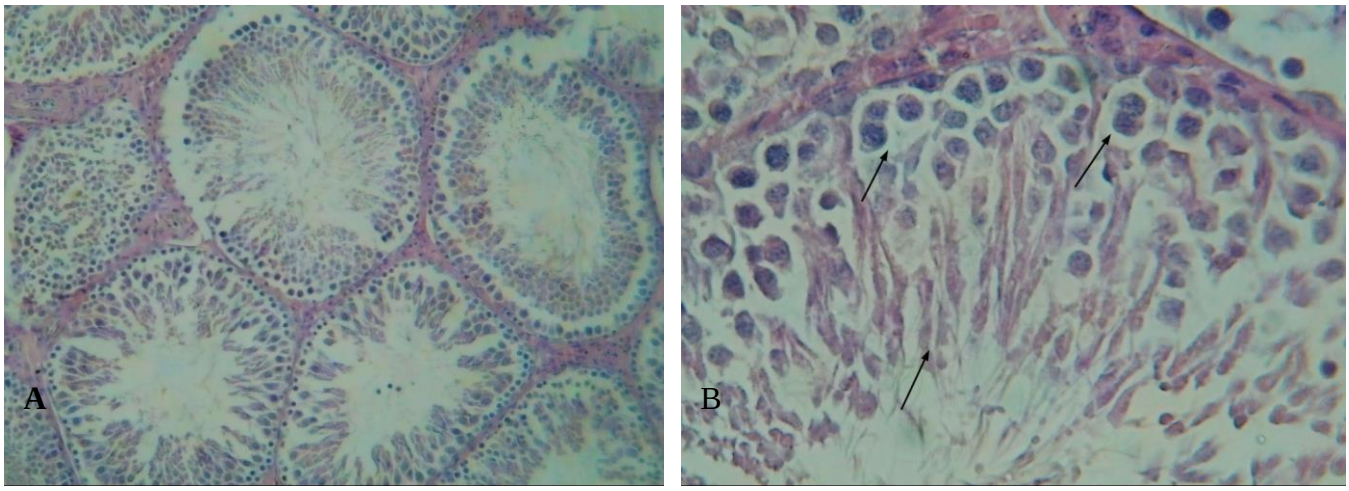


Figure 2:(A) Histopathological changes in rats receiving MTX showed a dilated seminiferous tubule with a reduced population of germinal epithelium (10x). (B) normal spectrum of germ cells is absent in seminiferous tubules owing to germ cell depletion (arrowhead) (40x).

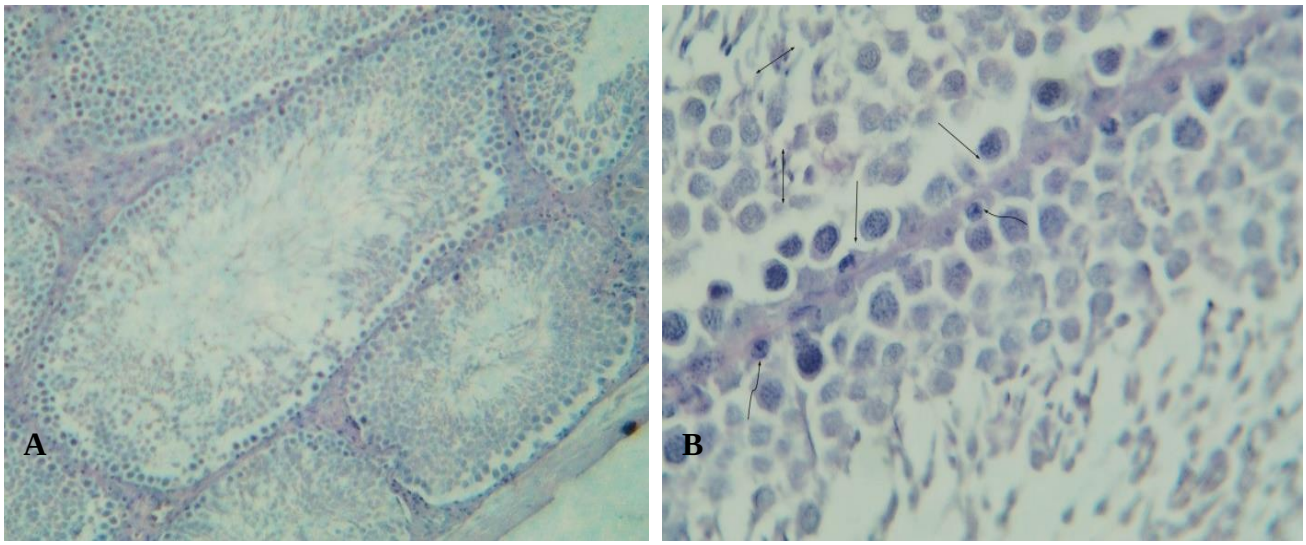


Figure 3: Histology of the testicular tissue of rats pre-treated with aqueous extract *of Matricaria chamomilla* before receiving methotrexate; (A) cytoplasmic vacuolization in spermatogonia (10x). (B) damaged testicular structure characterized by the disorganized arrangement of germ cells in seminiferous tubules (arrow double heads), vacuolation (*arrowheads*) and the necrotic cells (*curved arrow*) (40x).

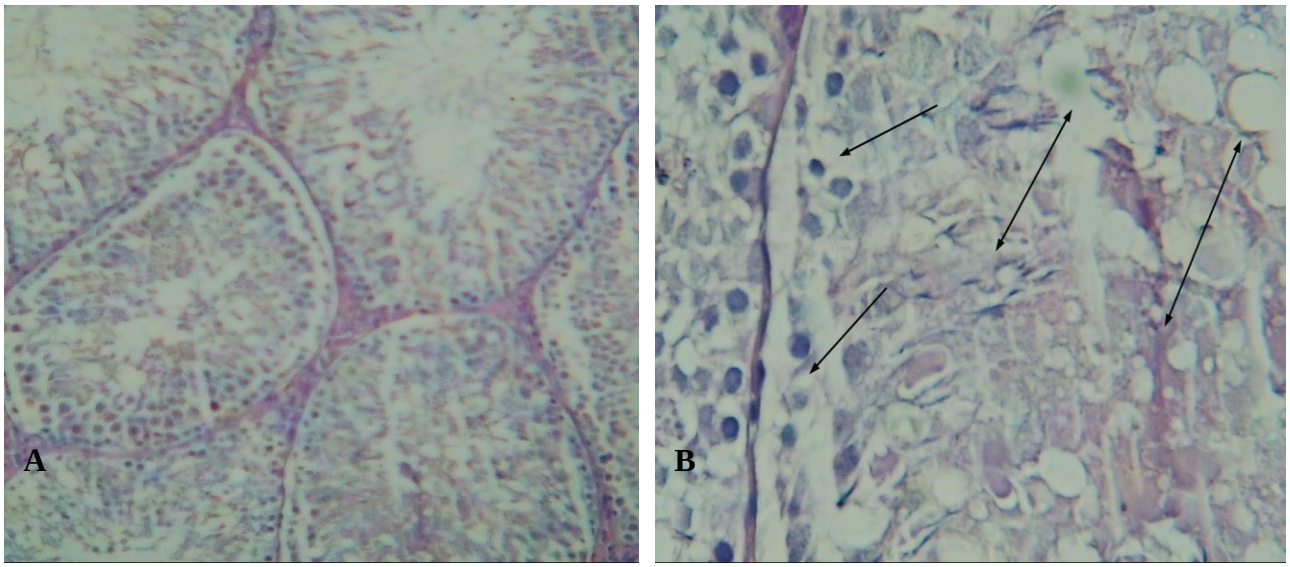


Figure 4: Histology of the testicular tissue of rats pretreated with methanolic aqueous extract *of Matricaria chamomilla* before receiving methotrexate. (A) germ cell depletion and Seminiferous tubules vacuolation (10x). (B) arrowhead shows the germinal epithelium atrophy and arrow double head shows mineralized seminiferous tubules (40x).

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