International Journal of Applied and Pure Science and Agriculture
ISSN Online - 2394-5532 Impact Factor : 5.295 by SJIF ISSN Print - 2394-823X

PROPHYLACTIC EFFECT OF *Tribulus terrestris* ON CYCLOPHOSPHAMIDE TOXICITY IN WISTAR RATS

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Abstract

Cyclophosphamide is a widely used antineoplastic and immunosuppressant agent with many adverse side effects which include myelosuppression. Tribulus terrestris is a medicinal plant with proven potency to reverse the adverse effects of many anticancer agents. The protective effect of Tribulus terrestris in cyclophosphamide induced hepato, nephro and haemotoxicity was experimentally evaluated using its alcoholic extract in Wistar rats. The preliminary phytochemical screening of ethanolic extrat of Tribulus terrestris was done for the identification of active principles. A total of 30 Wistar rats were divided in to five groups of six animals each. Group I received distilled water. Animals of groups II, III, IV and V were administered with cyclophosphamide twice weekly for 21 days @ 15 mg/kg bodyweight orally. Group III, IV and V were administered with ethanolic extract of Tribulus terrestris @ 100, 250 and 500 mg/kg body weight respectively, daily orally. Blood was collected on day 0 and 21 for estimation of the total RBC and WBC counts, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine levels. There was a significant increase in the levels of AST, ALT, RBC count and haemoglobin in cyclophosphamide control group (group II) where as no significant difference was observed in groups I, III, IV and V (administered with ethanolic extract of Tribulus terrestris @ 100, 250 and 500 mg/kg body weight respectively), on days 0 and 21. No significant difference was observed in blood urea nitrogen, creatinine and WBC count in all the groups on day 0 and 21. The active principles detected in the phytochemical screening of the plant extract like flavonoids, saponins etc might have prevented the increase in the levels of AST and ALT and decrease in RBC count and haemoglobin values in groups III, IV and V, which were significantly altered by cyclophosphamide administration. Hence it can be concluded that Tribulus terrestris has excellent protective effects on hepatotoxic, haemotoxic and myelotoxic properties of cyclophosphamide.

Key words: cyclophosphamide, Tribulus terrestris, RBC, WBC, Haemoglobin

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I. INTRODUCTION

Cyclophosphamide is a cytotoxic alkylating agent, commonly used as an anticancerous drug and an immunosuppressant. On the other hand, the clinical use of cyclophosphamide has been limited due to its potential to damage normal tissues which usually resulted in multiple organ toxicity

and attempts have been made to reduce the toxic effects of this drug. Recent research indicate that herbs and herbal products provide encouraging results in reducing toxicity of cyclophosphamide.

Tribulus terrestris, member of the *Zygophyllaceae* family, is an annual herb with pinnate leaves, yellow flowers and satellite shaped carpel fruits. The occurrence of glycosides, steroids, saponins, flavonoids, alkaloids, tannins, vitamins and unsaturated fatty acids has been reported in *T. terrestris.* The fruit extract prevents the cadmium induced hepato-renal toxicity in rats (1). Based on these reports, this study was designed to determine the possible protective effect of alcoholic extract of *T. terrestris* against oxidative damage of liver, kidney and myeloid tissue following oral administration of cyclophosphamide by ascertaining changes in serum biochemical and haematological parameters of male Wistar rats.

II. MATERIALS AND METHODS

The fresh fruits of *T. terrestris* were procured locally from Thrissur district of Kerala, identified and shade dried. The fruits were pulverised and methanolic extract was prepared using Soxhlet extraction apparatus, which was dried using rotary vacuum evaporator (R100, BUCHI) and stored under refrigerated conditions till further use. The ethanolic extract of *T. terrestris* was analysed qualitatively for the presence of active principles (2).

Thirty adult male Wistar rats weighing 150- 200 g were procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy and were divided in to five groups of six animals each. Group I received distilled water. All animals, except of group I, were dosed with cyclophosphamide at a dose of 15 mg/kg twice weekly orally in the morning. The animals of group III, IV and V were orally administered with methanolic extract of *T. terrestris* at 100 mg/kg, 250 mg/kg and 500 mg/kg respectively daily for 21 days along with cyclophosphamide treatment.

Blood was collected on days 0 and 21 from the retro orbital plexus of rats under ether anesthesia using heparinized capillary tubes, into sterile vials containing disodium salt of ethylene diamine tetra acetic acid (EDTA Sodium) at the rate of 1 mg/mL for estimation of haematological parameters. Blood was collected in sterile centrifuge tubes without anticoagulant, kept at refrigeration temperature for half an hour followed by incubation at room temperature for another half an hour. It was then centrifuged at 3200 rpm for 10 minutes and the clear serum obtained was pipetted out for the estimation of biochemical parameters.

2.1 Estimation of haematological parameters

Total erythrocyte count, total leukocyte count and haemoglobin concentration were estimated as per the standard technique (3).

2.2 Estimation of serum biochemical parameters

The serum biochemical parameters such as Alanine aminotransferase, Aspartate aminotransferase, blood urea nitrogen by UV kinetic method (4) and creatinine (Jaffe kinetic method) were estimated colourimetrically in semi automatic blood analyser (Hospitex) using analytical kits (Agappe diagnostics Ltd, India).

Data obtained from the experiment were statistically analysed using analysis of variance followed by Duncan's multiple range tests for comparison between groups. Results were expressed as Mean ± Standard error. A value of $P < 0.05$ was considered statistically significant.

III. RESULTS

The results were analysed statistically and are presented in table (1) and figures (1-7). The phytochemical investigation of ethanolic extract of fruits of *T. terrestris* revealed the presence of flavonoids, alkaloids tannins, saponins, polyphenols and steroids.

The initial values of ALT, AST, RBC count and haemoglobin recorded on day zero did not show any significant variation between the different treatment groups of rats. Serum ALT activity was found increased significantly ($P < 0.05$) in rats of the CP control group (group II) relative to the animals in other groups, on day 21. Co- treatment with extract in different doses showed no increase decreased its levels with maximum reduction observed in rats treated with 500 mg/kg extract, where the values were almost comparable to that of normal control. Similar observations were seen with serum AST, RBC count and haemoglobin concentration. There was a slight but not significant increase in serum creatinine and blood urea nitrogen in CP treated group on day 21. There was no significant change in these parameters in extract treated groups. Group II showed a slight decrease in WBC count while extract treated group showed slight but no significant increase in WBC count in compared to the control.

Table 1. Phytochemical Analysis of Ethanolic extract of *T. terrestris* fruits

Active principle	**Test**	**Observation**	**Inference**
Steroids	Salkowski test	Red colour	+
	Leiberman Burchardt test	Reddish ring at the junction of two layers	+
Alkaloids	Mayer's test	Creamy white precipitate	+
	Wagner's test	Reddish-brown precipitate	+
	Hager's test	Yellow precipitate	+
	Dragendroff's test	Reddish-brown precipitate	+
Phenolic compounds		Dark blue colour	+
Tannins	Ferric chloride test	Green colour	+
	Gelatin test	White precipitate	+
Flavonoids	Ferric chloride test	Green colour	+
	Lead acetate test	Yellow colour	+
Glycosides	Sodium hydroxide test	Yellow colour	+
	Benedict's test	Brown colour	+
Diterpenes		Green colour	+
Triterpenes	Salkowski test	Yellow colour on standing	
	Leiberman Burchardt test	Deep red ring at the junction of two layers	+
Saponins	Foam test	Presence of foam	+

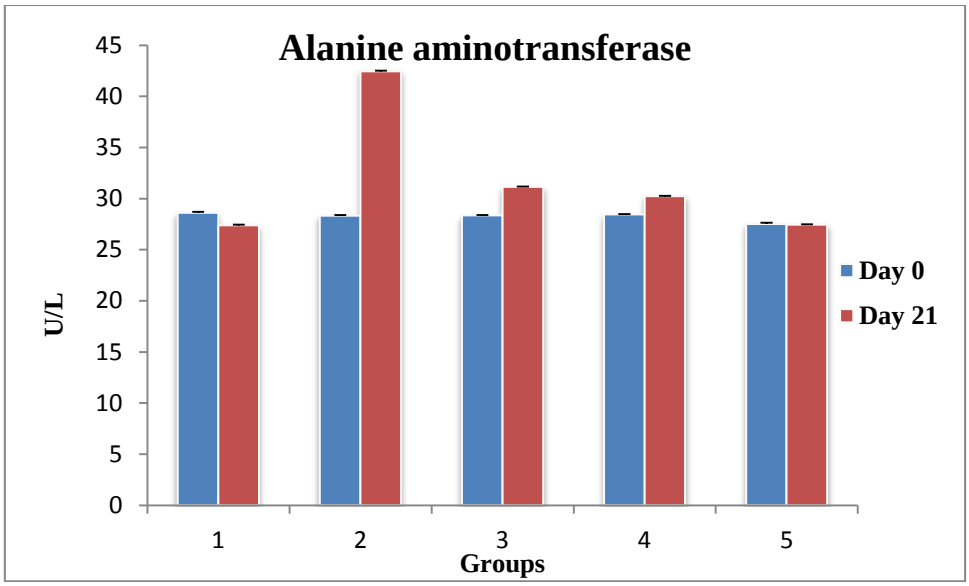

Figure 1. Effect of ethanolic extract of T. terrestris fruits on alanine aminotransferase on days 0 and 21

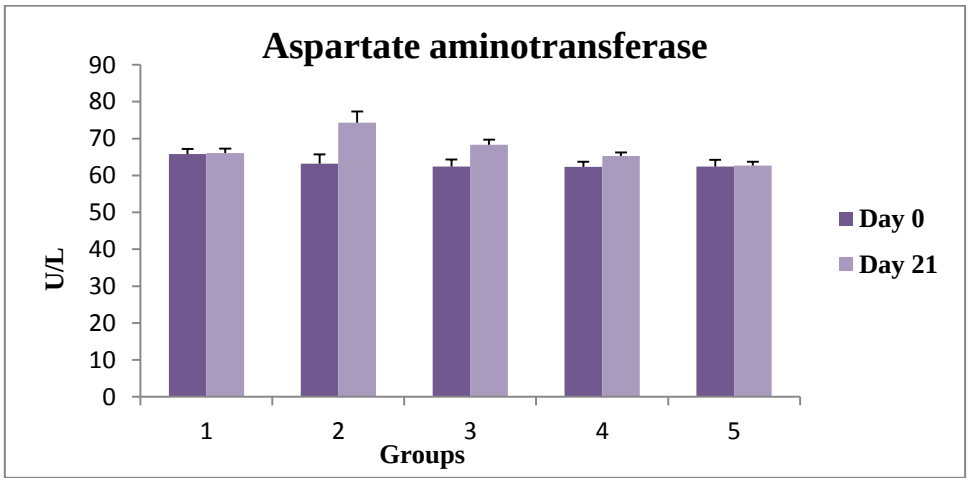

Figure 2. Effect of ethanolic extract of T. terrestris fruits on aspartate aminotransferase on days 0 and 21

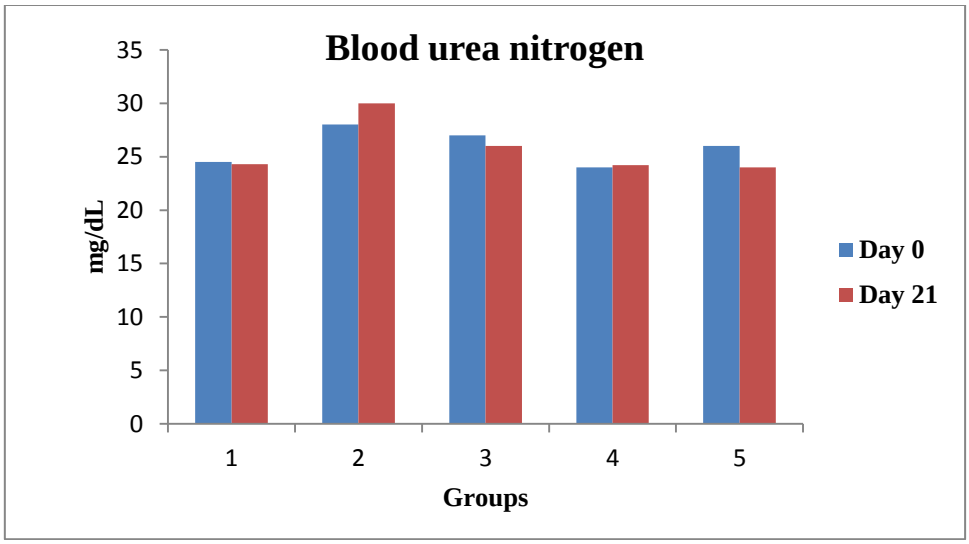

Figure 3. Effect of ethanolic extract of T. terrestris fruits on blood urea nitrogen on days 0 and 2

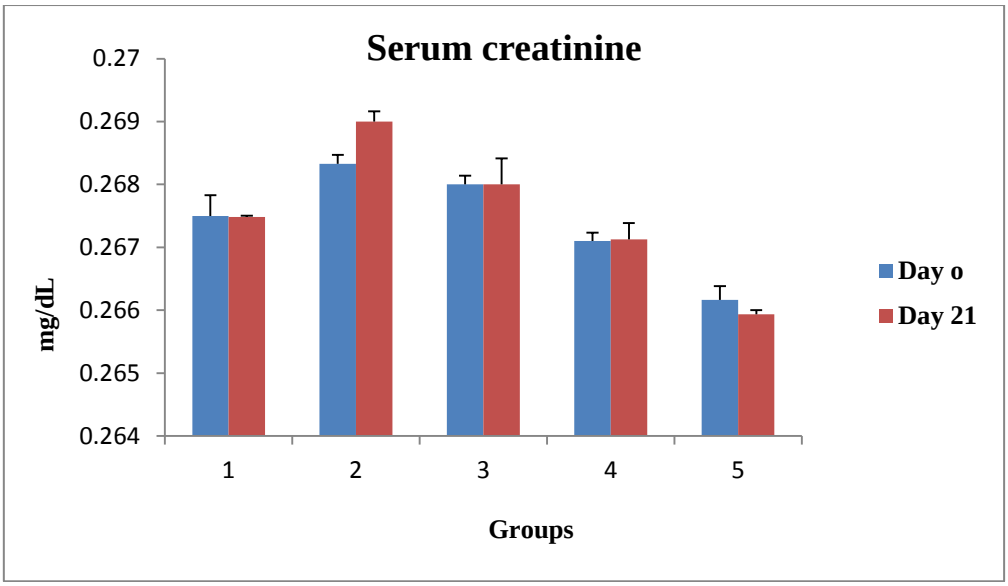

Figure 4. Effect of ethanolic extract of T. terrestris fruits on serum creatinine on days 0 and 21

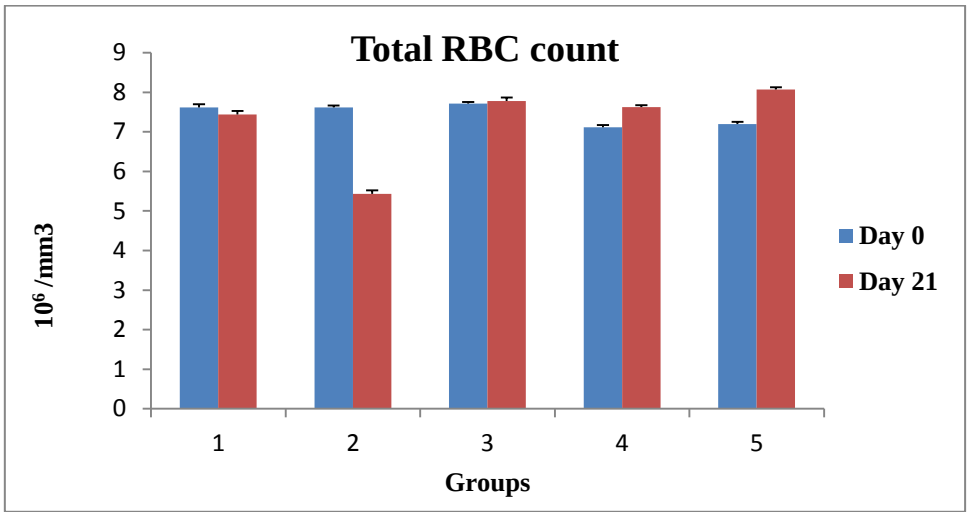

Figure 5. Effect of ethanolic extract of T. terrestris fruits on total erythrocyte count on days 0 and 21

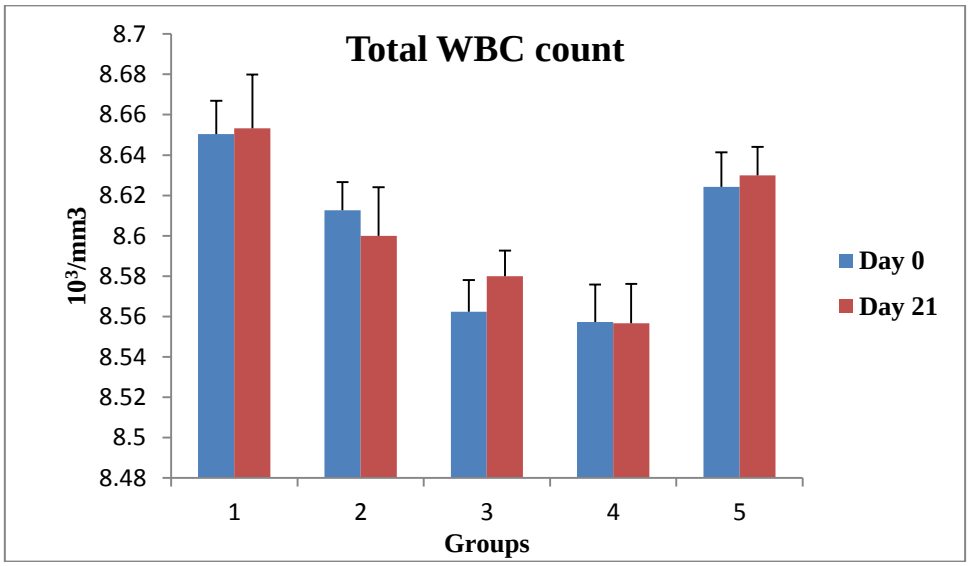

Figure 6. Effect of ethanolic extract of T. terrestris fruits on total leucocyte count on days 0 and 21

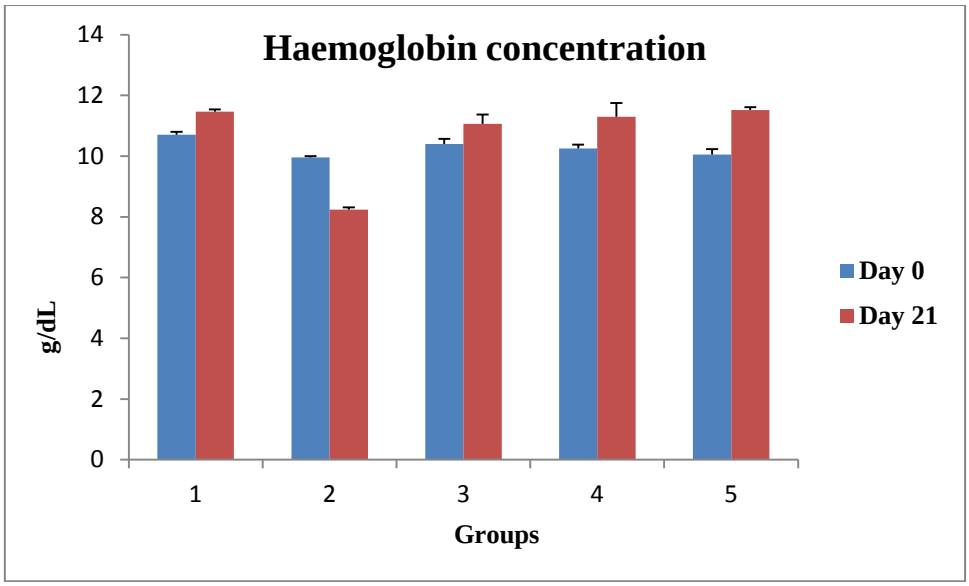

Figure 7. Effect of ethanolic extract of T. terrestris fruits on haemoglobin concentration on days 0 and 21

IV. DISCUSSION

The phytochemical investigation of ethanolic extract of fruits of *T. terrestris* revealed the presence of flavonoids, alkaloids tannins, saponins, polyphenols and steroids. Among the saponins isolated from the fruit extract of *T. terrestris*, protodioscin was the most popular active substance bearing aphrodisiac property (5). The existence of antioxidative flavonoid compounds in the ethanolic fruit extract of *T. terrestris* has also been well documented (6). Cytoprotective effects of diosgenin, a *T. terrestris* derived steroid is reported to increase biliary secretion of cholesterol and lipid vesicles in the rat liver (7).

ALT and AST are hepatospecific enzymes principally found in the cytoplasm (8). Hepatic activation of cyclophosphamide leading to the formation of toxic metabolite caused cellular damages and loss of functional integrity of hepatocyte membrane leading to their leakage into the serum or plasma (9). Oxidative stress produced by the metabolite acrolein is responsible for cyclophosphamide hepatotoxicity (10). In the present study, an increase in ALT and AST were observed in G_{II}. Similar increase in the levels of ALT and AST in the serum of cyclophosphamide injected mice was observed in mice (11).

From the result of this study, co-treatment with *T. terrestris* restored the activities of ALT and AST. The antioxidant properties of flavonoids and polyphenols present in *T. terrestris* have stabilized the cell membrane, thereby preventing the damage and thus suppress the leakage of enzymes through the cellular membrane (12).

Serum creatinine is considered only relatively specific but not very sensitive since its levels significantly increase only when more than 50% of the glomerular filtration rate is reduced. No statistically significant difference was observed in urea and creatinine in all the groups on day 0 and 21. Although, CP caused slight elevation in serum creatinine and urea, co- treatment with extract prevented this elevation.

Assessment of haematological parameters can be used to determine the extent of deleterious effect of a toxin on blood constituents (13). It can also be used to explain blood related functions of chemical compounds or phytochemicals. Significant decrease in RBC, platelets and Hb were observed on day 21 in G_{II} which was consistent with the previous findings in rats using cyclophosphamide (14). No significant difference was there in WBC count in all the groups on day 0 and 21.

The property of chemotherapeutic drugs is to kill rapidly dividing cells in the body including cancer cells and normal cells including red blood cells (15). Cyclophosphamide induces leucopenia

and thrombocytopenia which may be referred to its physiochemical properties such as lipophilicity, capacity to cross biological membranes and stability in aqueous solutions.

The positive effect of *T. terrestris* on haemopoeitic system could be due to the presence of protodioscin which increases blood testosterone level and its transformation into dihydrotestosterone (16). In turn, dihydrotestosterone stimulates erythropoiesis and haematopoiesis which leads to increase in erythrocytes, platelets and haemoglobin level.

V. CONCLUSION

This study demonstrated the protective potential of *T. terrestris* against cyclophosphamide-induced organ toxicity. We therefore suggest its possible use in chemotherapy and other stress-associated disorders as a supplementary/auxiliary therapy. However, further studies are required to clarify any potential interaction of *T. terrestris* with the chemotherapeutic activity of cyclophosphamide. Tissue damage due to cyclophosphamide might have been alleviated due to the antioxidant property like free radical scavenging, increased activity of antioxidant defense system or membrane stabilizing property of *T. terrestris*.

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