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EVALUATION OF *SELAGINELLA BRYOPTERIS* (L.) BAKER'S IMMUNOMODULATOR ACTIVITY ON ANIMAL MODELS

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Abstract:

Selaginellabryopteris (L.) (family: Selaginaceae), commonly known as Sanjeevni, is a magical herb which has the power to cure any malady. Immunomodulatory activity studies in animal models included haematological and serological tests to determine humoral antibody titre, T-cell population tests, sheep erythrocyte agglutination method, and drug-induced myelosuppression tests. In this study, different doses (100, 200, and 400 mg/kg body weight/day) of the methanolic extract and its bioactive fractions possessed promising immunostimulant activities.

Key Words: *Selaginella Bryopteris, Immunomodulatyory, Humoral Antibody Titer, T-cell Population test, Agglutination method, Myelosuppression*

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INTRODUCTION:

Selaginella bryopteris (L.) (Family: Selaginaceae), also known as Sanjeevni, is a lithophyticpteridophytic plant with exceptional recovery abilities[1]. *Selaginella bryopteris* is the first known life-giving herb in India as the name of a wonder herb identified as Sanjeevni is mentioned in the well-known epic by the Hindi poet Tulsidas[2].

The plants are growing on barren rocks and soil. Some of the xerophytic species (observed in the desert) remain curled up in a tight brown or reddish ball (folded) under dry conditions. As soon as they come in contact with water, they uncurl, becoming green and normal (unfolded). Such plants are called "resurrection plants." Some of the species prefer moist environments and can be found in shady areas of the hills. A few species are found in tropical forests; other species are found as epiphytes[3].

The species of *Selaginella* are widely distributed at different altitudes in different types of forest, viz., Himalaya, Western dry regions, Western Ghats, Eastern Ghats, Deccan plateau, Gangetic plains, and Andaman and Nicobar Islands. Although *Selaginella* grows luxuriantly in tropical and subtropical forests, none of the species ascends higher than temperate altitude and they are yet to be reported from subalpine and alpine regions in India[4].

The majority of species of *Selaginella* in India occur in rain forest ranging from low level to high altitude, usually preferring sandy alluvial soil, rock crevices or under the moist shady situation of the heavy rock boulders, among grasses, and even a few prefer open, dry sunny situations, and are xerophytic in nature[5].

In India, it is used as a major ingredient in local pills for the treatment of patients with spermatorrhoea, venereal diseases, constipation, colitis, indigestion, and urinary problems (diuretic). It is also used to treat patients who are unconscious and to lower the body temperature in patients with fever[6-8].

MATERIALS ANDMETHODS:

(i) Plant Collection: The whole plants of *Selaginellbryopteris* were collected from Budidagutta, (Mandal: Bheemadevarapally), Warangal Urban district, Telangana.Theplant was authenticated by Prof. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Warangal and the voucher specimens are preserved in the herbarium of University College of Pharmaceutical Sciences, Kakatiya University, Warangal. The plant material was shade dried and crushed to course powder. The crude powder drug was stored in air tight labeled containers

The powdered drug (1 kg) 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, it was filtered and concentrated under reduced pressure. The so obtained methnolic extract of the plant was kept in a desiccator to remove moisture and properly stored until used.

(ii) FRACTIONATION:

Methanol extract (15.49%) obtained by drug was dispersed in water and subjected to fraction with toluene (3.50%), ethyl acetate (2.50%) and n-butyl alcohol (1.50%) separately and successively. The solvent fractions were combined and concentrated under reduced pressure to afford the corresponding extracts.

Thin layer chromatographic and phyto chemical studies were performed with metabolic extract and its corresponding fractions of *S. brysopteris,* using various solvent systems and tests. The results were presented Table..1.1 &1.2

REAGENTS:

- **India ink:** It is the 1% w/v suspension of carbon black (Company. Veto.Co.Ltd., India).
- **Normal Saline:** 5% w/v of Nacl Claris Life Science Ltd., India
- **Alsever's Solution[9]:** Dextrose 2.05 gm, Sodium citrate 0.80 gm and sodium chloride 0.42 gm was dissolute and volume was made with distilled water up to 100 ml. It is routinely used as anticoagulant blood preservations, and permits the storage of whole blood for approximately two weeks in a refrigerator at 2-80^{0}C.
- **Levamsole[10]:** Levamsole (Khandelwal Laboratories, Mumbai, India) was used as a standard immune stimulating agent.
- **Cyclophosphamide[11]:** Tablets of endoxanAsta (Sigma Aldrich chemicals,India) 50 mg was triturated with a 100 ml slurry of 2% gum acacia in distilled water 0.6 ml of this suspension will contain 0.3 mg cyclophosphomids.
- Leishman's Reagent: Leishman'seosine methylene blue solution, E merk India Ltd, Mumbai
- Gower's Solution: RBC diluting fluid, Qualigens fine chemicals, Mumbai
- WBC Diluting Fluids: Qualigens fine chemicals, Mumbai.

ANIMALS:

Swiss albino mice weighing between 18 and 25g of both sex (for carbon clearence test) and Wister albino rats weighing between 180 and 250g of either sex were used for immunomodulatory activity. All the animals were procured from

Mahaveer Agency (Regd.No.146/1999/CPCSEA), Hyderabad and maintained in the hieghgenic environmental conditions (25±2ºC) 12h/12h light/dark cycles in the animal house of University College of Pharmaceutical Sciences, Warangal and provided a balanced diet with free access to mineral water accordingly. All the animal experimental protocols were duly approved by the institutional animal ethical committee (Regd. no. 166/1999/CPCSEA).

ACUTE TOXICITY STUDY[12] (Preparation of Doses and Determination LD_{50}):

Acute toxicity studies were carried out according to the method described in the literature. Separately weighed in doses of 100, 300, 500, 1000 and 2000 mg/kg b.w. of methanolic extract of each drug was triturated with 2% w/v slurry of gum acacia with distilled water and administered orally to albino mice of either sex. The animals were observed continuously for any change in behavioral, neurological, autonomic profile and mortality for first few hours and later 24h, 48h intervals for a period of 7 days. Based on this results obtained from this study, the doses were prepared (100,200 and 400 mg/kg b.w. per day) for the present pharmacological studies. The LD_{50} for each extract were calculated and it was found to be more than 500 mg/kg body weight.

ADMINISTRATION OF DOSES:

Doses of various extracts and its corresponding fractions were administrated orally to each animal using oral cannula fitted with a syringe of 5ml.

METHODOLOGY OF IMMUNOMODULATORY STUDIES

Immunological studies to evaluate immunomodulatory activity of crude extracts (Methanolic) and its fractions of *Selaginellabryopteris*, was carried out on swill albino mice and Wister rats of mixed population. Experiments were performed to determine Humora lAnitobodytiter (HAT) and cell mediated immune response of sheep erythrocyte agglutination method, T-cell population test and Drug induced myelosuppression test.

HUMORAL ANITOBODY TITRE (HAT)

To study humarol antibody response against antigens (Sheep erythrocyte aggluti nation test) to SRBC's was induced by injecting albino rates IP with SRBCs suspended in normal saline sensitizes them for induced antibody formation. Therefore this system has major advantages, i.e. 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[13-16]

SHEEP ERYTHROCYTE AGGLUTINATION TEST:

Sheep erythrocyte agglutination test (humarol antibody response) was performed against antigens[17-22].

The rats were divided into twelve groups, each having six rats, Group I was kept as control and received 2% suspension of gum acacia 1ml. Group II standard drug (Levamsole 50 mg/kg. bwt/day). Groups III, IV and V were fed with crude extract in 100, 200 and 400 mg/kg bwt/day. Groups VI amd VII were fed with Toluene Soluble fraction 50 and 100 mg/kg bwt/day. Groups VIII and IX were administered with ethyl acetate soluble fraction. Groups X and XI were gives with n-butanol soluble fraction 50 and 100mg/kg. bw t/day and Group XII was fed with aqueous fraction 100 mg/kg. bwt/ day. Respectively for 7 days.

All the animals were immunized by injecting 50µl of SRBCs suspension containing $5.2x10^6$ cells/ml intra peritoneal on day 0. Blood samples were collected in microcentrifuge tubes from individual animal by retro orbital puncture on seventh day and serum was separated by centrifugation technique. Antibody levels were determined by the haemagglunationtechnique. 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.

T-CELL POPULATION TEST:

The T-Cell has an affinity for bind spontaneously to sheep erythrocytes (SRBC). This binding can be visualized as a rosette and the red cells can be seen in a cluster around a central lymphocyte[23-26].

In this test, twelve groups of rats of either six were used, (six animals in each group). Methanolic extract groups III, IV and V 100, 200 and 400 mg/kg bwt/day and its fractions such as Toluene soluble fraction groups VI and VII 50 and 100 mg/kg. bwt/ day. Ethyl acetate soluble fraction 50 and 100 mg/kg bwt/day, and n-butanol soluble fraction groups VIII, IX, X and XI 50 and 100 mg/kg bwt/day, respectively. Group XII was fed with aqueous fraction 100 mg/kg bwt/day. Group I was kept as a control, fed with 2% gum acacia in distilled water and Group -II as standard levemsole 50mg/kg bwt./day, respectively 10 day

On 11th 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 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 minutes at 37^0C.

This mixed suspension was spanned at 200 rpm for 5 minutes and kept at 4^0C for 2 hr 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 erythrocyte considered as a E-rosette. By counting the number of rosette forming. Its percentage was determined and compared with control.

DRUG INDUCED MYELOSUPPRESSION TEST:

To determine the effect of drug induced myelosuppression, cyclophosphamide was used in albino rates[27-31].

Albino rates were divided in 12 Groups of six each. Group I was kept as control and given 2% gum acacia (1ml) suspension in distilled water. Group II as standard, treated with Cyclophosphamide 3mg/kg bwt/day. Groups III, V, VII and IX were administered with methanalic extract 200mg/kg. bwt/day, Toluene soluble fraction (100mg/kg bwt/day), Ethyl acetate soluble fraction (100mg/kg bwt/day) and n-butanol soluble fraction (100mg/kg bwt/day) Groups IV, VI, VII and X were fed with methanalic extract, Toluene soluble fraction, ethyl acetate soluble fraction and n-butanol soluble fraction 100mg/kg bwt/day along with cyclophosphamide 3mg/kg bwt/day, respectively for seven days.

On 7th day, blood was taken from retro-orbital pluxes and subjected to hematological studies. The cyclophosphamide was withdrawn from Group IV, VI,VIII and X fed only extracts and its corresponding fraction (100 mg/kg bwt/day) for next seven days (recovery studies). Blood samples of each animal collected on 15th day was again subjected to hematological studies, including hemoglobin count, RBC, WBC count, differential WBC count, platelet count and body weight. No mortality was observed after discontinuation of drugs till one month.

EXPERMENTAL WORK

(i) TLC STUDIES:

The studies were performed for methanolic extract and its fractions. Methanolic extract and its ethyl acetate soluble fraction of have shown best separation *Selaginellbryopteris* in chloroform: methanol (90:10) to give five spots (Rf – 0.88, 0.73, 0.52,0.44 and 0.32), and also n-butanol soluble fraction have shown two spot (Rf. 0.55, 0.38) in same solvent system, another solvent system is benzene: acetone to give six spots (70 : 30) to give five visible spots of methanolic extract and its ethyl acetate soluble fraction (Rf – 0.80, 0.62, 0.57,0.42,0.32 and 0.25) and n-butanol soluble fraction have shown two spots (Rf. 0.95, 0.77) in same solvent system benzene: acetone (70 : 30).

(ii) TEST TUBE REACTIONS

The preliminary micro chemical investigation of whole plant of ***Selaginellabryopteris L.,*** methanolic extract and its fractions were done by test tube reactions. By considering the above results the plant may contain phenolic compounds (Flavonoids), steroidal compounds and their glycosides.

Table 1.1 TLC profile for *Selaginellbryopteris.*

Solvent System	No.of Spots	Rf Value	Remark
Methanolic Extract			
Ethyl acetate: Benzene: (50:50)	5	-	Tailing
Methanol: ethyl acetate: (90:10)	3	0.70, 0.50 0.29	Good Separation
Chloroform: Methanol (90:10)	5	0.88, 0.73,0.52,0.44,0.32	Best Separation
Benzene: acetone (70:30)	5	0.87, 0.57,0.42, 0.32, 0.25	Good Separation
Ethyl acetate Soluble fraction			
Chloroform: Methanol (90:10)	5	0.85, 0.67,0.50,0.44,0.32	Best Separation
Benzene: acetone (70:30)	5	0.80, 0.62,0.57 0.42, 0.25	Good Separation
n-butanol Soluble fraction			
Chloroform: Methanol (90:10)	2	0.55, 0.38	Best Separation
Benzene: Acetone (70:30)	5	0.77, 0.37	Good Separation

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

Table 1.2 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.

III) 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 (Levamisole,50mg) Group I and II which were fed orally for 10 days. Human antibody titer and percentage of Agglutination of standard drug was found to be 85.333± 0.387 and 78.125 ±0.20% (P<0.001). Group III, IV and V were treated with methanolic extract orally for 10 days (100,200 & 400 mg/kg body weight) have shown HA titer values 20.00 ± 0.4906, 48.000 ± 0.365 and 96.000 ± 0.365 and % of Agglutinations 33.33 ±0.775, 60.417±0.577(P<0.05) and significant increase in agglutination (400 mg/kg) 91.667±0.035 (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 orally. They have shown their individual fractions of HA titer values 25.333 ± 0.420, 34.667 ± 0.454; 53.333 ± 0.310, 85.333 ± 0.387 and 69.33 ± 0.465, 96.000 ± 0.365 respectively. Where as in case of same doses of percentages of agglutinations were found to be 25.00±1.095, 45.833±0.536% (P>0.05); 60.417±0.275% & 86.45±80.106% (P<0.001) and 66.667±0.282%, 91.667±0.035% (P<0.001) respectively. Aqueous residue of methanolic extract has shown HA titer and %Agglutination 21.333 ± 0.387 and 25.000±1.095% with 100mg/kg body weight body weight orally for 10 days, Table 1.3, Figure 1.1.

(IV) 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 &II which were fed orally for 10 days. Percent increase in rosette formation of standard drug was found to be 35.29 ± 0.433. Percent increase in rosette formation was found to be 5.88± 0.481(P>0.05), 27.06± 0.603 44.71± 0.297 (P<0.001) when animals were administered with methanolic extract orally (100, 200 and 400 mg/kg body weight) for 7 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 5.88± 0.507 and 18.82± 0.496 (P<0.05); 28.29± 0.333 and 42.35± 0.222 (P<0.001) 31.76± 0.529 and 43.52± 0.257(P<0.001). Aqueous residue (100mg/kg body weight) has not shown significant increase, the value was found to be 5.88 ±0.514.

Lymphocyte formation of standard drug was found to be 18.298±0.053 (P<0.001), compared with control and test groups, significant increase in lymphocyte formation with methanolicextract 200 and 400mg/kg body weight, ethyl acetate soluble fraction and n-butanol soluble fraction of methanolic extract (Group IX and XI) in the doses 50 and 100mg/kg body weight aslo showed significant increase in lymphocyte formation as 16.560± 0.294&19.579± 0.191 and 17.658 ±0.224 and 21.5005± 0.107 (P<0.05). Table 1.4, Figure 1-2

(v) DRUG INDUCED MYELOSUPPRESSION TEST:

Myelosuppression were produced in animals with the treatment of cyclophosphamide 3mg/kg body weight orally for 7 days. Group I was kept as control and fed 1ml gum acacia (2%). Hematological studies shows the mean hemoglobin 11.47 ± 0.107%, mean RBC 5.24 ± 0.106 million/mm^3, W.B.C. 5.57 ± 0.096 thousand/mm3, Neutrophils 31.5 ± 0.164%, Eosinophils 2.30 ± 0.528%, Lymphocytes 65.67 ± 0.104% Monocytes 4.00 ± 0.354% Platelates 5.012 ± 0.129 lakhs/mm^3 and mean body weight was has to be 181.67 ± 0.028 gms (P<0.05). Group II (cyclophosphamide, 3mg/kg body weight) were shown a significant decrease in hemoglobin 5.13 ± 0.318 %, mean RBC 2.60 ± 0.396 millions/mm^3, WBC 2.53 ± 0.557 thousand/mm^3. Neutrophils 35.33 ± 0.159%, Eosinophils 3.33 ± 0.245%, Lymphocytes 54.17 ± 0.143%, Platelets 4.32 ± 0.177 lakhs/mm^3 and decrease in mean body weight was calculated as 161.67 ± 0.032 gms (P<0.05).

Protection against the effect of cyclophosphamide with methanolic extract was observed in Group IV and VI (200 and 400mg/kg body weight) as mean hemoglobin 16.88 ± 0.094 and 17.30 ± 0.069 %. mean RBC 9.55 ± 0.043 and 9.85 ± 0.036 million/mm^3 (P<0.001), WBC 9.25 ± 0.033 and 9.37 ± 0.037 thousand/mm^3, Neutrophils 36.00 ± 0.084 and 36.50 ± 0.051%, Eosinophils 1.50 ± 0.699% and 1.50 ± 0.699%, Lymphocytes 60.17 ± 0.067% and 60.17 ± 0.067%, Platelets 3.27 ± 0.014 lacks/mm^3 and 3.27 ± 0.014 lacks/mm^3 and mean body weight was found 172.50 ± 0.051 gms and 169.17 ± 0.047 gm (P<0.05). Toluene soluble fraction (100mg/kg body weight) had shown protection of cyclophosphamide as mean hemoglobin 18.42 ± 0.096% (P<0.01), mean RBC 10.70 ± 0.088 millions/mm^3, WBC 9.18 ± 0.092 thousand/mm^3, Neutrophils 42.33 ± 0.62%, Lymphocytes 51.33 ± 0.281% Platelets 7.57 ± 0.110 lacks/mm^3 (P<0.05) and mean body weight 167.50 ± 0.045 gm (P < 0.05). In group X the animals administered with chloroform insoluble fraction (100mg/kg body weight) and cyclophosphamide (3mg/kg body weight), the significant protection was calculated as mean hemoglobin 18.42 ± 0.096% (P<0.001), mean RBC 10.70 ± 0.088 millions/mm^3, WBC 9.18 ± 0.092 thousand/mm^3, Neutrophils 42.33 ± 0.62% (P< 0.01), Lymphocytes 51.33 ± 0-.281 (P < 0.001), Platelets 7.57 ± 0.110 lacks/mm^3 (P<0.001) and mean body weight 167.50 ± 0.045gm (P<0.001) (Table 3.7).

Recovery studies were carried out with withdrawal of cyclophosphamide from Group IV, VI, VIII, X and XII while the Group II was fed with cyclophosphamide for the next seven days. The results suggest and a significant decrease (Group II) in mean hemoglobin 4.80 ± 0.24%, mean RBC 2.27 ± 0.124 millions/mm^3, WBC 2.20 ± 0.323 thousand/mm^3 (P<0.01), 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 158.33 ± 0.052gm (P<0.05).

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

Toluene soluble fraction (100 mg/kg body weight) treated Group VIII showed mean hemoglobin 11.60 ± 0.0619%, mean RBC 6.60 ± 0.105 millions/mm^3, WBC 9.20 ± 0.0717 thousand/mm^3, Neutrophils 31.50 ± 0.148%, Lymphocytes 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) had shown protective effect significantly against cyclophosphamide in hematological parameters as mean hemoglobin 12.80 ± 0.053%, mean RBC 7.02 ± 0.083millions/mm^3, WBC 7.49 ± 0.126 thousand/mm^3 (P<0.001), Neutrophils 33.67 ± 0.024% Lymphocytes 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 ± 0.059gm (P<0.05).

n-butanol soluble fraction (100mg/kg body weight) treated Group XII showed mean hemoglobin 14.48 ± 0.0956%, mean RBC 8.11 ± 0.1356 millions/mm^3, WBC 7.63 ± 0.102 thousand/mm^3, Neutrophils 30.17 ± 0.1014%, Lymphocytes 65.00 ± 0.401%, Platelets 8.76 ± 0.145 lacks/mm^3 and mean body weight 181.67±0.038 gms (P<0.05), (Table 1-5).

STATISTICAL ANALYSIS [32-35]:

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.

Table 1.3HA titreand Sheep erythrocyte agglutination tests of *Selaginellabryopteris*.

Groups	Treatment doses mg/kg body wt/day	Mean of HA Titer ±SEM	Mean of % Agglutination ±SEM
I	Control (2% GA)	4.000 ± 0.548	--
II	Std. LML 50	85.333± 0.387[a]	88.125 ±0.201[a]
III	SBME 100	20.00 ± 0.490	16.667 ±1.549
1V	SBME200	48.000 ± 0.365[c]	50.417±0.577[b]
V	SBME400	96.000 ± 0.365[a]	82.292±0.212[a]
VI	SBTF50	25.333 ± 0.420	25.00±1.549
VII	SBTF100	34.667 ± 0.454	36.25±0.558[b]
VIII	SBEAF 50	53.333 ± 0.310[a]	68.083±0.275[a]
IX	SBEAF100	85.333 ± 0.387[a]	82.292±0.212[a]
X	SBn-BF 50	69.33 ± 0.465[a]	71.042±0.267[a]
XI	SBn-BF 100	96.000 ± 0.365[a]	83.333±0.113[a]
XII	AR 100	21.333 ± 0.387	23.333±0.775

n = six animals in each group;

Comparison with Control a=***p<0.001 very very significant; b=**p <0.01 very significant; c= *p <0.05 significant.

Comparison with standard x=***p<0.001 very very significant; y=**p <0.01 very significant; z= *p <0.05 significant.

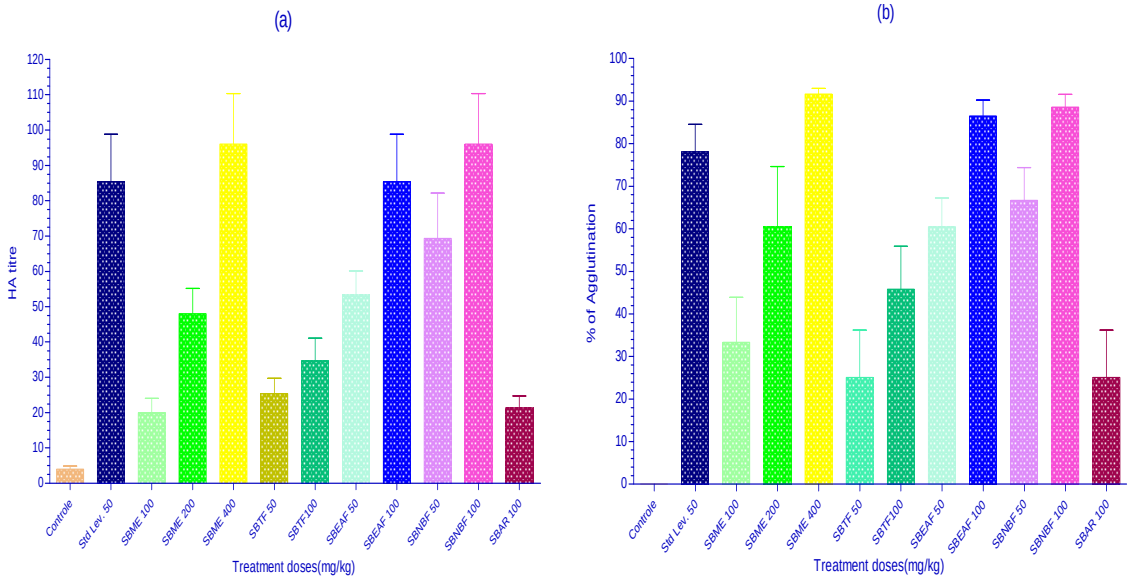

Figure 1.1 Sheep Erythrocyte agglutination Test of *Selaginellabryopteris*: (a) HA titre ; (b) % of Agglutination.

Table 1.4 T-Cell Population Tests of *Sellaginellabryopteris*

Groups	Treatment doses mg/kg body wt/day	ROSETTE(Mean ±SEM)		LYMPHOCYTE(Mean ±SEM)	
		Rosette Formation	% Increase	Lymphocyte formation	% Increase
I	Control (2% GA)	14.1667±0.094	----	182.1667±2.4833	----
II	Std. LML 50	19.1667±0.014	35.29 ± 0.433[a]	215.5±0.010	18.298± 0.053[a]
III	SBME 100	15.00 ± 0.112	5.88± 0.481	183.833± 0.015	7.223 ±0.096
1V	SBME 200	18.00 ± 0.079	27.06± 0.603[a]	205.833± 0.037	12.992± 0.372[a]
V	SBME 400	20.50± 0.067	44.71± 0.297[a]	222.670± 0.030	22.23± 0.194[a]
VI	SBTF 50	15.00±0.119	5.88± 0.507	187.1667± 0.025	2.745 ±0.715
VII	SBTF 100	16.8333± 0.069	18.82± 0.496[c]	196.000 ± 0.016	7.594 ±0.352[a]
VIII	SBEAF 50	18.1667± 0.081	28.29± 0.333[a]	212.333 ± 0.034	16.560± 0.294
IX	SBEAF 100	20.1667± 0.073	42.35± 0.222[a]	217.833± 0.029	19.579± 0.191[a]
X	SBn-BF 50	18.6667± 0.065	31.76± 0.529[a]	214.333± 0.027	17.658 ±0.224
XI	SBn-BF 100	20.3333± 0.060	43.52± 0.257[a]	221.333±0.015	21.500± 0.107[a]
XII	AR 100	15.00± 0.073	5.88 ±0.514	184.500± 0.014	1.281± 0.518

n = six animals in each group;

Comparison with Control a=***p<0.001 very very significant; b=**p <0.01 very significant; c= *p <0.05 significant

Comparison with standard x=***p<0.001 very very significant; y=**p <0.01 very significant; z= *p <0.05 significant.

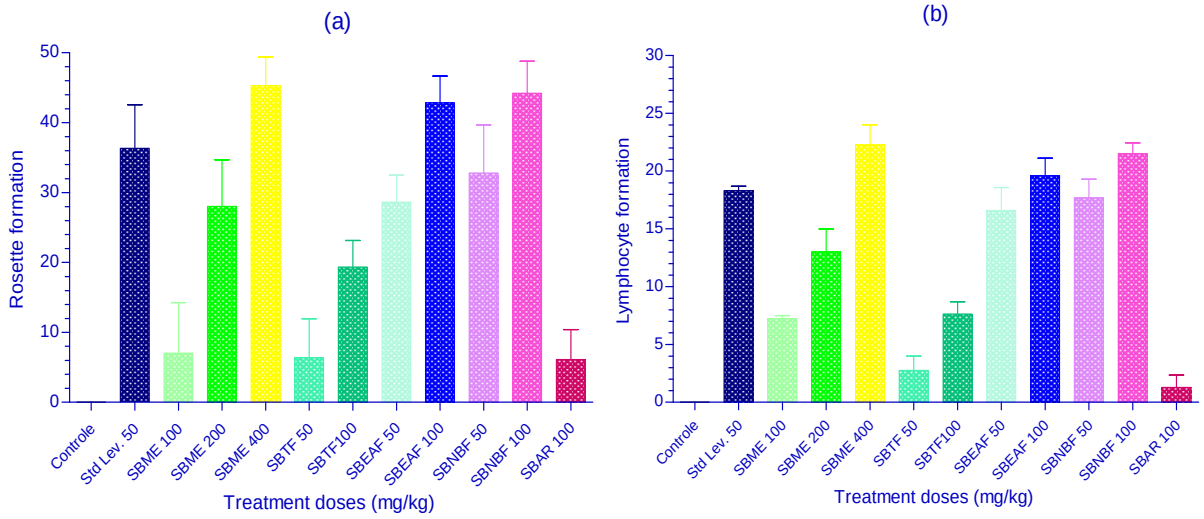

Figure 1.2 T-cell Population Test of *Sellaginellabryopteris*: (a) Rosette formation; (b) Lymphocyte formation.

Table 1.5 Drug induced myelosuppression test of *Sellaginellabryopteris* using Cyclophosphomide after 7 days.

Groups	Treatment dosesmg/kg body wt/day	Hb% Mean ± SEM	RBC Mean ±SEM (mill/mm^3)	WBC Mean ±SEM (thou/mm^3)	Neutrophils % Mean ± SEM	Eosinophils % Mean ± SEM	Lymphocytes% Mean ± SEM	Monocytes % Mean ± SEM	Platelates (lakhs/ mm^3) Mean ± SEM	Body/ Wt(gms) Mean ± SEM
I	Control(5% GA)	11.47± 0.107^x	5.24± 0.106^x	5.57 ± 0.096^x	31.5± 0.164	2.3± 0.528	65.67± 0.104^x	4.0± 0.354^y	5.012± 0.129^z	181.67± 0.028^x
II	**Std. CP 3mg**	**5.13± 0.318**	**2.60± 0.396**	**2.53 ± 0.55**	**35.33 ± 0.159**	**3.33± 0.245**	**54.17± 0.143**	**3.33± 0.490**	**4.32± 0.177**	**161.67± 0.032**
III	SBME 200 mg	11.88± 0.094^x	5.55± 0.043^x	5.25± 0.033^x	31.67 ± 0.158	1.50 ± 0.699	62.50 ±0.102^x	3.50± 0.433	4.63 ± 0.024	177.5± 0.043^y
1V	**SBME 200 + CP 3mg**	**10.63± 0.052^x**	**5.13± 0.126^x**	**4.40± 0.100^x**	**36.00 ± 0.084**	**2.50 ± 0.219**	**60.17± 0.067^y**	**2.33± 0.227±**	**±3.27 ±0.014**	**172.50 ±0.051**
V	SBME 400mg	13.30± 0.069^x	5.85± 0.036^x	5.37± 0.037^x	35.33 ± 0.113	1.83 ± 0.411	65.17± 0.067^x	3.17± 0.310	4.98 ± 0.199^z	185.00± 0.045
VI	**SBME 400 + CP 3**	**11.83± 0.114^x**	**5.39± 0.065^x**	**5.82± 0.098^x**	**36.50 ± 0.051**	**2.83 ± 0.266**	**60.17 3± 0.076^y**	**2.33± 0.350**	**3.69 ± 0.218**	**169.17 ±0.047**
VII	SBTF 100	12.42± 0.096^x	5.70± 0.088^x	5.18 ± 0.092^x	28.67 ± 0.139^y	3.67± 0.411	62.50± 0.063^x	5.00± 0.179	7.92 ± 0.092^x	179.17± 0.045^x
VIII	**SBTF 100 +CP 3**	**11.37± 0.137^x**	**5.42± 0.164^x**	**6.22± 0.195^x**	**42.33 ± 0.062^x**	**2.33 ± 0.238**	**51.33± 0.281**	**2.67± 0.387**	**3.88 ± 0.246**	**167.50 ±0.045**
IX	SBEAF 100	13.27± 0.092^x	6.52± 0.057^x	6.15 ± 0.034^x	35.17 ± 0.087	1.83± 0.638	61.00 ± 0.074^x	2.50± 0.219	7.57 ± 0.110^x	181.67± 0.041^x
X	**SBEAF 100 + CP 3**	**12.10± 0.093^x**	**5.65± 0.051^x**	**5.32± 0.231^x**	**36.33 ± 0.075**	**1.00± 0.365**	**55.33± 0.099**	**1.33± 0.387**	**3.50 ± 0.170**	**174.17± 0.028^z**
XI	SBBF 100	14.58± 0.060^x	5.63± 0.038^x	6.50 ± 0.050^x	38.33 ± 0.067	1.83± 0.411	61.67 ± 0.077^x	2.67± 0.194	8.49 ± 0.060^x	183.33± 0.056^x
XII	**SBBF 100 + CP 3**	**12.80± 0.077^x**	**5.33± 0.03^x**	**6.05 ± 0.207^x**	**39.17 ± 0.118**	**1.00± 0.843**	**57.83 ± 0.046^z**	**2.00± 0.316**	**5.89± 0.133^z**	**176.67± 0.056^z**

n = six animals in each group;
Comparison with Control a=***p<0.001 very very significant; b=**p <0.01 very significant; c= *p <0.05 significant.
Comparison with standard x=***p<0.001 very very significant; y=**p <0.01 very significant; z= *p <0.05 significant.

DISCUSSION:

A significant increase in humoral immune response was also seen in the sheep erythrocyte agglutination test. When animals were compared with control, the titer and % of agglutination to SRBC were increased significantly (P<0.001) at the serum dilution up to X: 64 and X : 128 dilution, with methanolic extract and its ethyl acetate soluble fraction. It was also observed that the n-butanol soluble fraction showed a significant increase in the dose of 100 mg/kg body weight orally.

The effect on cell mediated immunity of *Selaginellabryopteris* supported by the T-Cell population test resulted in an increase in rosette formation and lymphocyte formation. The methanolic extract of *Selaginella bryopteris has* shown a dose-dependent profile in rosette formation as 927.06 ± 0.603% (200 mg/kg body weight) and 44.71 ± 0.296% (400 mg/kg body weight) increases (P<0.001).

The influencing effect was also seen in a dose dependent manner with the ethyl acetate and n-butanol soluble fractions, which showed a significant increase in rosette formation up to 28.29 ± 0.333%, 42.35 ± 0.222, 31.76 ± 0.529% and 43.52 ± 0.257% (P<0.001) and lymphocyte formation up to 17.658 ± 0.224% and 21.500 ± 0.107% (P<0.001) with 50 and 100 mg/kg/body weight, respectively. These studies suggest *Selaginellabryopteris* has a significant influence on cell-mediated immune responses in experimental animals.

The findings indicate that the drug may activate CD4 and CD8 cells, which influence the mechanism of T-cell immunity, resulting in a significant increase in T-cell immune response.

In another experimental model, i.e. drug-induced myelosuppression test, the myelosuppression was produced by administration of cytotoxic drug, cyclophosphamide which produced significant myelosuppression in experimental animals. Cyclophosphamide produces significant bone marrow suppression resulting in cytopenia and subsequent suppression of humoral and or cellular as well as non-specific and specific cellular immune response. Cyclophosphamide treatment resulted in significant lowering of haemoglobin concentration, RBC, platelet and total WBC counts, as well as lymphocyte percentage. The suppressive effect of cyclophosphamide was protected by administration of methanolic extract of *Selaginellabryopteris*in 200mg/kg body weight (P<0.05). Studies on hematological parameters indicate that the methanolic extract and its ethyl acetate and n-butaonol soluble (100 mg/kg body weight P<0.05) protect the effect of cyclophosphamide. Recovery studies also suggests that the myelosuppressive drug withdrawal can be supported by the treatment with extracts of *Selaginellabryopteris*which results in a complete restoration of immune response or even in improved immunological parameters[27].

The experimental studies suggest that the drug is capable to influence the role of immunoglobulins resulting in activation of pre-B cell and or dendritic cells resulting in activation of antibodies which gives the higher agglutination titer against sheep red blood corpuscles.

Studies on immunomodulatory activity of *Selaginella bryopteris* reveals that the drug is capable of influencing the phagocytic activity, activating cell mediated immune and humoral immune responses in dose dependent manner. These findings justify its use in various ailments of diversed physiological conditions.

CONCLUSION:

In view of all the parameters assessed, *Selaginella bryopteris was* found to, even in improving the immunological parameters and even completing the immune response. This finding justified its use in various ailments of diverse physiological conditions. It may be, therefore, a useful drug in this task, and the drug may be considered to possess the immunostimulant property.

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