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Original Research Article

IMMUNOMODULATORY ACTIVITY OF ETHANOL EXTRACT OF *SARCOSTEMMA SECAMONE* (L.) BENNET (ASCLEPIADACEAE) IN MICE

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Abstract:
Sarcostemma secamone (L.) Bennet an ethnomedicinal plant was studied for its immunomodulatory activity. Immunomodulatory activity of different doses of ethanol extract of *Sarcostemma secamone* was evaluated in Swiss albino mice. Mice were treated with two doses (150 and 300mg/kg body weight) for 5 days. Body weight, relative organ weight, delayed type hypersensitivity (DTH) response and Haemagglutionation titre (HT) were studied in various groups of animals. The results obtained show a significant increase (p<0.05) in body weight and relative organ weight of spleen, liver and kidney at dose of 300mg/kg. The *Sarcostemma secamone* extract eliciated a significant increase (p<0.05) in the DTH response at dose of 300mg/kg. In the HT test, the plant extract showed a stimulatory effect at all doses. The doses of 300mg/kg significantly (p<0.05) increases the WBC count, compared with the control group. Overall, *Sarcostemma secamone* showed a stimulatory effect on both humoral and cellular immune functions in animal models.

Keywords: *Sarcostemma secamone,* Delayed type hypersensitivity, Haemagglutionation Titre, Immunomodulation.

Introduction:

Immunomodulation is a process, which alters the immune system of an organism by interfering with its functions. This interference results in either immunostimulation or immunosuppression. An immunomodulator is substance that helps to regulate the immune system. This regulation is a normalization process, so that an immunomodulator helps to optimize immune response. Immunomodulators are become very popular in the Worldwide natural health was as these do not tend to boost immunity, but no normalize it [1]. Keeping in this view, efforts have to be directed to modulate the immune responses, to permit effective treatment of various ailments associated with immune system and thus the development of a safe and effective immunomodulator for clinical use.

Sarcostemma secamone (L.) Bennet, is an important medicinal plant belonging to the family Asclepiadaceae. It is used in the

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traditional systems of medicine for various ailments. The decoction of the plant is useful to gargle for throat and mouth infection. The latex is bitter and used as a vulnerary. Fresh roots are prescribed for jaundice[2-5]. The milky sap forms a wash for ulcers. In combination with turpentine, it is prescribed for itch[6]. The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leucoderma and bronchitis. The juice is used in gleet, gonorrhea, pain in the muscles, cough and given to children as an astringent[7]. Leaf powder stimulates arculatory system, increases secretion of urine and activates uterus[8]. The fruit juice is used in gonorrhea and pain in muscles[7]. The leaves, roots and latex of *Sarcostemma secamone* are employed in treating many diseases like mouth ulcer, sour throat, jaundice and ulcers [9-11]. Realizing the importance and common use of the roots of *Sarcostemma secamone* in the treatment of liver disorder by several tribes in India. Literature reviews indicated that the immunomodulatory activity of whole plant of *Sarcostemma secamone* has not been scientifically evaluated so far. In view of this, the present study was aimed at evaluating the immunomodulatory activity of whole plant of *Sarcostemma secamone* in mice.

Materials and Methods

Plant material

The whole plant of *Sarcostemma secamone* (L.) Bennet was collected from Natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu and identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Animals

Study was conducted in Swiss albino female mice (20 - 25 g). The animals were bred and maintained under standard laboratory conditions (temperature 25 ± 2°C and light period of 12 h). The rats were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Study was carried out as per IAFC approval No: 82/ PHARMA/SCRI, 2010.

Treatment protocol

The plant extract was administered i.p. for 5 days at doses of 150 and 300 mg/Kg body weight. The dose volume was 0.2 ml. The control animal group received the same volume of normal saline and left untreated. The animals were divided into five groups (Groups I - V). Each group comprised of a minimum of five animals. The control group (Group I) was given normal saline and the treatment groups were given the whole plant extract of *S. secamone* at the doses of 150 mg/Kg and 300 mg/Kg body weight (Groups II and III) for five days, respectively and were humanized 24 h after the last dose. Body weight gain (percentage) and relative weight of kidney, liver and spleen (organ weight/100 g of body weight) were determined for each animal.

Assessment of humoral immune functions

Animals within the experimental groups were challenged with 0.2 mL of 10% sheep red blood cells (SRBC), i.p., on the 10th day of the initiation of experiment. The haemagglutinin titre was also studied in these animals.

Haemagglutinin titre assay

Haemagglutinin titre (HT) assay was performed as per the procedure given by Bin-Hafeez *et al.* (2001). On the fifth day after immunization, blood was collected from the heart of each mouse for serum preparation. Serial two fold dilution of serum was made in PBS (pH 7.2) in 96 - well microtitre plates and mixed with 50 µL of 1% SRBC suspension in PBS. After mixing, the plates were kept at room temperature for 2 h. The value of antibody titre was assigned to the highest serum dilution showing visible haemagglutination.

Delayed type hypersensitivity response

The delayed type hypersensitivity (DTH) response was determined using the method of Raisuddin et al.[12]. On the day of termination of the treatment with plant extract, animals were immunized with $1x10^8$ SRBC, subcutaneously. On the fifth day of immunization, all the animals were again challenged with $1x10^9$ cells in the left hind footpad. The right footpad was injected with the same volume of normal saline, which served as the trauma control for non specific swelling. Increase in footpad thickness was measured 24 h after the challenge by using a dial clipper.

Statistical analysis

All values were expressed as mean ± standard error of mean (S.E.M) and comparison between the groups were made by Analysis of Variance (ANOVA). The Data were analysed using the statistical analysis system SPSS (SPSS Software for windows release 10.0; SPSS Inc., Chicago IL, USA).

Results

After treatment with two different doses (150 and 300 mg/Kg body weight) of whole plant ethanol extracts of *S. secamone* for 5 days, the Swiss albino female mice were evaluated for immunomodulatory activity. Body weight, relative organ weight, delayed type hypersensitivity (DTH) and haemagglutination titres (HT) were studied in all the treated animal groups.

Effect of plant extract on Body weight and Relative organ weight

In the present study treatment with the whole plant ethanol extract of *S. secamone* was effective in increasing the body weight and also the weight of spleen, liver and kidney (Table 1).

Table 1: Effect of whole plant extracts of *Sarcostemma secamone* on the Body weight and Relative Organs weight

Parameter	Body weight and relative organs weight (mean± SEM) in gram				
Treatment	**Dose mg/Kg**	**Body weight**	**Spleen**	**Liver**	**Kidney**
Control - (Group-I)	Normal saline	20.45±1.24	0.48±0.12	4.23±0.19	1.14±0.05
Group - II	150	20.85±1.22	0.59±0.12	4.65±0.11	1.29±0.07
Group – III	300	21.65±1.45	0.66±0.14	5.92±0.16*	1.38±0.03

Each Value is SEM of 5 individual observations * $p < 0.05$ Compared Normal Control vs Treated groups

Effect of plant extract on humoral immunity parameters

In the haemogglutinin titre (HT) (Table 2), doses 150 mg and 300 mg/kg showed titre value of 3.83 and 5.31 respectively, while the titre value of control was 2.91, thus showing a significant increase in the titre values with doses of 150 and 300 mg/kg in the treated groups ($p< 0.05$).

Table 2: Effect of whole plant extracts of *Sarcostemma secamone* on DTH response compared with dexamethasone and on HT titre by using SRBC as an antigen in mice.

Treatment Groups	Parameter		
	Dose (mg/Kg)	Foot Pad Edema (mm)	HT titre
Control (Group-I)	Normal saline	0.29±0.021	2.91±0.022
Group - II	150	0.30±0.020	3.83±0.031
Group - III	300	0.34±0.12	5.31±0.022*
Dexamethasone Group- IV	10 mg	0.11±0.01	ND

Each Value is SEM of 5 individual observations * P < 0.05 Compared Normal Control vs Treated groups; ND - Not Done

Effect of plant extract on cell mediated immunity parameters

The plant extract at dose of 300mg/kg elicited a significant (p< 0.05) increase in DTH response (Table 2), compared to the control animals. In this study, dexamethasone (Group IV) decreased DTH response, compared to the control group.

Effects of plant extract on blood parameters and liver enzymes

There was no significant elevation in the levels of SGOT, SGPT and ALP as a result of treatment with *S. secamone* (Table 3). Total bilirubin content was slightly increased. No significant difference in blood parameters was recorded in various test groups. The doses of 150 and 300mg/kg increased the WBC count, compared with the control group.

Table 3: Effect of whole plant extracts of *Sarcostemma secamone* on the Hematological and Serum Liver marker enzymes.

Parameter		**Hematological (Blood)**			**Biochemical (Serum)**			
Treatment	**Dose mg/Kg**	**Hb (g/dl)**	**RBC (X10 ^{6}/mm^{2})**	**WBC (X10 ^{6}/mm^{2})**	**Total Bilirubin (mg/dl)**	**SGOT (U/L)**	**SGPT (U/L)**	**ALP (U/L)**
Control (Group - I)	Normal saline	13.36±0.84	4.86±0.11	5.35±0.81	0.51±0.04	46.55±3.56	39.14±2.84	123.56±5.74
Group - II	150	10.86±0.17 *	3.98±0.15*	6.59±0.57	0.88±0.04	49.33±4.19	50.36±2.51	145.66±6.43
Group - III	300	11.55±0.13	4.09±0.12	7.11±0.32	0.91±0.02*	48.16±3.91	52.99±2.63*	151.22±4.94*

Each Value is SEM of 5 individual observations * $P < 0.05$ Compared Normal Control vs Treated groups

Discussion

Immunomodulatory agents of plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. However these agents and the polyhedral formulations should be subjected to systematic studies to substantiate the therapeutic claims made with regard to their clinical utility.

The presence study showed an overall stimulatory effect of whole plant ethanol extracts of *S.secamone* on the immune function in mice. Stimulatory effects were observed on both humoral and cellular immunity. In the HT test, the whole plant extracts of *S.secamone* showed an increased response with all the tested doses, but this increase was only significant at 300 mg/Kg dose. This activity could be due to the presence of flavonoids or coumarins, which can augment the humoral response by stimulating the macrophages and B-lymphocytes involved in antibody synthesis[13]. The treatment with whole plant ethanol extracts of *S.secamone* improved the haemagglutinin antibody titre reflecting an overall elevation of humor immune response. The DTH response which directly correlates with cell mediated immunity (CMI) was found to be the highest at the maximum dose (300 mg/Kg) tested. The mechanism behind this elevated DTH during cell mediated immunity response could be due to sensitised T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lympokines, attracting more scavenger cells to the site of reaction[14]. The infiltrating cells are probably immobilized to promote defensive (inflammatory) reaction[15]. Treatment with *S.secamone* extracts enhanced DTH reaction, which is reflected from the increased foot pad thickness compared to control group suggesting heightened infiltration of macrophages to the inflammatory site.

Recent reports indicate that several types of flavonoids stimulate human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T-cells, cytokines, interlukin-2, gama interferon and macrophages and are there by useful in the treatment of several diseases caused by immune dysfunction[16]. Some reports have suggested that these compounds affect the health as immunostimulating agents i.e. directly enhancing the lymphocyte activation and / or secretion recreation of multipoint cytokine IFN-r. Some of these constituents also possess antioxidant properties and they may induce the immunostimulant effect, as several antioxidants have been reported to possess immunomodulatory properties[17-19]. This study may be supporting the possible role of *S.secamone* in assisting cell mediated immune response.

The ethanol extracts of *S.secamone* enhanced the production of WBC and SGPT, SGOT and ALP. Results of the present study also revealed no significant difference in the other blood parameters. Findings of the present study establish that *S.secamone* also have appreciable immunostimulatory activity. Their reported immunomodulatory effects warrant further investigation for their use in the cases of clinical immunosuppression.

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