

# **RESEARCH ARTICLE**

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# Protective Effects of Tribulus terrestris Extract on Methotrexate-Induced Liver Damage in Male Rabbits: An Experimental Study

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Submitted: 13<sup>th</sup> January 2025 Accepted: 12<sup>th</sup> April 2025 Published: 30<sup>th</sup> April 2025 ID: Orcid ID

### Abstract

**Objective**: Methotrexate (MTX), a commonly utilised chemotherapeutic agent for malignant tumours, adversely impacts and has side effects on blood parameters and some liver enzymes. This study sought to examine the protective role of TTE against hepatotoxicity initiated by Methotrexate (MTX) in adult male rabbits.

**Methods**: Twenty adult male rabbits were distributed into four groups. Each group comprised five rabbits. The initial group served as a control, the second group received T. terrestris extract at a dosage of 250 mg/kg, the third group was administered Methotrexate (MTX) at 0.03 mg/kg, and the fourth group was treated with both 0.03 mg/kg of MTX and T. terrestris extract at 250 mg/kg, orally, for 42 days.

**Results**: Methotrexate (MTX) resulted in a significant decrease ( $P \le 0.05$ ) in red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), packed cells volume (PCV) and significant increase in Alkaline phosphatase (ALP), Alanine transaminase (ALT) and Aspartate transferase (AST). The administration of T. terrestris extract contributed to restoring the parameters to their normal standard.

**Conclusion**: Methotrexate tends to decrease RBC, WBC, Hb, and PCV levels, contributing to anaemia and increased susceptibility to infections due to bone marrow suppression, and there was an increase in liver enzymes that was considered an indicator of liver damage due to the administration of methotrexate.

Keywords: Tribulus terrestris, Methotrexate, Liver enzymes, Male rabbits, Anaemia

### **Plain English Summary**

Methotrexate, a cancer treatment antimetabolite, affects blood and liver enzymes. This study examined the preventive role of Tribulus terrestris extract against Methotrexate-induced hepatotoxicity in adult male rabbits. Twenty mature male rabbits were split into four groups. The study found that methotrexate significantly decreased RBC, WBC, Hb, and packed cell volume. Methotrexate lowers RBC, WBC, Hb, and PCV, causing anaemia and bone marrow suppression, making patients more susceptible to infections. The MTX-treated group had considerably higher AST, ALT, and ALP levels than the control group.

### Background

Most chemotherapy treatments disrupt spermatogenesis, resulting in a significant drop in sperm count and quality cells (1). Several different

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chemotherapy treatments cause different general side effects and defects in spermatogenesis (2).

Testicular injury with Leydig cell harm related to

chemotherapy is one of the most general

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cytotoxicity consequences of treatment in males (3). Male infertility is an extremely worrying problem, as a result, there is a growing need to address the issue and study preventive strategies (4).

There are increasing numbers of males that hoped to have or wanted children after chemotherapy treatment (5). This was attributed to the improvements in cancer therapies, which increased the possibility of survival. Methotrexate (MTX) is an anti-metabolite predominantly utilised in chemotherapy and as an immunosuppressant for autoimmune disorders. This activity outlines the indications. mechanism of action. and contraindications for Methotrexate as an effective treatment for many neoplastic disorders (6). There is limited research on the direct effects of Tribulus terrestris on haemoglobin and haematocrit levels. Some studies suggest potential benefits, but more research is needed to confirm these effects. Also, Tribulus terrestris is believed to have antioxidant properties, which could potentially help in reducing oxidative stress in the blood. This might have various indirect benefits on blood parameters (7). The effect of Τ. terrestris extract. immunohistochemical and pathophysiological, against induced toxicity on the reproductive system in male rabbits, and its effect seems to be refereed by its antioxidant action, and it also stimulates the production of testosterone from the Leydig cell (8). This study aimed to examine the impact of T. terrestris on some physiological and biochemical indicators in male rabbits that were exposed to the administration of MTX and its role in moderating the side effects of MTX.

T. terrestris has been reported to have the ability to protect the liver from damage and restore normal functions. The aqueous extract of T. terrestris and silymarin had a significant protective effect against liver damage induced by the hepatotoxin carbon tetrachloride in rats. The T. terrestris extract was found to have cytoprotective effects and to restore hepatic architecture by lowering the levels of liver enzymes such as ALT, AST, ALP, and lipid peroxidation while increasing the levels of antioxidants such as glutathione and superoxide dismutase (SOD) in the liver tissue (9).

# Materials and methods

This research employed a cohort of twenty male rabbits. Their ages ranged from 4 to 5 months. Their body weight ranged from 1 to 1,200 kilogrammes. All animals were kept in consistent climatic settings of 25-27 °C with a daily photoperiod of 12 hours. The animals were contained in plastic enclosures measuring

90x60x30 cm. The cages underwent weekly sanitation. The meals (pellets) and water (tap water) were supplied without limitation. The animals were held for at least two weeks for acclimatisation before the examination began. The experiment's duration was six weeks.

# Experimental Design

Dose of Methotrexate: The MTX dosage was established at 0.03 mg/kg body weight by dissolving one tablet of MTX (2.5 mg) in 833 ml of distilled water, yielding a concentration of 0.003 mg/kg body weight per ml (0.003 mg/100 g body weight/ml).

# Preparation Tribulus terrestris

The following methodology was utilised to generate the extract: The fruits of Tribulus terrestris were sourced from their native habitats in Iraq, and the extraction process involved several stages. Plant samples were air-dried, rinsed with water, and then dried again. Following the evaporation of water, a powdered residue weighing 250 g was obtained from 500 millilitres of 70% ethanol using a Soxhlet apparatus. The extracts were then dried with a rotary evaporator set at 65°C, Ethanol extracts were accomplished according to established protocols by le Grand et al. (10). Each dried plant sample was ground and extracted in a percolator with 95% ethanol. About 10 ml of ethanol per gram of plant sample was used. The ethanol extract was dried under reduced pressure at 40 °C. The dried extract was stored in sterile bottles until further use. A total of 20 male rabbits were evenly distributed into 4 groups as follows (11):

1. Control (C) group: Five rabbits were administered distilled water.

2. Tribulus terrestris group: Five rabbits were given Tribulus terrestris (250 mg/kg/BW) orally three times per week (12).

3. Methotrexate (M) group: Five rabbits were administered methotrexate (0.03 mg/kg body weight) three times per week (13).

4. Tribulus terrestris and Methotrexate Group: Five rabbits got Tribulus terrestris (250 mg/kg/BW) daily and methotrexate (0.03 mg/kg body weight) three times weekly. The medications were administered by intubation.

# Blood collection

Blood samples were collected from each animal using a 5 ml disposable syringe with a 22G needle via the heart puncture procedure. The blood was divided into two parts; the first part was used to measure haematological parameters by haematology coulter. The other samples were deposited in a gel tube devoid of anticoagulant and allowed to stand at room temperature for approximately 30 minutes to facilitate adequate agglutination to separate the serum to be used in biochemical parameters. The serum was subsequently isolated in an Eppendorf tube via centrifugation at 3000 rpm for fifteen minutes and then preserved at -20 °C.

### Studied parameters

1. Serum Aspartate Aminotransferase determination: The aspartate aminotransferase (AST) activity in the serum is measured using an aspartate aminotransferase test kit (Agappe diagnostic company).

2. Serum Alanine Aminotransferase (ALT) determination: Serum levels were measured by using an Alanine Aminotransferase Test Kit (Agappe diagnostic company).

3. Serum Alkaline Phosphatase (ALP) determination: Serum alkaline phosphatase levels

are measured using an alkaline phosphatase test kit (Agappe diagnostic company).

### Statistical Analysis

One-way analysis of variance was used to examine the data in order to determine how the groups differed from one another by using the SPSS software. For every group, the significant difference was found to be less than the probability ( $P \le 0.05$ ).

### Results

# Haematological parameters

Experiments were performed to evaluate the levels of erythrocytes, leucocytes, haemoglobin, and packed cell volume following the injection of TTE and MTX in male rabbits, revealing significant variations among the groups ( $P \le 0.05$ ). Also, the TT group showed the protective effect on the value of blood parameters, which values were near the control group as showed in Table 1.

Table 1: Haematological Parameters in Male Rabbits Treated with Tribulus Terrestris Extract
(TTE), Methotrexate (MTX), and Their Combination

Groups	Control (C)	Tribulus terrestris	Methotrexate (M)	Tribulus terrestris and
parameters	group	group (250 mg/kg/D44)	group (0.05 mg/kg)	Metholiexale Oroup
Red blood cell 10⁰∖µl	5.11 ± 1.21	6.13 ± 1.21	3.21 ± 1.11	4.87 ± 1.63
	а	а	b	а
White blood cell 103\µl	7.11 ± 1.43	8.15 ± 1.43	5.32 ± 1.42	6.63 ± 1.72
	а	а	b	а
Haemoglobin g/dl	13.54 ± 2.21	14.21 ± 1.21	10.87 ± 2.35	13.88 ± 2.43
	а	а	b	а
Packed cell volume %	37.32 ± 3.32	38.11 ± 2.32	31.31 ± 2.54	$36.56 \pm 2.75$
	а	а	b	а

Values are expressed as the mean  $\pm$  SD. Distinct letters in each row and column signify the existence of significant differences at the probability level (P $\leq$ 0.05)

### **Biochemical Parameters**

Assessment of liver function enzymes: Experiments were conducted to assess the level of serum liver enzymes following the administration of TTE and MTX in male rabbits. The level of serum liver enzymes, i.e. AST, ALT, and ALP, were significantly increased in the MTX-treated group compared to the control group ( $P \le 0.05$ ). Also, the TT group showed the protective effect on the value of liver parameters, which values were near the control group, as shown in Table 2.

# Table 2: Liver Function Enzymes (AST, ALT, ALP) in Male Rabbits Treated with Tribulus Terrestris Extract (TTE), Methotrexate (MTX), and Their Combination

Groups	Control (C)	Tribulus terrestris	Methotrexate (M)	Tribulus terrestris and
parameters	group	group (250 mg/kg/BW)	group (0.03 mg/kg)	Methotrexate Group
AST (U/L)	147.6±4.17	154.3±3.22	211±5.22	166.5±4.22
	В	b	а	b
ALT (U/L)	76.85±3.22	79.44±2.21	131.2±4.22	83.55±5.11
	В	b	а	b
ALP (U/L)	86.32±2.44	91.14±3.53	135.2±3.23	88.66±3.32
	В	b	а	b

Values are expressed as the mean  $\pm$  S.D. Distinct letters in each row and column signify the existence of significant differences at the probability level ( $P \le 0.05$ )

# Discussion

Methotrexate (MTX) is widely used as an immunosuppressive and chemotherapeutic agent, but it can impact several physiological parameters, including red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and packed cell volume (PCV). Van De Meeberg et al. (14) explained that the MTX can reduce RBC count over time, leading to anaemia. This is because of the interference of folate metabolism with Methotrexate, which is necessary to produce erythrocytes, and this drug also may decrease leukocytes count, which can increase the risk of infectious disease because of its immunosuppressive roles.

However, methotrexate may be distributed in certain tissues. indicating selective immunosuppression in certain physiological compartments. Methotrexate decreases the concentration of Haemoglobin (Hb) and Packed Cell Volume (PCV) because of its effects on the production of erythrocytes. Anaemia would occur with continuous usage of methotrexate, as a side effect of the drug, particularly linked to bone marrow suppression (15). Our study findings revealed substantial protective effects linked to the usage of Tribulus terrestris extract. The study indicates a significant decrease in the components of blood in animals exposed to methotrexate. This may be due to the direct destruction of mature circulating blood cells, the depletion of blood cells from circulation due to haemorrhage or capillary leakage, and a decrease in the synthesis of blood cells (16).

Our study results indicate that administration with Tribulus terrestris extract gives protection against haematopoietic damages (17). On the other hand, the reduction of Glutathione increases the generation of reactive oxygen species and increases oxidative stress phenomena, leading to many effects that threaten the functional and structural integrity of cellular and organelle membranes (18). The reduced depletion of hepatic Glutathione in rabbits' pretreatment with Tribulus terrestris extract can be related to the increased availability of Glutathione, hence improving the ability to neutralise free radicals produced by oxidative stress. The increased Glutathione level proposes that the protective effects of Tribulus terrestris extract may be improved by the influence of cellular antioxidant levels.

Glutathione functions as a complex defender, revealing antioxidant characteristics by neutralising free radicals, repairing damaged molecules through hydrogen donation, decreasing peroxides, and maintaining protein thiols in their reduced form (19). The liver is considered the principal organ affected by pharmaceuticals, xenobiotics, and other hazardous substances due to its intricate anatomical structure and various metabolic functions (20). The primary functions of the liver are to metabolise and eliminate risky ingredients. The partial alteration of its normal function may lead to hepatotoxicity, revealed by changes in the liver enzvmes. particularly Alkaline phosphatase, aminotransferase Alanine and Aspartate Transaminase, showing hepatic injury and failure (21). This study examines the impact of Tribulus terrestris extract on modifying Methotrexateinduced hepatotoxicity in rabbits, revealing that Methotrexate administration may lead to a decrease in the levels of the studied liver enzymes (Alkaline phosphatase, Alanine aminotransferase and Aspartate Transaminase).

Some studies indicate that several plant extracts, functioning as natural antioxidants, can diminish the detrimental effects and toxicity linked to Methotrexate (22). The current study indicates the injection of Methotrexate in rabbits significantly increased liver enzymes (Alkaline phosphatase, aminotransferase and Aspartate Alanine Transaminase) levels. These findings align with those of Jan et al. (23). The increase in liver phosphatase, enzvmes (Alkaline Alanine aminotransferase and Aspartate Transaminase) levels may result from an increase of Methotrexate in hepatic tissues, causing a rupture of the cell membrane and the subsequent release of enzymes into the bloodstream. Rabbits in the Tribulus terrestris + Methotrexate cohort exhibited significantly reduced levels of Alkaline phosphatase. Alanine aminotransferase and Aspartate Transaminase enzymes. Many different compounds with a variety of biological properties and chemical structures have been identified from TT. including steroidal saponins. flavonoids. glycosides, phytosterols, tannins, terpenoids, amide derivatives, amino acids, and proteins. Among the different types of constituents, steroidal saponins and flavonoids are the most important metabolites with various bioactivities (24).

Tribulusterrestris extract contains alkaloids, resins, flavonoid oil, and nitrates. which have antihypertensive, anti-inflammatory, hypolipidemic, and anti-diabetic activities. The ethanolic extract of T. terrestris has been reported to possess substantial antioxidant activity that leads to the modulation of Glutathione (25). In the liver of Wistar rats that had undergone experimental fibrosis induced by carbon tetrachloride. Malondialdehyde, NF-kb, collagen 1, nuclear factor erythroid-2related factor 2, and tumour necrosis factor all showed numerical declines in the treatment groups

when compared to the control, but aspartate aminotransferase and alanine aminotransferase levels were unaffected (9).

Some studies indicate that TT supplementation modulates the androgen profile through an increase in serum testosterone levels and the testosterone/oestradiol ratio. Steroidal saponins are the main TT phytochemical compound responsible for the rise in the levels of testosterone and related hormones, such as luteinizing hormone. dehydroepiandrosterone, and dehydroepiandrosterone sulphate. However, the mechanism by which TT elevates testosterone levels is not fully understood. Saponins from TT also act directly as a neurosteroid, increasing dehydroepiandrosterone levels, which may exert an antagonistic effect on gamma-aminobutyric acid (GABA) and thereby enhance sexual function regardless of testosterone levels (26).

Akbary et al. (27) showed that the administration of the extract of Tribulus terrestris at doses of 0.5, 1, and 1.5 g/kg diet enhanced hepatic and digestive enzyme activity and ameliorated the lipid profile (28). To corroborate this, our findings suggest that the Tribulus terrestris extract and its polyphenolic bioactive substances may mitigate Methotrexateinduced hepatic injury by preserving the integrity of liver cell membranes. We suggested future human studies and the study of the long-term safety of the *Tribulusterrestris*.

# Conclusion

In summary, this study shows that Tribulus terrestris extract can help protect against the harmful effects of methotrexate, particularly when it comes to blood and liver health. When methotrexate is administered, it greatly lowers levels of red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), and packed cell volume (PCV) while increasing oxidative stress and liver enzyme levels. This points to issues like bone marrow suppression and liver toxicity. However, taking Tribulus terrestris extract beforehand seems to counteract these negative effects. It boosts glutathione levels, cuts down on oxidative damage, and helps stabilise liver enzyme activity. The extract's active components, especially steroidal saponins and flavonoids, likely play a big role in its antioxidant and anti-inflammatory effects. These insights suggest that Tribulus terrestris could be a useful complement to traditional treatments for managing methotrexate-induced toxicity. More clinical trials are needed to confirm these benefits in humans and to evaluate long-term safety.

# List of abbreviations

- MTX: Methotrexate
- TTE: Tiliacora triandra extract
- RBC: Red blood cells
- WBC: White Blood Cells
- Hb: Haemoglobin
- PCV: Packed cells volume
- ALP: Alkaline phosphatase
- ALT: Alanine transaminase
- AST: Aspartate transferase

# Declarations

### Ethical approval and consent to participate

The inquiry was governed by the ethical standards outlined in the Declaration of Helsinki. Before the collection of the sample, the patient provided verbal and analytical consent. The study protocol, subject information, and permission form were reviewed and approved by a local ethics committee under document number 3022 (dated 20/9/2024).

# Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

# Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

# Competing interests

The writers of this article affirm that they have no potential conflicts of interest related to writing or publishing it. Author's contributions. The entire project was created by the authors, who also worked in tandem to gather data and perform statistical studies on it.

# Funding

There is no funding source for this study.

# Author contributions

RHH: contributed to the conceptualisation and supervision of the study, as well as reviewing and editing the final manuscript.

HSD: was responsible for data collection, laboratory analysis, and drafting the original

manuscript.

MTA: assisted in methodology development, statistical analysis and contributed to the interpretation of the results

# Acknowledgement

Not applicable.

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