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Effect of Tribulus Terrestris Extract on male mice with Cyclophosphamide

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Abstract

Background: This study explores the protective effect of Tribulus terrestris, a well-known medicinal plant traditionally used to enhance physical strength and reproductive health, against liver and reproductive damage caused by Cyclophosphamide, a chemotherapeutic agent. **Method:** In the experiment, male albino mice were divided into four groups. The first group (control) received only distilled water. The second group was injected with Cyclophosphamide alone. The third group received only the Tribulus terrestris extract, while the fourth group received Cyclophosphamide followed by treatment with the plant extract for 14 days. At the end of the experiment, blood samples were collected to measure liver enzymes (ALT, AST, ALP) and testosterone levels. Histological examination of liver and testis tissues was also performed. **Results:** The results showed that the group treated only with Cyclophosphamide had significantly high levels of liver enzymes, indicating liver damage, along with a sharp drop in testosterone levels, reflecting reproductive toxicity. In contrast, the group treated with Tribulus terrestris alone showed normal enzyme levels and healthy testosterone values, indicating the safety of the plant and its potential benefits. Most importantly, the group that received both Cyclophosphamide and the plant extract showed notable improvement. Although the levels were not fully restored to normal, liver enzyme levels decreased, and testosterone levels increased compared to the Cyclophosphamide-only group, suggesting that the plant extract provided partial protection against the drug's harmful effects. **Conclusion:** Tribulus terrestris extract demonstrated promising hepatoprotective and fertility-supporting properties in this experimental model. It could potentially be used as a natural supplement to reduce the side effects of chemotherapy drugs like Cyclophosphamide.

Keywords: Tribulus terrestris, Cyclophosphamide, ALT, AST, ALP, testosterone.

INTRODUCTION

Most plants are an important food source, in addition to their high nutritional value, because they contain sources of vital energy, carbohydrates, proteins, and fats. It has a medical benefit. Therapeutic, as it has a role in treating many medical conditions, including asthma and infections, such as Bronchitis, whooping cough, and skin allergies. Many plants are

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pharmacologically effective because they contain chemical compounds and have clear biological activity against bacteria. And various pathogenic fungi (1).

Tribulus terrestris is a plant commonly used in traditional medicine, particularly in Ayurvedic and Chinese medicine. It is known for its potential benefits in enhancing athletic performance, improving libido, and supporting overall health.

Tribulus terrestris is a small, spiny plant found in many parts of the world, including Europe, Asia, and Africa. The plant contains various bioactive compounds, including saponins (such as protodemetil saponin), flavonoids, alkaloids, and glycosides, which are believed to contribute to its therapeutic effects, Enhancing Libido and Sexual Function (2).

Tribulus terrestris is widely used as a natural aphrodisiac. Several studies have shown that it may help improve sexual desire and performance, particularly in individuals with low libido or sexual dysfunction (3).

Materials And Methods

1. Aqueous extraction of Tribulus terrestris
2. Preparation of Cyclophosphamide ($C_7H_{15}ClN_2O_2P$).
3. dosing mice(four group each group contain 4 mice) for 14 days, according to the experiment.

4. Measurement of liver enzymes and testosterone hormones, and histological section of the testis and liver.

Plant extract

The leaves of the Tribulus terrestrial plant were taken and washed well to get rid of bacteria and pollution, dried well, then ground to a powder and then 100 grams of the plant were taken and placed in filter paper, closed well, and placed in a Reflex device with an amount of 500 ml of distilled water and leave it in the incubator at 70 C° for a three day, then filter it with filter paper and gauze, pour it into dishes, leave it for a day, and after it dries the plant is scraped to obtain the extract.

Reflex extractor

The Reflex extractor is used to extract active compounds from raw materials using solvents. It works by heating the raw material with a suitable solvent to evaporate the desired compounds, then condensing the vapor to separate the extract. The device includes a heating vessel, a condenser for cooling vapors, and a collection tank for the extract. It is commonly used in pharmaceutical, food, and chemical industries, offering high efficiency and precise control(4).

Preparation of Cyclophosphamide

Cyclophosphamide, all other chemicals, and reagents were purchased from Zydus. It was broken and then prepared in a dose (25mg/kg bw) was administered intraperitoneally only once and dissolved in distilled water.

Laboratory Animals

Albino male mice were the laboratory animals. They were supplied by the Biotechnology Research Centre (Al-Nahrain University). Their ages at the start of the experiments were 8-10 weeks, and their weight was 25-30 grams. They were distributed into groups, and each group was kept in a separate plastic cage. The animals were maintained at room temperature and had free access to food (standard pellets) and water (ad libitum).

THE METHOD OF WORK

We need four groups, as follows:

- Group I(n=4 mice): Mice were administered a single daily dose (1 ml) of distilled water for 14 days (Control I).
- Group II (n=4mice): Mice were (Intra-abdominal) with a single dose (1ml) of cyclophosphamid for 14 days.
- Group III (n=4mice)I: Mice were administered (Intra-abdominal) with a single dose (1ml) of the plant for 14 days.

- Group IV(n=4mice): Mice were administered (Intra-abdominal) with a single dose (1ml) of CP for 1 day and then administered with (1ml) the extract of the plant from 2 days to 14 days. On the 14th day, the autopsy is done to take a tissue section of the liver and testis, and take a blood sample for each group. Put it in a tube gel and then in a centrifuge to get the serum to measure liver enzymes and testosterone in commercial kits.

Tissue culture mechanics

a) Tissue sample: We get a small sample of tissue through the ablation diagnostic or surgical removal must be cut the fabric carefully with a sharp tool so as not to deform the microscopy showing. Even installation is good, you should not tissue mass of more than one centimeter, and the sample immersed in the installer immediately after you take it.

b) Fixation: The fixation process hardens soft tissue to prevent damage and chemical changes that occur as a result of the activity of proteins in the tissue. The fixation process binds the protein to the tissue. The use of thermal installation can not cause distortions in the tissue. For this, chemical fixation is a coagulation variant.

c) Dehydration: The goal of the paraffin etching process is to replace all the water in the tissue mass with paraffin wax so that the tissue can be easily cut later. However, since paraffin does not dissolve in water, the water must be disposed of first. The tissue is passed successively in rising alcohol solutions, leaving the tissue in each solution long enough for the water to replace the alcohol

d) Clearing: Using Alzailol, usually, the leaching tissue passes through a mass saturated with alcohol several changes with Alzailol and results in the replacement of all Alzailol alcohol and then becomes a textile block ready for embedding

e) Embedding:- Mass Saturated pass through Balzailol several changes of warm paraffin and as soon as it becomes saturated, fabric paraffin, which fills in the blanks that were originally containing water, paraffin wax in the fabric, which facilitates the cutting process.

f) Sectioning: Then, prove tissue mass in the cutting device, which is called a micotome, and cut into thin slices attached to each other, so they can be removed from each other later with ease.

g) Staining and mounting: For dyes to be added to the tissue slide, the paraffin wax must be removed and replaced with water for the dye to appear effectively.

To this end, the slide is passed through alcoholic solvents and then, in a few steps, Alzailol even replaces all paraffin with water. Then add a drop or two of the coloring, and the process of converting water to wax begins again. Finally, the examination of the tissue remains.

Statistical Analysis

To determine if there were any statistically significant differences between the treatment groups, the data were reported as mean values ± SE and subjected to an unpaired t-test statistical analysis. The statistical significance criterion of $p<0.05$ was selected for the biochemical data. All statistical analyses were performed using the SPSS statistical version 21 software package (SPSS® Inc., USA).

Result

After performing histological sections of the testis, measurement of Testosterone and Liver Enzymes for each group, which includes:

1- Glutamic pyruvate (GPT) or alanine aminotransferase (ALT).

2- Glutamic aminotransferase (GOT) or aspartate aminotransferase (AST).

3- Alkaline phosphatase (ALK) or (ALP).

Table (1):. level of liver function test in different mouse groups:

Groups	GPT mean±SD	GOT mean±SD	ALP mean±SD
GroupI(control)	58±2.1	45±1.5	78±2.5
groupII(Cp)	70±1.5	82+2.9	195±4.2
groupIII(tribulus terrestris)	53±2.5	43±2.3	88±1.5
GroupVI(Cp+tribulus terrestris)	66±2.3	58±1.1	150±2.1

Table (2):. show the level of testosterone hormones in different mouse groups:

Groups	Testo hormone mean±SD
GroupI(control)	14±1.2
groupII(Cp)	5±1.5
groupIII(tribulus terrestris)	13±2.1
GroupVI(Cp+tribulus terrestris)	9±0.6

Histopathological Examination of liver Differential groups in male mice

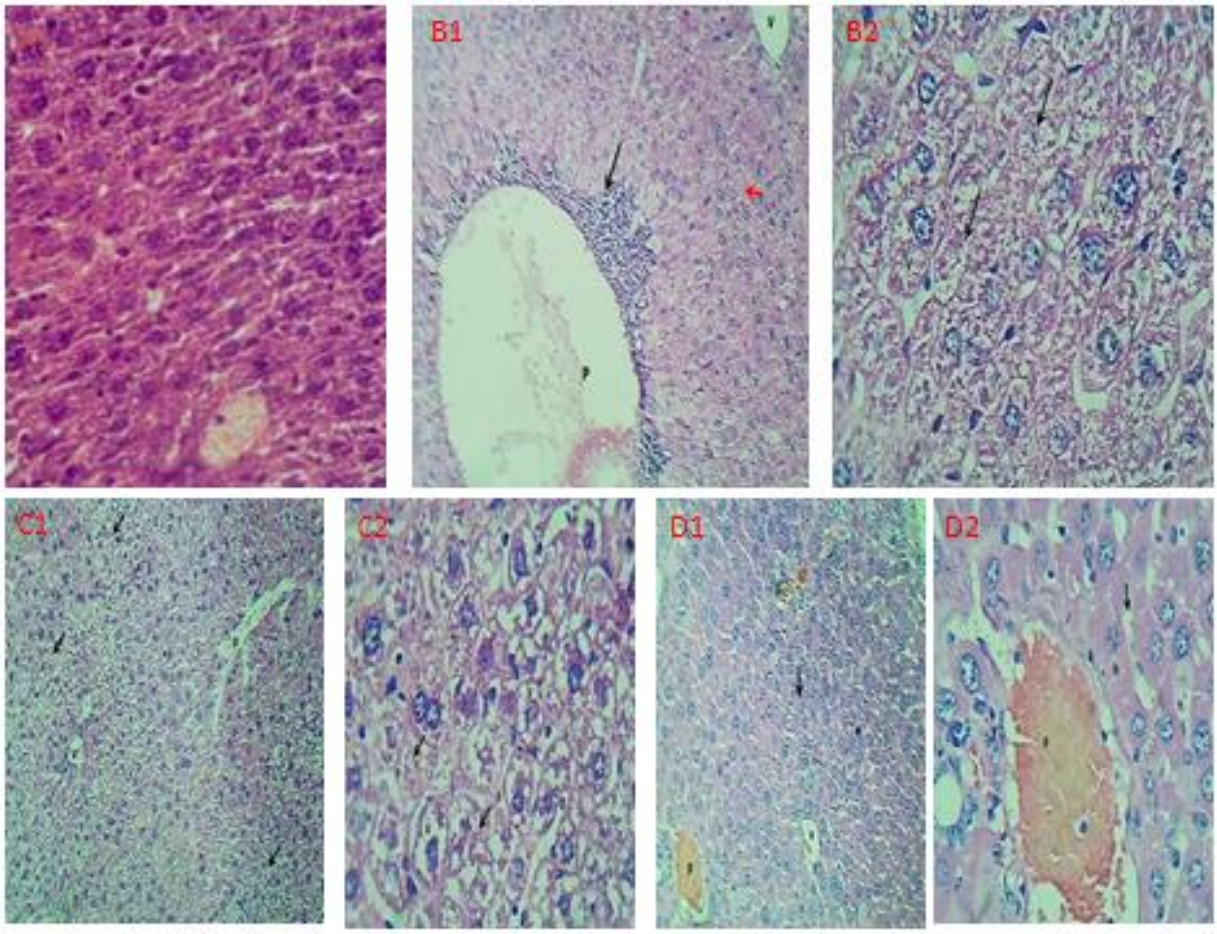

Figure (1):. Histopathological Examination of liver Differential groups in male mice.

A:. The histopathological figures of the liver showed a normal appearance(Fig A1).

B:.The histopathological figures of the liver revealed normal arrangement of the hepatic cords, marked dilation of the portal vein that showed perivascular cuffing of mononuclear leukocytes, dilation of the central vein, and marked hypertrophy with vascular degeneration and necrosis of hepatocytes (figs.B1 & B2).

C:.The histopathological figures of the liver revealed normal arrangement of the hepatic cords, normal portal vein and central vein, the hepatocytes revealed marked generalized vacular degeneration with necrosis (fig.C1 & C2).

D:.The histopathological figures of the liver revealed normal arrangement of hepatic cords, normal central vein with normal hepatocytes, and little congestion of the portal vein (fig.D1 & D2).

Histopathological Examination of Testes: Differential groups in male mice

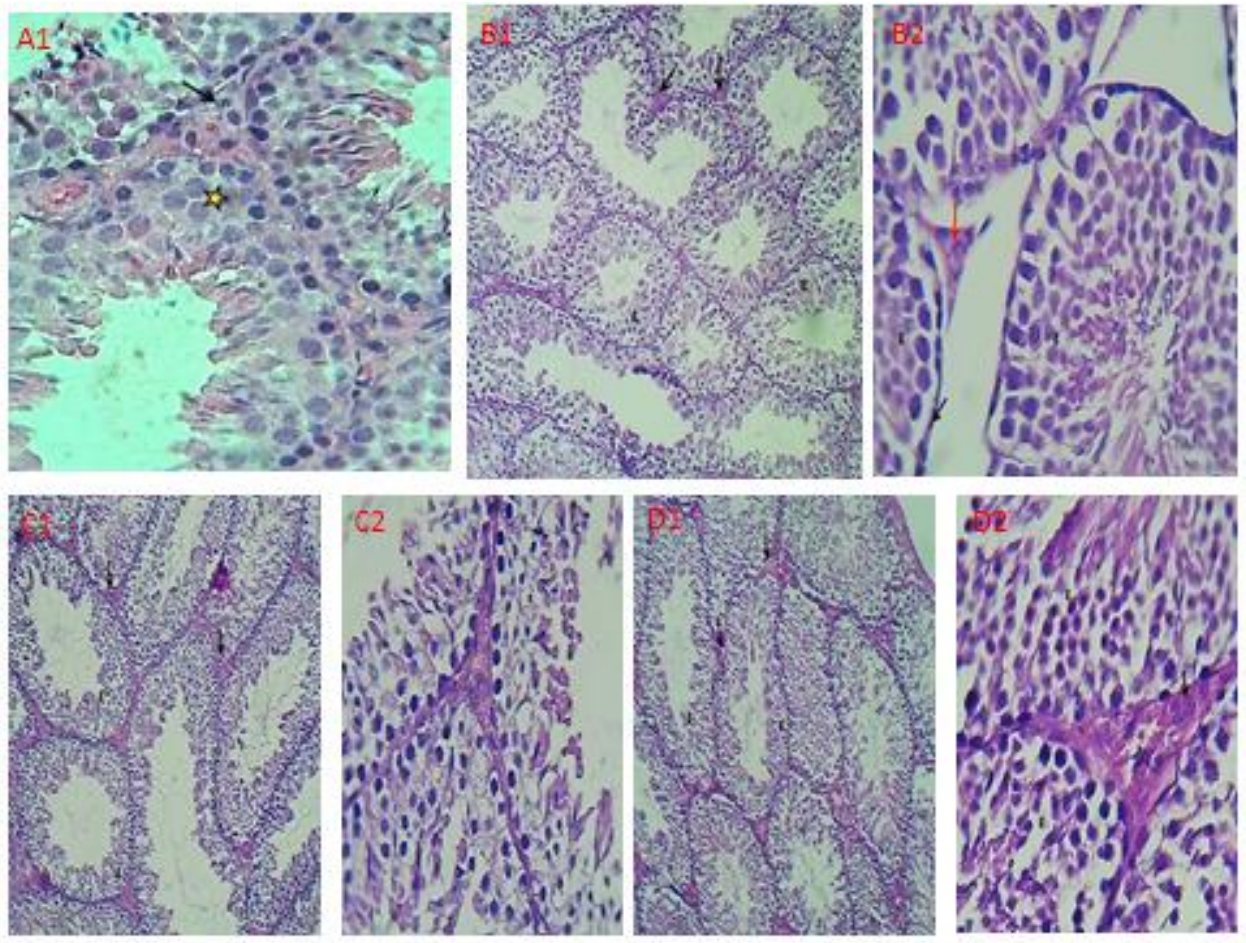

Figure (2): Histopathological Examination of Testes Differential groups in male mice.
A :. The histopathological figures of the testis showed normal appearance and size of the Seminiferous tubules with normal testicular interstitum(Fig A1).
B :.The histopathological figures of the testis revealed normal appearance of the seminiferous tubules with normal appearance of all stages of germinal epithelium cells, normal interstitial cells, and myoid cells (B1 & B2).
C:.The histopathological figures of the testis revealed normal appearance of seminiferous tubules with a few figures of cellular swelling in all stages of germinal epithelial cells, normal interstitial cells, and myoid cells (C1 & C2).
D:.The histopathological figures of the testis revealed normal appearance of seminiferous tubules with marked thickening of normal germinal epithelial cells, normal interstitial cells, and myoid cells (D1 & D2).

Discussion

In this study, the hepatoprotective potential of Tribulus terrestris extract was evaluated against liver toxicity induced by Cyclophosphamide (Cp), a widely used chemotherapeutic agent known for its hepatotoxic side effects. The study involved albino male mice divided into four groups: control, Cp-only, plant-only, and a combination of Cp and the plant extract.

The biochemical results clearly demonstrated that the Cyclophosphamide group exhibited a significant increase in liver enzymes: GPT (70±1.5), GOT (82±2.9), and ALP (195±4.2), compared to the control group. This elevation strongly indicates liver cell damage, as these enzymes are released into the bloodstream when hepatocyte membranes are compromised. These findings are consistent with earlier studies such as (5,6), which reported that Cp generates oxidative stress and free radicals (ROS), leading to cellular damage.

Moreover, a marked reduction in serum testosterone levels (5±1) was observed in the Cp group. This suggests that Cp might negatively affect hormonal balance and testicular function by impairing Leydig cells or disrupting the hypothalamic-pituitary-gonadal axis. Similar hormonal suppression effects have been documented in chemotherapeutic studies, including those by (7.8).

In contrast, the plant-only group showed stable enzyme levels: GPT (53±2.5), GOT (43±2.3), and ALP (88±1.5), along with a relatively normal testosterone level (13±2.1). This indicates that Tribulus terrestris extract is safe for the liver and hormonal functions. Previous literature, such as (9-11), has confirmed that this plant possesses stimulating effects on testosterone production due to the presence of steroidal saponins like protodioscin.

Most notably, the combination group (Plant + Cp) demonstrated significant biochemical improvement compared to the Cp-only group. Liver enzyme levels were lowered to GPT (66±2.3), GOT (58±1.1), and ALP (150±2.1), and testosterone levels improved to 9±0.6. Although not fully normalized, these results suggest a strong protective effect of the plant extract (12-14).

The protective action is likely attributed to the antioxidant compounds in Tribulus terrestris, such as flavonoids and saponins, which can scavenge free radicals, stabilize cell membranes, and reduce inflammation. These mechanisms play a crucial role in minimizing oxidative damage caused by Cp (15,16).

These findings align with other studies highlighting the hepatoprotective and antioxidative effects of Tribulus terrestris. For instance, (17) found similar liver protection effects in mice exposed to other hepatotoxins(18).

In conclusion, Tribulus terrestris extract demonstrated a promising hepatoprotective effect and partially restored testosterone levels in Cp-induced hepatotoxicity(19). It may serve as a beneficial complementary therapy for patients undergoing chemotherapy, pending further research including histopathological assessments to confirm tissue-level recovery(20,21).

Conflict of interest: NIL

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