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Role of Methotrexate and Thyme in Physiological Changes of Male Rat

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Abstract

Forty adult male rats weighting (300±50) g were used and divided into 4 equal groups (10 rats/each); Group I: normal control rat group which received no treatment. Group II: control rat treated orally with thyme leave extract only at a dose (500mg/kg b.w/d) for a month, Group III: rat treated (i.p) with methotrexate (MTX) only at a dose (1mg/kg b.w/d) one per week for four weeks and dissolved in (2ml) of saline solution, Group IV: rats co-treated with methotrexate at a dose (1mg/kg b.w) and thyme extract at a dose (500mg/kg b.w) daily for a month at the same time. Thyme leave extract enhanced reproductive hormones (testosterone and estradiol E2) after inhibition of MTX. MTX induced increment in MDA level but thyme improved it. Antioxidants enzymes (GR, SOD and CAT) inhibited by MTX and thyme enhanced their depression. Moreover, HB, Platelets and WBCs recorded significant decrease in group of MTX but improved in MTX and thyme treatment group. In conclusion, thyme extract is more effective in the improvement of physiological abnormalities of testicular of rat after injection with MTX.

Keywords: testis, rat, MTX, Thyme, physiological parameters.

Introduction

In all species, the testicle or testis refers to the male reproductive gland or gonad. It's the same as a woman's ovary **(Duffus *et al*., 2016).** The functions of the testicles are to produce both sperm and androgens, mainly testosterone. Effects of LH on testosterone release. The presence of testosterone and FSH is required to support spermatogenesis **(Oduwole *et al*., 2018).** Tests can be identified by detecting damage caused by exposure to both chemical and toxic chemicals **(Altwaijry *et al*., 2020).** In general, chemotherapy cannot differentiate between cancer cells and non-cancerous cells, and toxic side effects can occur. However, chemotherapy is effective in treating various types of cancer; it causes the death of normal growing cells, including male immune cells **(Tousson *et al*., 2014).**

Methotrexate (MTX; 4-amino-10-methylfolic acid) considers folic acid antagonist with anti-pulmonary symptoms **(Tousson *et al*., 2018).** MTX achieves its chemotherapeutic effect in competing with folic acid in cancer cells, leading to cellular folic acid deficiency

and subsequent cell death. There are concerns about MTX poisoning. It is used to treat a number of diseases including cancer, rheumatoid arthritis, psoriatic arthritis, systemic lupus erythematosus, and dermatomyositis. However, this use has some side effects, such as low blood cell count, hair loss, mouth sores, and diarrhea, as well as damage to the liver, lungs, nerves and kidneys **(Ozogul *et al*., 2013).**

The most important consequence of MTX is damage to the testicles which can lead to male infertility. MTX leads to an increase in the level of malondialdehyde (MDA) and the number of apoptotic cells **(Asci and Ozer, 2011).** Azoospermia and impotence in men caused by chemotherapeutic chemicals are reported in previous studies. Also, MTX has been shown to damage the seminiferous testicles of the testicles, reduce sperm count, and cause genetic mutations (DNA) in sperm. Excessive testicles tissue damage is caused by increasing the formation of free oxygen radicals caused by MTX **(Daggulli *et al*, 2014).**

Active oxygen types (ROS), include hydroxyl radicals (OH), peroxyl radicals (ROO), superoxide radicals (O2), hydrogen peroxide (H2O2), and singlet oxygen **(Cao *et al*., 2018)** play an important role in human health and are an integral part of the cell structure of living organisms. When ROS is low, ROS can effectively play many beneficial roles in the human body, for example it acts as an intracellular signaling factor. Also, tissues, organs, and other biological macromolecules will be damaged by oxidative stress as ROS increases the acceptance of the human body **(ko *et al*., 2012).**

Cells basically contain a series of complex intracellular antioxidant immune systems, mainly composed of two components, the enzymatic and non-enzymatic antioxidant systems. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) are examples of antioxidant enzymes. However, glutathione (GSH), carotenes and ascorbic acid are examples of a non-enzymatic antioxidant system. Under normal circumstances, cells can prevent and reduce oxidative stress by two antibodies. For example, SOD can remove free radicals and reduce oxidative damage **(Poljsak *et al*., 2013).** However, sophisticated antioxidant systems do not completely prevent the damage caused by oxidative stress **(Olagunju *et al*., 2018).**

Thyme is a plant rich in essential oils that contain more than 60 anti-oxidative and phenolic compounds with anti-oxidant and anti-microbial activity. Thyme was used as part of human nutrition as a stimulant and a stimulant and to strengthen cells in the treatment of various ailments. Also, it has been used in animal and poultry diets as an anti-oxidant and growth stimulant **(Haselmeyer *et al*., 2015).**

Thyme is said to have many medicinal effects such as lowering blood sugar, lipid, and high blood pressure; kidney protection; and antiviral and antifungal agents due to its antioxidant and anti-inflammatory properties **(Banerjee *et al*., 2019).** Thyme has antioxidant properties that can protect male reproductive factors from the effects of oxidative damage. If you look at the antioxidant properties of thyme extract it can protect male reproductive barriers against oxidative stress **(Salahshoor *et al*., 2020).**

The present study aims to investigate the role of thyme leave extract in reproductive hormonal changes and oxidative stress and antioxidants enzymes and hematological measurements on rat testis induced by MTX.

Material and methods

Animal selection and care :

Forty male adult rats (300 ± 50 g) were used in the current study and found in Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt. The animals were kept in plastic cages (2 per cage) for one week to adapt to the same temperature conditions as the natural dark light cycle. Food and tap water were available free of charge to animals throughout the study. All care and producers accepted in the current investigation were in line with the approval of the Institution Animal Ethics committee of the National Research Center and in line with the recommendation for proper care and use of laboratory animals.

Chemicals:

MTX was acquired from Ebewe Pharma Company (Egypt) and dissolved in 0.9% salt. Dried thyme leaves (*Thymus vulgaris*) are purchased at the local market and obtained by the Egyptian Institute for Herbs. Thyme leaves are ground into a powder using an electric grinder. One hundred grams of fine-powder was

diluted with 200 ml of boiling water in a covered flask and left for 30 minutes. After that, the extract was cooled and filtered through a filter paper to remove the filtrate material dried in the vacuum. The required dose is then measured and reconstituted in 5 ml of distilled water the minute before oral administration.

Experimental design :

Forty male adult rats were divided into 4 equal groups, housed in cages, all were kept under the same conditions and received the same diet. Group I: normal control rat group received 0.2 ml of 0.9% saline solution four weeks. Group II: control rats treated orally by thyme only at a dose 500 mg/kg b.w/d for four weeks. Group III: rats treated (i.p) by MTX at a dose (1mg/kg b.w) per week for four weeks that dissolved to saline 0.9 % to induce testicular changes. Group IV: rats co-treated with 1mg/kg b.w per week for four weeks MTX and thyme extract at a dose 500mg/kg/bw/d for four weeks at the same time.

Serum separation and samples collection:

After the 24th hour of the end of each test, the mice were killed. Blood samples were collected from all groups and incubated at 3000 rpm for 10 min, while sera samples were collected and stored in a clean plastic vial at -20 ° C to measure the following parameters: testosterone and estradiol E2 hormone, plasma samples collected MDA enzymes and antioxidants and blood protected by complete blood cells.

Estradiol and testosterone hormones determination:

Acetonitrile-certified materials in acetonitrile 1 mg / ml 17β-Estradiol (**Thienpont *et al*., 1988)** (purity 98.4%, National Metrology Institute of Japan, NMIJ CRM 6004-a, Japan) and testosterone **(Singer, 1992)** (purity 99.4%, Australian National Measurement Institute, ANMI M914, Australia) were used as scales, and [2,3,4-13C3] -17β-Estradiol (13C3-E2, ≥98%) and [2,3,4-13C3] - Testosterone (13C3-TT, ≥98%) obtained from IsoSciences (King of Prussia, PA) was used as internal standards (IS). Ammonium acetate, ammonium fluoride, ethyl acetate, glacial acetic acid, hexane, methanol, sodium chloride and water were purchased from Fisher Scientific (Suwannee, GA) and ammonium bicarbonate, ammonium hydroxide and ethanol from Sigma-Aldrich. All solvents were HPLC grade and chemicals were reagent. Steroids used for dementia testing have been found in Steraloids (Newport, RI), Sigma-Aldrich, and Cerrilliant (Round Rock, TX). They were prepared at 20% methanol in water at a concentration of 200 ng / dL per TT disturbance steroid and 200 pg / mL per E2 concentration test.

Oxidative stresses determination:

Thiobarbituric acid (TBA) reacts with MDA in acidic medium a temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm **(Satoh, 1978).**

Antioxidants determination:

Glutathione reductase (GR) determined by using biodiagnostic kit UV method. However, catalase (CAT) and Superoxide Dismutase (SOD) were determined by biodiagnostic colorimetric method. GR catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADPH^{+}$. The decrease in absorbance at 340 nm is measured **(Goldberg and Spooner, 1983).** CAT reacts with a known quantity of H2O2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining H2O2 reacts with 3,5 Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample **(Aebi, 1984).** SOD level were determined by assay relies on the ability of the enzyme to inhibite the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye **(Nishikimi *et al*., 1972).**

Hematological (Complete blood count) measurements:

At the end of the test, blood samples were collected immediately. Blood samples are collected from dry, clean, and labeled tubes containing EDTA (1mg / ml of fresh blood). Anticoagulated blood samples are used to obtain Hemoglobin counts, platelets and white blood cell counts. All measurements tested 2hr after collection by SYSMEX 800I.

Results

Effect of thyme extract on testosterone hormone levels in testicular abnormalities rats induced by MTX:

Testosterone levels were measured in blood sera. The values were 2.82 ng/ml in control rats, 4.02 ng/ml in administrated with thyme leave extract only, 1.58 ng/ml in rats injected (i.p) with MTX only, and 2.01 ng/ml in rats injected (i.p) with MTX and administrated with thyme extract.

Table 1: Effect of MTX and thyme leave extract administration on the levels of testosterone hormone:

Parameters	G I	GII	GIII	GIV	(p)
Testosterone	2.82 ±1.23	4.02 ±1.94	1.58 ±1.25	2.01 ± 0.67	0.029*
$p_{Control}$		0.491	0.395	0.738	
Sig. bet. grps		p_1=0.024*, p_2=0.087, p_3=0.936			

Effect of thyme extract on estradiol E2 hormone levels in testicular abnormalities rats induced by MTX:

Estradiol E2 levels were measured in blood sera. The values were 17.08 ng/ml in control rats, 21.94 ng/ml in administrated with thyme leave extract only, 15.27 ng/ml in rats injected (i.p) with MTX only, and 16.80 ng/ml in rats in rats injected (i.p) with MTX and administrated with thyme extract.

Table 2: Effect of MTX and thyme leave extract administration on the levels of estradiol E2 hormone:

Parameters	G I	GII	GIII	GIV	(p)
Estradiol E2	17.08 ±3.46	21.94 ±1.05	15.27 ±3.74	16.80± 3.21	0.014*
$p_{Control}$		0.102	0.761	0.999	
Sig. bet. Grps		p_1=0.009*, p_2=0.063, p_3=0.818			

Effect of thyme extract on MDA enzyme levels in testicular abnormalities rats induced by MTX:

MDA levels were measured in blood plasma. The values were 18.27 nmol/ml in control rats, 24.52 nmol/ml in administrated with thyme leave extract only, 54.36 nmol/ml in rats injected (i.p) with MTX only, and 17.0 nmol/ml in rats injected (i.p) with MTX and administrated with thyme extract.

Table 3: Effect of MTX and thyme leave extract administration on the levels of MDA enzyme:

Parameters	G I	GII	GIII	GIV	(p)
MDA	18.27 ± 1.75	24.52 ± 1.63	54.36 ± 5.56	17.0 ± 2.67	(<0.001*)
$p_{Control}$		0.106	<0.001*	0.956	
Sig. bet. grps		p_1<0.001*, p_2=0.044*, p_3<0.001*			

Effect of thyme extract on GRD enzyme levels in testicular abnormalities rats induced by MTX:

GRD levels were measured in blood plasma. The values were 94.21 U / L in control rats, 76.03 U / L in administrated with thyme leave extract only, 19.07 U / L in rats injected (i.p) with MTX only, and 22.91 U / L in rats injected (i.p) with MTX and administrated with thyme extract.

Table 4: Effect of MTX and thyme leave extract administration on the levels of GRD enzyme:

Parameters	G I	GII	GIII	GIV	(p)
GRD	94.21 ± 10.74	76.03 ± 14.71	19.07 ± 4.46	22.91 ± 4.37	(<0.001*)
$p_{Control}$		0.057	<0.001*	<0.001*	
Sig. bet. grps		p_1<0.001*, p_2<0.001*, p_3=0.907			

Effect of thyme extract on SOD enzyme levels in testicular abnormalities rats induced by MTX:

SOD levels were measured in blood plasma. The values were 325.0 U / L in control rats, 350.0 U / L in administrated with thyme leave extract only312.50 U / L in rats injected (i.p) with MTX only, and 349.99 U / L in rats injected (i.p) with MTX and administrated with thyme extract.

Table 5: Effect of MTX and thyme leave extract administration on the levels of SOD enzyme:

Parameters	G I	G II	GIII	GIV	(p)
SOD	325.0 ± 0.0	350.0 ± 0.0	312.50 ± 43.30	349.99 ± 0.0	(0.073)
$p_{Control}$		0.398	0.845	0.398	
Sig. bet. grps		p_1=0.120, p_2=1.000, p_3=0.120			

Effect of thyme extract on CAT enzyme levels in testicular abnormalities rats induced by MTX:

CAT levels were measured in blood plasma. The values were 666.23 U / L in control rats, 952.38 U / L in administrated with thyme leave

extract only, 635.91 U / L in rats injected (i.p) with MTX only, and 894.25 U / L in rats injected (i.p) with MTX and administrated with thyme extract.

Table 6: Effect of MTX and thyme leave extract administration on the levels of CAT enzyme:

Parameters	G I	G II	GIII	GIV	(p)
CAT	666.23 ± 13.51	952.38± 18.45	635.91 ± 27.90	894.25± 70.62	(<0.001*)
$p_{Control}$		<0.001*	0.640	<0.001*	
Sig. bet. grps		p_1<0.001*, p_2=0.077, p_3<0.001*,			

Effect of thyme extract on hemoglobin (Hb), platelets and white blood cells (WBCs) levels in testicular abnormalities rats induced by MTX:

CBC levels were measured in whole blood. The values of Hb were 14.83 U / L in control rats, 14.60 U / L in administrated with thyme leave extract only, 12.44 U / L in rats injected (i.p) with MTX only, and 13.96 U / L in rats injected (i.p) with MTX and administrated with thyme extract. Platelets values were 895.0 U / L in control rats, 908.0 U / L in administrated with thyme leave extract only, 743.0 U / L in rats injected (i.p) with MTX only, and 1074 U / L in rats injected (i.p) with MTX and administrated with thyme extract. Furthermore, WBCs values were 9.47 U / L in control rats, 15.80 U / L in administrated with thyme leave extract only, 6.80 U / L in rats injected (i.p) with MTX only, and 12.41 U / L in rats injected (i.p) with MTX and administrated with thyme extract.

Table 7: Effect of MTX and thyme leave extract administration on the levels of Hb, platelets and WBCs enzyme

Parameters	G I	GII	GIII	GIV	(p)
Hb	14.83 ± 0.57	14.60 ± 0.47	12.44 ± 0.98	13.96 ± 0.76	(0.004*)
$p_{Control}$		0.974	0.006*	0.431	
Sig. bet. grps		p_1=0.007*, p_2=0.612, p_3=0.057			
Platelets	895.0 ± 5.57	908.0 ± 74.22	743.0 ± 44.09	1074.0 ± 89.94	(<0.001*)
$p_{Control}$		0.994	0.047*	0.019*	
Sig. bet. grps		p_1=0.031*, p_2=0.030*, p_3<0.001*			
WBCs	9.47 ± 2.97	15.80 ± 1.96	6.80 ± 1.39	12.41 ± 1.12	(<0.001*)
$p_{Control}$		0.005*	0.298	0.228	
Sig. bet. grps		p_1<0.001*, p_2=0.104, p_3=0.007*			

All Data were expressed by using mean ± SD by using ANOVA test. Data was expressed by using mean ± SD, p= comparing between the four groups, pControl: each other group against control, p1: GII against GIII, p2: GIV against GII and p3: GIV against GIII. *: Statistically significant at $p \leq 0.05$ and highly significant at $p \leq 0.001$.

Discussion

Today, there are many types of chemotherapy used as anticancer drugs. It is important to look for therapies that can reduce the side effects of anticancer treatment without altering their effectiveness or increasing toxicity or injury to target organs such as testis **(Schirrmacher, 2019).** In addition, thyme contains steroids, saponins, flavonoids, terpenoids, alkaloids, carvacrol thymol, tannins, resin potassium nitrate, aspartic acid, glutamic acids and vitamins. These compounds have the ability to break down the chain reaction of free radicals due to oxidation from free radicals and are sought after by several types of free radicals such as superoxide and peroxy and hydroxyl radial. Polyphenolic compounds also inhibit enzyme oxidation, which inhibits the formation of free radicals **(Meeran *et al*., 2017).**

Testosterone supplementation plays important roles in sexual function. The synthesis of testosterone is in Leydig cells and estradiol is in Sertoli testis cells. Testosterone secretion is responsible for spermatogenesis, sperm maturation, and reproduction. Under normal circumstances, testosterone is directly related to the number and size of Leydig cells and the volume of the endoplasmic reticulum is an excellent precursor to Leydig's ability to produce testosterone (Zirkin and Papadopoulos, 2018). However, testosterone deficiency causes erectile dysfunction and lowers sexual fertility. The cause of MTX is a decrease in the level of testosterone serum **(Sarihan *et al*., 2020).**

The current result recorded decrease in testosterone level in group of MTX compare to control group and increment in group that treated with MTX and thyme extract compare to MTX group. Also, significant decrease in testosterone in MTX group compared to thyme group. However, insignificant statically increased of testosterone in group that treated MTX with thyme compare to MTX group, but increase in testosterone concentration .

Similarly, a decrease in testosterone hormone secretion is due to enzymatic impairment and a

defective state of the axothalamo-pituitary-gonadal axis that regulates syntheses **(Oyola and Handa, 2017).** Testosterone levels are reduced due to the enzymatic impairment of gonadal steroid synthesis in MTX-treated rabbits. MTX in testes inhibited testosterone synthesis may be due to exposure to gonadotropin secretion from the pituitary gland **(Al-Azawi and Asker, 2017).** The group that received MTX stimulates a decrease in testosterone levels due to enzymatic impairment in gonadal steroid synthesis, MTX antiestrogen and anti-digestive functions **(Karri *et al*., 2010).**

In addition, the release of ginseng acts as a phenolic chemical that causes an insignificant increase in serum testosterone levels which is greatly reduced by MTX. Pre-treatment increased hormone levels compares to the MTX group but was statistically significant **(Kamel *et al*., 2019).** It prevents toxic effect in male reproduction and an insignificant increase in serum testosterone hormone levels **(Jang *et al*., 2011).** Also, many natural products have proven its anti-toxin effect produced by MTX and raises testosterone levels **(Sherif *et al*., 2020).**

Leydig cells can produce estradiol. Estrogen has been found to be important in spermatogenesis in animals. However, estrogen insensitivity syndrome (ERα-deficient) in man produces sperm with a normal sperm count. High estrogen levels are indicative of spermatogenesis due to gonadotropin suppression inhibition **(Edmund *et al*., 2010).**

The present study recorded decrease in E2 level in group of MTX compare to control group and increment in group that treated with MTX and thyme extract compare to MTX group. Also, significant decrease in E2 in MTX group compared to thyme group. However, insignificant statically increase of E2 in group that treated MTX with thyme compare to MTX group, but increase in E2 concentration.

Similarly, testicular estradiol was decreased markedly by oxidative stress effect in rats, as also recorded **(Farrell *et al*., 2008).** The decrease in testicular 7-ethoxycoumarin O-deethylase activity which could be a reflection of decrease of the activity of steroidogenic P450 enzyme induced by oxidative damage which damage the testicular Leydig cells **(Huang *et al*., 2008).** A significant increase in E2 level in male rats that fed thyme leave against toxic rats was recorded **(Ahmed *et al*., 2015**). In agreement, herbal extracts induced increment in E2 after toxic **(Karbalaei *et al*., 2019).**

MTX has caused reproductive toxicity, oxidative stress, and testicular damage and changes in testicular structure in men. MDA is a hallmark of lipid peroxidation and free radial production **(Sarihan *et al*., 2020).** MTX increased MDA concentration in tissue and plasma. This increase in MDA levels is similar to many previous studies showing that MDA levels are increased by MTX causing oxidative sress. Similarly, Oxidative stress is one of the major causes of the damage caused by MTX in many organs **(Roghani *et al*., 2020).**

The current result recorded highly significally increased in MDA levels in MTX group compared to normal control group. MTX and thyme treatment group recoded improvement in MDA level compared to MTX group.

Some reports indicating that MTX induced oxidative stress by raising levels of MDA **(Belhan *et al*., 2017).** Moreover, the marked raise of MDA in serum and testicular tissue in MTX group attributed to oxidative stress in MTX-induced toxic injury **(Uyar *et al*., 2018).** Also, **Neslihan *et al* (2018)** illustrated that MTX caused severe destruction in testicles tissues by increment the formation of ROS and cause raises in MDA. Also, betamethasone-that has ROS induced oxidative damage, increase MDA levels in rat **(Hassan *et al*., 2020).**

Phenolic phytochemicals have many active ingredients such as antioxidant and free radicals to break down structures **(Meeran et al., 2017).** Thyme extract contains thymol and carvacrol which are common antioxidants. Ways to protect thyme from testis are important in declinig lipid peroxidation ad is a semen that protects against ROS. It can promote the prevention of reduced antioxidant and oxidative stress and therefore MDA. On the other hand, thyme treatment alone revived the activity of antioxidant enzymes and lowered MDA levels; and suppressed oxidative stress to make changes **(Hoseini *et al*., 2019).**

Apparently, antioxidants play an effective role in reducing free radicals and destroying cells and tissue damage **(Nimse and Palb, 2015).** Thyme vulgaris has protective effects on certain male reproductive parameters and a decrease in oxidative stress by indicating a decrease in MDA levels **(Salahshoor *et al*., 2020).** Antioxidants can play an important role in

increasing the body's ability to fight ROS. Thyme extraction inhibited the oxidative stress response caused by MTX and led to a reduction in MDA concentration **(Swayeh *et al*., 2014).**

Methotrexate causes an increase in lipid peroxidation causing metabolites and an increase in free radicals (Sarihan et al., 2020) which can lead to serious problems in the immune system to prevent oxidative stress, including intracellular antioxidant enzymes and levels of GSH (So and Aouacheri, 2017). These defenses consider the significant importance of the gonads and enzymes involved including SOD, GSH.Px, GR and CAT, all of which provide harmless oxidative stress products (Bihari et al., 2016). MTX reduces the activity of the antioxidant enzyme system causing cells to be more sensitive to ROS and more cellular damage. On the other hand, Oxidative stress is triggered by a decrease in MTX activity in GSH, CAT and SOD activities and the formation of lipid peroxidation **(Daggulli *et al*., 2014).**

Antioxidant protective mechanisms in the testis play an important role in protecting sperm from ROS **(Asadi *et al*., 2017).** Hydrogen peroxide (H2O2) is less effective than the superoxide group and is less effective by converting it into products for weak effects, for example, oxygen and water, with catalase enzymes and glutathione present in tissues, **(Callaghan *et al*., 2013).** SOD enzymes and CAT antioxidants have a protective effect that lowers free radicals. MTX has caused a decrease in SOD and CAT activity in the testicular tissues. SOD and CAT enzyme increase the imbalance with the use of renewable energy equipment **(Daggulli *et al*., 2014).**

In this result, MTX induced decrement in antioxidants enzymes concentration (GRD, SOD and CAT) and improved by thyme leave extract. However, SOD is insignificant statically decrease by MTX and increased by thyme extract.

Significant decrease in CAT activity was shown in response to MTX treatment, indicating that cells are sensitive to oxidative stress **(Ekinci-Akdemir *et al*., 2018).** MTX effects caused decrease in SOD level and cause elimination of cytotoxic superoxide radicals to H2O2 and cellular oxygen depleting polyunsaturated proteins and fatty acids **(Moghadam et al., 2015).** Some scientists have recorded significant reductions in SOD, CAT, GRD levels in which MTX is included. Those reductions account for the inhibition of the reaction or oxidative action of the protein enzyme due to free radicals **(Moodi *et al*.,** 2020).

The antioxidant properties of thyme extract is capable to protect reproductive parameters of the male against oxidative stress **(Salahshoor *et al*., 2020).** Similarly, Thyme enhances antioxidants enzymes activity against hydrogen peroxide that induced oxidative stress **(Hassan *et al*., 2020)**. Thyme has protective effects against oxidative stress by its antioxidant defense to convert reactive oxygen species into safe compounds to protect biological materials from oxidation **(Hoseini and Yousefi, 2019).** The phenolic compounds have antioxidant property and stimulate improvements in the antioxidant enzymes activities (SOD, CAT and GRD) after oxidative stress **(Mabrouki *et al*., 2020).** Similar results, thyme stimulate enhancement of SOD, CAT and GRD concentration **(Sobhy *et al*., 2020).**

In the present study, Hb, platelets and WBCs recorded decrement by MTX compared to control group and improved by thyme leave extract.

Oxidative stress is caused by MTX. The cell membrane is made up of polyunsaturated fatty acids that are sensitive to oxidative damage. MTX polyglutamates affect 5, 10-methylene-THF reductase which inhibits pyrimidine inhibition and purine metabolism resulting in inhibition of RNA and DNA synthesis **(Amézaga *et al*., 2018).** MTX toxicity is due to its action in the S-phase of the cell cycle, affecting highly beneficial tissues such as bone marrow. The risk of using MTX in the hematological system is high **(Conway and Carey, 2017).** The approach to phenolic remedies may be to reduce active oxygen species, which can prevent blood cells from damage caused by MTX. Platelet replacement, WBC, and a number of variables suggest that the remedies may mask the hematological abnormality caused by methotrexate. Thyme has the ability to sow active oxygen species and prevents oxidative damage to many blood cells **(Banji *et al*., 2011).**

Similarly, Activation in proliferating progenitor cells of the bone marrow are sensitive to methotrexate anticancer agents **(Patel and Ghodasara, 2014).** The hematological effect of MTX including decrement in platelets, WBC, and RBC. The death that occurs in RBCs as a result from

oxidative injury can also contribute to the decrement in RBCs count and in Hb level **(Gonzalez-Ibarra *et al*., 2014).** Furthermore, The anticoagulation effect of thyme prevents the accumulation of platelets, atherosclerosis, and thrombosis **(Al-Khalaf and Ramadan, 2013).** In agreement**,** Treatment with *Morinda citrifolia* showed beneficial effect by enhancing the levels of RBCs, WBCs, and Hb according to its ameliorative effect of preventing MTX-induced bone marrow suppression **(Mhatre *et al*., 2016).**

In conclusion, injection of MTX caused elevation of oxidative stress; inhibit antioxidant enzymes and abnormalities in sex hormones and hematological parameters. However, after administration of thyme leave extract with MTX injection may be improve testicular functions and hematological parameters by prevent oxidative damage due to the antioxidants property of thyme

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