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EVALUATION OF IMMUNOMODULATORY ACTIVITY OF *TRIBULUS TERRESTRIS* IN ANIMAL MODEL

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ABSTRACT

Tribulus terrestris L. (Zygophyllaceae), an herb commonly known as "palleru" in Telugu. In this present study, immunomodulatory activity of Tribulus terrestris L was evaluated by using immunological studies like phagocytic test, Carbon clearance test, humeral antibody titer (hat) and delayed type hypersensitivity (dth) response, humeral antibody titer (hat), delayed type hypersensitivity (dth) response, t-cell population (rosette and e-rosette formation) test, drug induced myelosuppression test. Results were shows that Tribulus Terrestris L. has potent immunomodulatory activity.

KEY WORDS

Tribulus terrestris L. immunomodulatory, Carbon clearance test.

INTRODUCTION

Tribulus terrestris L. (Zygophyllaceae), a procumbent herb commonly known as "palleru" in Telugu. It is found throughout India up to 3,600 ft from Kashmir to Sri Lanka, and all warm regions in the both the hemispheres. The plant is cultivated in a few places of India. It is a procumbent herb; stems and branches pilose; young parts villous. *Leaves* opposite, abruptly pinnate, one of each pair usually smaller than the other, or wanting altogether; stipules lanceolate hairy; leaflets 3-6 pairs, oblong, mucronate, villous, base oblique, petioles brief, hairy. Axilary, leaf opposed, yellow, solitary, hairy, pedicillate; sepals lanceolate, acute, hairy; petals oblong, claw short, hairy; stamens 10, inserted on the base of the disk, alternately longer and shorter; ovary sessile, hirsute, 5-12 lobed; style short, stigmas 5-12; ovules superimposed. *Fruits* dry, woody, sub-globose, schizocarpic, with spines present all over. *Seeds* 4-6 in each of the five parts of the fruit that separate on maturity. Flowering and fruiting occur during the hot and rainy seasons. Leaves diuretic, tonic, increases the menstrual flows, cures gonorrhoea; decoction used to gargle for mouth trouble and painful gums and reduce inflammation. Fruit diuretic, removes gravel from the urine and stones in the bladder, regarded as cooling, tonic, asperient and aphrodisiac; used in painful micturition, urinary disorders, cough, heart diseases and impotency. In some countries it is a reputed tonic and astringent, used for coughs, scabies, anaemia and ophthalmia. The diuretic property of the drug is due to the presence of large quantities of nitrates present and the essential oil which occurs in the seeds. The root is a good stomachic and appetizer, diuretic and carminative. The entire plant, but more particularly the fruits are used in medicines. It was given a good trial in brights diseases with dropsy. Ayurveda describes *Tribulus* as diuretic, aphrodisiac, tonic and rejurvenating herb with the ability to build muscle and strength. The fruit and root of *Tribulus* contains pharmacologically important metabolites like steroidal saponins, flavonoids, alkaloids and glycosides. The active constituents have a stimulating effect on the immune, sexual and reproductive systems. [1-3].

MATERIALS AND METHODS

Plant Collection: The roots of *T. terrestris* were collected from the village Koppur of Bheemadevarapally mandal of Karimnagar district, Andhra Pradesh. The powder drug 1.5kg was macerated with methanol in round bottomed flask for seven days. The flask was shaken intermittently to ensure the efficiency of extraction. After a weak, the extract was filtered and concentrated under reduced pressure. The methanolic extract obtained (brown flakes) of the plant was kept in a dissector to remove moisture and properly store until used. [4]

ii) Fractionation:

Methanol extract (8.45%) of the drug was dispersed in water and subjected to fraction with toluene (1.50%), ethyl acetate (2.45%) n-butyl alcohol (1.80%) and aqueous residue (9.50%), separately and successively. All the individual solvent fractions were combined and concentrated under reduced pressure.

The methanolic extract and its corresponding fractions of *Tribulus terrestris* were studied in TLC using various solvent systems.

Experiments were carried out to determine phagocytic activity, humoral antibody response and cell-mediated immune response using Carbon clearance test, Delayed type of hyper sensitivity reaction, T-Cell population test, Sheep erythrocyte agglutination method and Drug-induced myelosuppression tests. [5]

Methodology of Immunomodulatory studies

Immunomodulatory activity of methanolic extract and its fractions of *Aristolochia indica* were under taken in Swiss albino mice and Wister rats of mixed population. Experiments were carried out to determine phagocytic activity, humoral antibody response, delayed type of hyper sensitivity reaction, T-cell population and Drug induced myelosuppression.

Phagocytic Activity Phagocytic activity of Reticulo Endothelial System (RES) was assayed by carbon clearance test.

Carbon Clearance Test Phagocytic index was calculated as rate of carbon elimination by reticulo endothelial system by carbon clearance test. The animals divided into twelve groups of six in each were administered with respective samples orally for 7 days (Table 1). At the end of seven days the mice were injected with 0.1ml Indian ink intravenously through tail vein to all the animals. Blood samples were collected from retro orbital plexus immediately at 0 minute after 5, 10, and 15 minutes interval and transferred to centrifuge tube containing 0.15% w/v disodium ededate. Then it was centrifuged and to a 50µl serum sample 0.1% sodium carbonate solution (2ml) was added. The absorbance was measured at 660 nm taking 0.1% sodium carbonate solution as blank. The carbon clearance was calculated using the following equation.

$$\text{Carbon Clerance} = \frac{\text{LogOD}_1 - \text{LogOD}_2}{t_2 - t_1}$$

(Where OD_1 and OD_2 are the optical densities at t_1 and t_2, respectively. t_1 and t_2 are time at different intervals).

Humeral Antibody Titer (HAT) and Delayed Type Hypersensitivity (DTH) Response

To study humeral antibody response and DTH response against SRBCs antigens, which was induced by intra peritoneal administration of SRBCs suspended in normal saline on albino rats, sensitizes them for elicitation of DTH and also induced antibody formation. Therefore, this system has major advantages as it enables two components of immune response to be measured in the same species under ideal conditions and is relatively simple and inexpensive to perform. [6]

Humeral Antibody Titer (HAT)

Sheep erythrocyte agglutination test or humeral antibody response was performed against antigens. The rats were divided into twelve groups and they were immunized by injecting 50µl of SRBCs suspension containing $5.2x10^6$ cells/ml intra peritoneal on day zero. They were administered with doses mentioned in Table 1. Blood samples were collected in micro centrifuge tubes from individual animal by retro orbital puncture on eighth day and serum was separated by centrifugation technique. Antibody levels were determined by the haemagglunation technique. Briefly, equal volumes of individual serum samples of each group were pooled. To serial two-fold dilution of pooled serum samples made in 50µl volume of normal saline, in u-bottomed micro titration plates were added 50 µl of freshly prepared 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37^0C for 2h and examined visually for agglutination. The reciprocal of

the highest dilution of the test serum causing visible heamagglutionation was taken as the antibody titer.

Table 1. TLC profile for *Tribulus terrestris.*

Solvent System	No.of Spots	Rf Value	Remark
Methanolic Extract:			
Toluene:Ethyle acetate (80:20)	5	0.95,0.82,0.71, 0.62,0.20	Good Separation
Benzene: acetone (50:50)	4	0.95, 0.80 0.66, 0.23	Best Separation
Chloroform:Methanol (90:10)	3	0.77, 0.45,0.22	Good Separation
Ethyl acetate Soluble fraction			
Toluene:Ethyle acetate (80:20)	6	0.95,0.82,0.67, 0.57,0.37,0.25	Good Separation
Benzene: acetone (50:50)	4	0.95, 0.90 0.76, 0.22	Good Separation
Chloroform:Methanol (90:10)	3	0.80, 0.45,0.20	Good Separation
n-butanol soluble fraction			
Chloroform:Methanol (90:10)	2	0.95, 0.47	Good Separation

Adsorbent : Sillica gel G
Detection : Vanillin (1% w/v) in Sulphuric acid.

Table 2. Results of chemical tests

Sl.No.	Name of the Test	Methonolic extract	Toluene fraction	Ethyl acetate	N-butanol	Aqueous residue
1	Alkaloids					
	a) Dragendorffs test	+	+	+	+	-
	b) Mayers test	+	+	+	+	-
	c) Wagner's test	+	+	+	+	-
	d) Hager's test	+	+	+	+	-
2	Carbohydrates/ Glycoasides					
	Molisch's test	+	-	+	+	+
3.	Steroids/Triterpenoids					
	Liebermann-Burchard Reaction:	+	+	+	+	-
4.	Saponins (Form test)	+	+	+	+	+
	Lead Acetate	+	-	*	+	-
5.	Flavonoids Shinoda's	+	-	-	+	-
	Ferric Chloride	+	-	-	+	-

'+': Present, '-': Absent; * Violet ppt.

Delayed Type Hypersensitivity (DTH) Response

Delayed Type of Hypersensitivity response to SRBCs was induced in albino rats. Twelve groups of rats of six in each was immunized by 50 µl of 5.2 x 10^6 SRBC/ml (i.p) to each animal of each group (Primary challenge) similarly in HA titer. The thickness (ml) of the right hind

foot pad was measured using plethismometer. The rats were then challenged by injection of 25µl of $5.2x10^6$ SRBCs/ml (s.c) into right hind foot pad (secondary challenge). Foot thickness was measured again +24hrs after this challenge. The difference between the pre- and post-challenge foot thickness expressed in ml was taken as a measure of DTH. [7]

T-Cell Population (Rosette and E-rosette Formation) test

The T-Cell has an affinity for bind spontaneously to sheep erythrocytes. This binding can be visualized as a rosette and the red cells can be seen in a cluster around a central lymphocyte.[13] In this test, twelve groups of rats of either sex of six animals in each group were used. The calculated drug doses given up to 10 days. On 11^{th} day blood was collected from retro orbital plexus and heparinized with 50 IU in heparin in micro centrifuge tube. The tube containing blood left in a sloping position 45^0 angle at 37^0C for one hour. Red cells settled to the bottom and the supernatant which contains lymphocytes and leucocytes were collected by using micro pipette. An amount of 50 µl of this lymphocyte suspension was taken in eppendorf's tube and mixed with 50 µl of 0.5% sheep erythrocytes and incubated for 5 min at 37^0C. This mixed suspension was spunned at 200 rpm for 5 minutes and kept at 4^0C for 2 h in a refrigerator. After incubation and refrigeration, one drop of the cell suspension placed on a neubar slide and covered with cover slip and seated. Two hundred lymphocytes were counted and a lymphocyte binding with three or more sheep erythrocytes considered as a E-rosette. Its percentage was determined and compared with control.

Drug Induced Myelosuppression Test

In study of drug induced myelosuppression, the cyclophosphamide 3mg/kg b.wt. was used in albino rats.[14] Rats were divided in to twelve groups of six each dosage regimen given in Table 5. After completion of seven days administration of doses, on eighth day, blood was taken from retro-orbital plexus and subjected to hematological studies. The cyclophosphamide was withdrawn from Group IV, VI, VIII and X which were fed only extracts and its corresponding fraction for next seven days (recovery studies). Blood samples of each animal collected on 15^{th} day was again subjected to hematological studies, including hemoglobin count, RBC, WBC count, differential WBC count, platelet count and body weight. [8-10]

Statistical Analysis

Results of immunomodulatory activities in various animal models have been presented as Mean ± SD (Standard deviation) or Mean ± SEM (Standard Error of Mean). The significant difference was analyzed using student't' test. The variation present in a set of data was analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keul Multiple Comparison test p values < 0.05 were considered significant. [11]

RESULTS AND DISCUSSION

i) TLC Studies:

The studies were performed for methanolic extract and its fractions. Methanolic extract of *Tribulus terrestris* has shown best separation in chloroform: methanol (90:10) by developing six spots (Rf – 0.80, 0.45 and 0.22) followed by n-butanol soluble fraction with two spots (Rf. 0.95, 0.20). Another solvent system is benzene: acetone (50:50) revealed five visible spots of methanolic extract and its ethyl acetate soluble fraction (Rf – 0.95, 0.80, 0.66 and 0.23) and n-butanol soluble fraction showed two spots (Rf. 0.95, 0.76) in the same solvent system, benzene: acetone (70:30).

ii) Test Tub Reactions:

The preliminary phytochemical screening methanolic extract and its fractions of fruits of *Tribulus terrestris* was performed through test tube reactions. To discover glycosides, steroidal and triterpinoidal compounds, flavonoids and traces of alkaloids.

iii) Pharmacological Studies: Pharmacological studies of *Tribulus terrestris* were carried out by using methanolic extract and its corresponding fractions on albino mice and rats of mixed population to determine phagocytic activity, cell mediated immune response and humoral antibody response using Carbon clearance test, Delayed type of hyper sensivity reaction, Sheep erythrocyte agglutination method, T-cell population test and Drug-induced myelo suppression tests.

a) Carbon Clearance Test: Effect of methanolic extract and its corresponding fractions of *T. terrestris* on phagocytic activity in Carbon clearance test were

assayed on albino mice. The treated groups of animals were compared with control and standard drug (Groups I and II). The rate of carbon clearance as mean phagocytic index, was calculated as 0.0076 ± 0.001 (P>0.05) and 0.0127±0.001 (P<0.001). The Groups III, IV and V were treated with methanolic extract 100mg, 200mg, 400mg/kg body weight orally for 7 days. These groups showed an increase in phagocytic index as 0.0107 ± 0.0009(P>0.05), 0.0115 ± 0.0007 (P <0.01) and 0.0126 ± 0.0010 (P<0.001). Groups VI, VII, VIII, IX, X and XI animals were treated with Toluene soluble, ethyl acetate soluble and n-butanol soluble fractions 50 and 100mg/kg body weight respectively. The 100mg/kg of these respective groups showed an increase in phagocytic index of 0.0116 ± 0.0006 (P<0.01), 0.0101 ± 0.0009 (P<0.01) and 0.0120 (P<0.001), respectively. Aqueous residue of methanolic extract showed phagocytic index as 0.0090 ± 0.0003 (P>0.05) with 100mg/kg body weight body weight treated orally for 7 days.

b) Delayed type of Hypersensitivity response:

Delayed type of hypersensitivity response to sheep red blood erythrocyte were calculated as a measure of paw volume (in ml) for each animal which compared with control (1ml 2%gum acacia - Group-I) and standard drug (Levemisole,50mg – Group II) were fed orally for 7 days. Paw volume was measured 0 hour and +24 hrs and calculated percent increase of paw volume after 24 hrs. The DTH response of standard drug was found to be 17.77 ± 0.421 (P<0.001). Animals of Group III, IV and V were treated with methanolic extract (100mg, 200mg, and 400 mg/kg/body weight) the percent activity for these groups was calculated at 24 hrs. and was found to be 13.750 ± 0.143, 15.407 ± 0.306 (P<0.01), and 19.0572 ± 0.320 (P<0.01). Animals treated with Toluene, ethyle acetate) and n-butanol soluble fractions of methanolic extract with 50mg and 100mg/kg body weight treated orally for 7 days. The DTH response was respectively 11.88 ± 0.620, 12.942 ± 0.368 (P>0.05); 8.635 ± 0.401, 9.040 ± 0.310 and 11.25 ± 0.2713.59 ± 0.27(P>0.05). Aqueous residue of methanolic extract has shown DTH response as 9.034 ± 0.25 with 100mg/kg body weight body weight treated orally for 7 days.

c) Sheep Erythrocyte Agglutination Test:

Heamoagglutination titer to sheep red blood erythrocyte and percent agglutination of were calculated and compared with control (1ml 2%gum acacia) and standard drug which were fed orally for 7 days. Heamoagglutination titer and percent agglutination of standard drug was found to be 80.000±0.490 and 92.188±0.042 (P<0.001). Groups III, IV and V treated with methanolic extract orally for 10 days (100,200 & 400 mg/kg body weight) showed HA titer values 18.667±0.350, 42.667±0.387 &85.333±0.387 and % Agglutinations 68.75±0.223, 85.417±0.0.100 (P<0.001) and significant increase in agglutination (400 mg/kg) 92.708±0.046 (P<0.001). Animals treated with Toluene, ethyl acetate and n-butanol soluble fractions of methanolic extract with 50mg and 100mg/kg body weight per 7 days orally. They have shown their individual fractions of HA titer values 12.000±0.365 & 21.333±0.387; 42.667±0.387 & 85.333±0.387 and 48.000±0.365 & 90.667±0.469 (P<0.001) where as in case of same doses of %Agglutinations were found to be 54.167±0.188 & 70.833±0.060; 85.417±0.060 & 92.708±0.046 (P<0.001) and 85.417±0.096 & 91.146±0.096 (P<0.001) respectively. Aqueous residue of methanolic extract has shown HA titer and % Agglutination 8.000±0.548 and 25.000 ±01.095 (P<0.05) with 100mg/kg body weight body weight orally for 10 days.

d) T-Cell Population Test:

Rosette formation and lymphocyte formation were assayed in this test and compared with control (1ml 2%gum acacia) and standard drug (Levamisole, 50mg) Group I and II they were fed orally for 10 days Percent increase in rosette formation of standard group was found to be 97.768 ± 0.035%, 35.0889 ± 0.07, 54.450 ± 0.094 and 87.401 ± 0.861 and (P<0.001) when animals were administered with methanolic extract (100mg, 200mg and 400mg/kg body weight) orally for 10 days. Remaining Groups VI to XI treated with Toluene, ethyl acetate and n-butanol soluble fractions of methanolic extract with 50mg and 100mg/kg body weight per orally. They were shown up to 5.052 ± 0.77 & 7.135 ± 0.114 (P>0.05); 48.409 ± 0.087 & 81.76 ± 0.082 (P<0.001) 73.15 ± 0.066 & 87.51 ± 0.063 (P<0.001).

Aqueous residue (100mg/kg body weight) has not shown and significant increase the value 8.18 ± 0.27.

Lymphocyte formation of standard drug was 20.02 ± 0.108 (P<0.001), compared with control and test groups, there was significant increase in lymphocyte formation with methanolic extract 200 and 400mg/kg body weight were given 11.443 ± 0.191 and 20.135 ± 0.165 P<0.001), ethyl acetate and n-butanol soluble fractions of methanolic extract in the doses 50 and 100mg/kg body weight also showed significant increase in lymphocyte formation as 7.844 ± 0.312 & 12.75 ± 0.186 and 16.84 ± 0.146 & 19.39 ± 0.125 (P<0.001).

e) Drug induced Myelosuppression Test:

In this test, myelosuppression was produced in animals with the treatment of cyclophosphamide 3mg/kg body weight orally for 7 days. Group I was kept as control and feed 1ml gum acacia (2%). Haematological studies showed the mean hemoglobin 13.50 ± 0.1622%, mean RBC 4.76 ± 0.135 million/mm^3, WBC 9.80 ± 0.065 thousand/mm^3, Neutrophils 54.46 ± 1.044%, Eosinophils 2.30 ± 0.445%, Lymphocytes 41.33 ± 0.545%, Monocytes 2.5 ± 0.54%, Platele 4.347 ± 0.034 lakhs/mm^3 and mean body weight was found to be 163.25 ± 0.028 gm (P<0.05). Group II (cyclophosphamide, 3mg/kg body weight) showed significant decrease in hemoglobin 5.13 ± 0.224%, mean RBC 2.60 ± 0.183millions/mm^3, WBC, 4.54 ± 0.046thousand/mm^3, Neutrophils 60.33 ± 0.822%, Eosinophils 3.33 ± 0.26%, Lymphocytes 34.17 ± 0.240%, Platelets 4.32 ± 0.177 lacks/mm^3 and decrease in mean body weight was calculated as 161.67 ± 0.032 gm (P>0.05).

Protection against the effect of cyclophosphamide with methanolic extract were observed in group IV&VI (200 &400mg/kg body weight) as mean hemoglobin 13.40 ± 0.250 and 13.63 ± 0.381% mean RBC 4.60 ± 0.82 and 4.74 ± 0.053 million/mm^3 (P<0.01), WBC 9.72 ± 0.145 and 9.68 ± 0.167 thousand/mm^3 (P<0.001), Neutrophils 52.66 ± 0.520 and 53.13 ± 1.72%, Eosinophils 1.4 ± 0.32 and 2.8 ± 0.60% Lymphocyte 53.832 ± 0.706 and 45.33 ± 0.332% Platelets 4.37 ± 0.14 and 4.268 ± 0.032 mean body weight was 162.32 ± 0.623gms and 158.30 ± 1.55gm (P<0.01). Toluene soluble fraction (100mg/kg body weight) showed protection of cyclophosphamide as mean hemoglobin 10.25 ± 0.246% (P<0.001), mean RBC 3.27 ± 0.128 millions/mm^3, WBC, 8.10 ± 0.088 thousand/mm^3, Neutrophils 55.66 ± 0.642%, Lymphocytes 35.62 ± 0.94%, Platelets 3.832 ± 0.022 lacks/mm^3 (P<0.05) and mean body weight 151.50 ± 1.75gms (P < 0.05). In Group X the animals administered with ethyl acetate soluble fraction &n-butanol soluble fraction (100mg/kg body weight) and cyclophosphamide (3mg/kg body weight), the significant protection was calculated as mean hemoglobin 10.18 ± 0.267 &10.32 ± 0.187% (P<0.001), mean RBC. 3.83 ± 0.98 & 3.56 ± 1.54 millions/mm^3, WBC 8.91 ± 0.263 &8.12 ± 0.47 thousand/mm^3 (P < 0.001), Neutrophils 56.23 ± 0.192 &51.68 ± 0.766% (P< 0.01), Lymphocytes 36.79 ± 0.522 and 42.13 ± 0.203% (P < 0.05), Platelets 3.97 ± 0.278 and 3.927 ± 0.922lacks/mm^3 (P<0.01) and mean body weight was calculated as 159.97 ± 1.85gm (P<0.001).

Recovery studies were carried out with withdrawal of cyclophosphamide from group IV, VI, VIII, X and XII while the group II fed with cyclophosphamide for next seven days. The result suggests there was a significant decrease (Group II) in mean hemoglobin 4.80 ± 0.24%, mean RBC 2.27 ± 0.124 millions/mm^3, W.B.C. 2.20 ± 0.323 thousand/mm^3 (P<0.01), decrease in Neutrophils 34.83 ± 0.0172%, Lymphocyte 50.83 ± 0.087% Platelets 4.16 ± 0.118 lacks/mm^3 (P<0.05) and mean body weight as be 158.33 ± 0.052gms (P<0.05).

The animals treated with methanolic extract (group IV &VI, 200 &400 mg/kg body weight) have shown the significant increase in mean hemoglobin 15.95 ± 0.0716% &17.13 ± 0.0494%,mean RBC 9.37 ± 0.0480 &10.68 ± 0.132millions/mm^3 (P<0.001), W.B.C., 8.97 ± 0.108 &11.57 ± 0.0718 thousand/mm3 (P<0.001), Neutrophils 34.00 ± 0.089% &22.83 ± 0.128%, Lymphocyte 64.00 ± 0.0541% &73.00 ± 0.0312%(P<0.01), Platelates 4.10 ± 0.151 &7.88 ± 0.0102lacks/mm^3 (P<0.01) and mean body weight 180.83 ± 0.0371&181.67 ± 0.041gms (P<0.05).

Toluene soluble fraction (100mg/kg body weight) treated group VIII were shown mean hemoglobin 11.60 ± 0.0619% mean RBC 6.60 ± 0.105 millions/mm3, W.B.C. 9.20 ± 0.0717 thousand/mm3, Neutrophils 31.50 ± 0.148%, Lymphocyte 64.50 ± 0.082% Platelets 6.72 ±

0.127 lacks/mm3 and mean body weight 176.67±0.034gms (P<0.05).

Recovery studies suggest that the ethyl acetate fraction (Group VIII 100 mg/kg body weight) have shown protective effect significantly against cyclophosphamide in hematological parameters as mean hemoglobin 12.80 ± 0.053%, mean RBC 7.02 ± 0.083millions/mm^3, W.B.C. 7.49 ± 0.126 thousand/mm^3 (P<0.001), Neutrphills 33.67 ± 0.024% Lymphocyte 62.33 ± 0.016%, Platelets 7.70 ± 0.084 lacks/mm^3 (P<0.05) and significant increase in mean body weight as 182.50 ± 0.059gms (P<0.05) were also observed.

N-butanol soluble fraction (100mg/kg body weight) treated group XII were shown mean hemoglobin 14.48 ± 0.0956% mean RBC 8.11 ± 0.1356 millions/mm^3, W.B.C. 7.63 ± 0.102 thousand/mm3, Neutrophils 30.17 ± 0.1014%, Lymphocyte 65.00 ± 0.401% Platelets 8.76 ± 0.145 lacks/mm3 and mean body weight 181.67±0.038gms (P<0.05).

CONCLUSION

Tribulus terrestris L. (Zygophyllaceae), has shown significantly immunostimulatory effect and also the drug well recovers the hematological parameters when administrated withdrawal of cytotoxic drug cyclophosphemide. Conversely, the drug is an immune stimulant. But, it exhibits quite immuno-suppressive activity in some parameters due to its additive effect its phyto-constituents. Further detailed investigations on the species are necessary to isolate the unknown of active compounds and study them to justification of the effects.

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