

Study on immunomodulatory activity of ethanolic extract of *Spilanthes acmella* Murr. leaves

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The leaves of *Spilanthes acmella* Murr. have been used traditionally as tonic and in the treatment of rheumatism, gout, sailagogene and claimed to possess immunostimulant activity. In the present study, the ethanolic extract (500mg/kg bwt p.o.) of its leaves was evaluated for immunomodulatory activity using various models like modulation of macrophage function (morphometric and functional changes in mice), carbon clearance assay with the help of Indian ink dispersion (0.5ml/100g bwt i.v.) in mice and immunoprophylactic effect with the help of *Escherichia coli* (0.5ml/100g bwt i.p.) in mice. The extract (500mg/kg b.wt. p.o.) exhibited significant ($P < 0.01$) peritoneal macrophage stimulation and 25 to 50% mortality as compared to control mice, indicating its prominent immunostimulant activity.

Keywords: *Spilanthes acmella*, *Escherichia coli*, Indian ink dispersion, Macrophage, Immunoprophylactic, Immunomodulation.

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Introduction

Immunomodulatory agents of plant and animal origin increase the immune responsiveness of the body against pathogens by activating primarily the non-specific immune system i.e. stimulation of the function and efficiency of the macrophages and other complements. However, these drugs should be subjected to systematic studies to substantiate the therapeutic claims made with regard to their clinical utility.

Spilanthus acmella Murr. (Family-Asteraceae), commonly known as 'Akarkara' is an annual hairy herb, up to 32-60 cm tall with numerous stems of marigold yellow flowers. Stems are glandular and hairy with pungent taste. The whole plant is acrid in taste¹. The leaves are used as immunomodulatory, adaptogenic, diuretic, tooth paste, lithotriptic, antiscorbutic, sailagogene, antibacterial, tonic and digestive²⁻⁷. The leaves are reported to contain alkaloids, carbohydrates, pungent amide tannins, steroids, carotenoids, essential oils, sesquiterpenes, amino acids, etc⁸⁻¹². So far no systematic study has been reported to evaluate the immunomodulatory

potency of its leaves extract. In the present study, an effort was made to validate and exploit the immunostimulant potential of ethanolic extract of leaves using various experimental models.

Materials and Methods

Plant material

The fresh leaves of *S. acmella* (SA) were collected from local areas of Hubli, Karnataka, India and authenticated by Dr. G R Hegde, Professor & Head, Department of Botany, Karnataka University, Dharwad, Karnataka.

Preparation of extract

The leaves were separated, shade dried and then powdered (40 mesh size). The coarse powder of shade dried leaves of SA was extracted with ethanol (95%) in a Soxhlet extractor. The ethanolic extract was then concentrated on a rotary flash evaporator to obtain a greenish brown colored residue, yield 3.6%(w/w) respect to the dry starting material. It was stored in a desicator. This extract, referred to as SA was subjected to preliminary phytochemical investigations^{9, 13, 14}.

Preparation of test sample and other solutions

Test sample

It was prepared by suspending the dried ethanolic extract, SA (20mg) in distilled water (100ml), using

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emulsifying agent 2% Tween 80 for oral administration.

Phosphate buffer salt solution (PBS)

Taken 50ml of 0.2M of KH_2PO_4 (27.218g KH_2PO_4 in 1000ml water) in 200ml of volumetric flask and added 34.7ml of 0.2 Na_2HPO_4 and make the volume to 200 ml.

Indian ink dispersion

Taken 1.6ml of Camlin (black) ink and diluted it with 10ml distilled water. A dose of 0.5ml/100g b. wt. i.v. *Escherichia coli* (0.5%) solution in PBS (pH-7.2), dose-0.5ml/100g b.wt. i.p.

Animals

Healthy male albino rats (200-250g) of Wistar strain were obtained from the Central Animal Faculty, Indian Institute of Science, Bangalore. The animals were housed under controlled conditions of temperature (25°C) and humidity (55%). They were fed with pellet diet (Amrut rat and mice pellet sagli) and water *ad libitum*.

Acute toxicity study

Healthy albino mice of either sex weighing 25-30g were maintained under controlled conditions of temperature (20-25°C) and humidity (55%), were used for toxicity study as per Up & Down or Staircase method¹⁵. The effective oral dose of ethanolic extract of SA was found to be 500mg/kg body weight.

Screening of immunomodulatory activity

*Modulation of macrophage function, morphometric and functional changes*¹⁶⁻²⁰

Albino male mice (20-25g) of either sex were housed under standard laboratory conditions prior to experimentation. They were usually fasted for a period of 24-39h. Allowing free access to drinking water, prior to drug administration orally. Group-1: (Control, n=4) received only clean tap water. Group-2: (SA extract treated, n=4) received 500mg/kg body weight ethanolic extract of SA.

Peritoneal macrophages were isolated from the treated mice (n=4) on consecutive days (5, 10, 15 days) and also from a set of control mice (n=4). Peritoneal fluid was collected in phosphate buffer saline (PBS, pH-7.2) and the macrophage count was determined.

Carbon clearance assay in mice¹⁸

The term clearance has been employed for studies that measure the disappearance of a particle or

immune complex from the circulation, depending upon the formation of intravascular complexes, following injection of antigen, which are then more readily removed by the Reticulo Endothelial System (RES). Albino mice (20-25g) were divided into two groups, each group containing four animals. Group-1 (control, n=4) received only clean tap water and Group-2 (SA extract treated, n=4) received daily 500mg/kg body weight of SA extract for 9 days. On day 10, Indian Ink was given orally to both groups of mice. Blood samples were collected after 5, 10, 15 and 30 min. from tail vein and lysed in distilled water (3ml). The optical density was measured spectrophotometrically at 650nm. Using pre-injection blood sample as blank and correlation co-efficient was calculated by correlation-regression co-efficient test (Statistical method).

Evaluation of immunoprophylactic effect¹⁹

This parameter was studied with the help of *E. coli* (0.5ml/100kg b. wt. i.p.), SA extract (500mg/kg p.o.) was administered and effect of *E. coli* injection in mice and protection by ethanolic extract was evaluated. Male albino mice (20-25g) were divided into two groups: Group-1 (control, n=6) received only clean tap water. Group-2 (extract treated, n=6) received 500mg/kg body weight of ethanolic extract (SA) orally every day for 15 days. On 15th day, 3h after the last dose of SA extract, *E. coli* (0.5ml/100kg b. wt. i.p.) was injected to both groups of mice and percentage of mortality was observed after 24h.

Statistical analysis

The values are expressed as mean \pm SEM and results were analyzed by using ANOVA and Student's 't' test. Statistical significance on comparison with control group are indicated by *mark, * $P < 0.01$, highly significant.

Results and Discussion

The yield of the ethanolic extract of leaves was found to be 16.62% w/w and preliminary phytochemical investigation revealed the presence of alkaloids, carbohydrates, tannins, steroids, carotenoids, sesquiterpenes and pungent amide. The total ethanolic extract of leaves showed a significant immunomodulatory activity. The time dependent effect of SA extract on morphometric functional changes in mice (peritoneal macrophages) were evaluated., SA extract treated (500mg/kg b. wt. p.o.) showed a significant increase in the macrophage

count and the maximum number of cells was found to be on the 15th day of extract administration (Table 1)

In the carbon clearance assay in mice, clearance of carbon in SA extract treated mice was rapid (~0.082/60min) compared to control (~0.159/60min) and the stimulation rate was found to be significant ($P<0.01$) in the concentration range of 500mg/kg b. wt. p.o. (Table 2). Immunoprophylactic effect was significantly enhanced in animals treated with the SA extract (500mg/kg b. wt. p.o.) before administering *E. coli* (0.5ml/100kg b. wt. i.p.). Animals treated with the SA extract in mice, showed only 25 to 50% mortality as compared to 100% mortality in control mice (Table 3).

The result obtained with SA leaves extract treatment were significantly ($P<0.01$) comparable with that of the control. The kinetics of the clearance depended on particle size, opsonic factor in serum, relative weight of RES organs, activation and proliferation of RES, blood flow through the liver and spleen and nature of the vehicle used to suspend the particles. Clearance techniques have also been extensively exploited for the measurement of circulating antibody. These depend upon the formation of intravascular complexes, following injection of antigen, which are then more readily removed by the RES. The elimination of carbon in response to SA treatment was found to be

Table 1: Effect of ethanolic extract of *Spilanthes acmella* on morphometric and functional changes of macrophages in male albino mice

Macrophage count (cell/mm ³)	Days after treatment with SA (500mg/kg b.wt. p.o.)		
	5 days	10 days	15 days
Control group	5,700±147.20	6,075±179.70	5,775±188.75
Extract treated	6,925±94.69	8,675±125.00*	9,100±129.10*

The values are expressed as Mean±SEM.

The significance on comparison with control group is indicated by * mark. * $P<0.01$

Table 2: Mean absorbance, correlation co-efficient and carbon clearance stimulation rate in male albino mice

Groups	15min	30min	45min	60min	C.C.	S.R.
Control (Saline)	0.0841±1.2047	0.0829±1.1108	0.0837±1.1080	0.082±1.2136	-0.7651	
Extract (500mg/kg b.wt. p.o.)	0.128±0.4270	0.174±0.3162	0.167±0.3320	0.159±0.4176	-0.9998	1.306*

The values are expressed as Mean ± SEM.

The significance on comparison with control group is indicated by * mark; * $P<0.01$. C. – Carbon Clearance assay; S. R. – Stimulating Rate

Table 3: Effect of ethanolic extract of *Spilanthes acmella* on *E. coli* induced in male albino mice

S. No.	Total no. of animals died		Protection (%)
	Control group	Treated group	
1	4/4	2/4	50
2	4/4	1/4	75
3	6/6	3/6	50
4	6/6	3/6	50

significantly enhanced compared to the control. *E. coli* treatment caused 100% mortality of the animals, due to abdominal peritonitis in untreated mice and extract treated mice. However, treatment with SA reduced the mortality to 25 to 50% showing SA has immunomodulatory activity¹⁸.

Conclusion

The time dependent effects of ethanolic extract of *S. acmella* treatment on morphometric and functional changes of macrophages in mice showed significant increase in the number of macrophage cells on the 15th day of drug administration. Thus it significantly activated macrophages and enhanced their function as compared to control, suggesting this herb as a potential natural drug for immunostimulant effect.

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