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Study of Immunomedulatory Activity of Tanacetum Balsamita in Mice

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ABSTRACT: The immunomodulatory potential of Tanacetum balsamita, commonly known as Costmary or Balsamita, was evaluated through a series of in vivo experiments in mice. The present research study designed to explore the pharmacological effects of Tanacetum balsamita extract on the immune response by assessing various immunological parameters. The mice were administered with different doses of the extract, and subsequent analyses included measurements of spleen and thymus weights, white blood cell (WBC) count, and cytokine production. Additionally, the delayed-type hypersensitivity (DTH) response and humoral antibody response were evaluated to determine the extract's influence on humoral and cell-mediated immunity, respectively. Results showed that the extract of Tanacetum balsamita significantly enhanced both the DTH response and antibody production, suggesting a stimulatory effect on both cellular and humoral immune responses. Furthermore, increased spleen and thymus weights, as well as elevated WBC counts, supported the immunostimulatory activity of the extract. These findings suggest that Tanacetum balsamita holds potential as a natural immunomodulatory agent, warranting further investigation for its therapeutic applications in immune-related disorders.

Keywords: Tanacetum balsamita, immunomodulatory activity, mice, immune response, cytokine production, delayed-type hypersensitivity, humoral immunity

1. INTRODUCTION

The immune system shares a key role in maintaining homeostasis and protecting the body against pathogenic infections and diseases. Immunomodulation, the adjustment of immune responses to desired levels, is a convincing strategy for the treatment and prevention of various immune-related conditions, including autoimmune diseases, infections, and cancer. Natural products, particularly medicinal plants, have been explored for their immunomodulatory properties due to their diverse bioactive compounds and relatively low toxicity. Tanacetum balsamita, commonly known as Costmary or Balsamita, is a perennial herb belonging to the Asteraceae family. Traditionally used in folk medicine, this plant has been valued for its antimicrobial,antioxidant and anti-inflammatory properties. Despite its extensive use in traditional remedies, scientific investigation into its immunomodulatory potential remains limited.

Preliminary studies suggest that the bioactive constituents of Tanacetum balsamita, such as flavonoids, terpenoids, and phenolic acids, may contribute to its therapeutic effects. However, comprehensive studies examining its impact on the immune system are lacking. The present study designed with the objective to bridge this gap by evaluating the immunomodulatory activity of Tanacetum balsamita extract in an in vivo mouse model. The primary objective aims to assess the effect of Tanacetum balsamita on both humoral and cellular immune responses. Specifically, we investigated its influence on the delayed-type hypersensitivity (DTH) response, humoral antibody production, and cytokine levels. Additionally, changes in spleen and thymus weights and white blood cell (WBC) counts will be measured to provide further insight into the extract's overall impact on immune function. By elucidating the immunomodulatory properties of Tanacetum balsamita, this study pursues to the growing body of evidence supporting the therapeutic potential of medicinal plants in immunoregulation. Ultimately, our findings could contribute indesigning and development of novel natural immunomodulatory agents derived from Tanacetum balsamita, offering new avenues for the treatment and management of immune-related disorders.

PLANT PROFILE: **Botanical Name:** Tanacetum balsamita
Family: Asteraceae
Common Name: Bible leaf, Costmary
Description: Plants belonging to Tanacetum genus (Asteraceae family) are widely distributed

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throughout the temperate zone of the northern hemisphere. They are perennial, herbaceous plants, native to Europe and Asia, but introduced in other parts of the world, as well. Some Tanacetum species have been known for ages as important medicinal plants, e.g. feverfew (Tanacetum parenthium L. Schultz Bip.) is listed in European Pharmacopeia as a traditional herbal remedy used for prophylaxis of migraine. Recently, special attention has been paid on two other species of this genus − tansy (Tanacetum vulgare L.) and costmary (Tanacetum balsamita L.). The herb of these plants has been used in traditional medicine as anthelmintic, antibacterial, digestive, and diuretic agent. The studies on the extracts obtained from these plants confirm different biological activities. Tansy exhibits antioxidant, antibacterial, antifungal, antihypertensive, diuretic, and anthelmintic properties as well as acaridical and repellent activity. Costmary reveals mainly antibacterial, antioxidant and astringed activity.

II. MATERIALS AND METHODS

Selection and Authentication:

The freshly collected leaves of Tanacetum balsamita from the near region market of Jaipur, Rajasthan. Plant will authenticate in Botany department, Rajasthan University, Jaipur.

Extraction of Leaves:

The coarsely powdered leaves material was extracted with methanol by cold maceration technique.The leaves of Tanacetum balsamitawas made to mechanical grinding and powdered by electrical blender. Ten grams of the powder was soaked in 100 ml of methanol for 48 hrs followed by filteration through whattman filter no.1. The filtrate wasthen dried at 60°Cthrough rotary evaporator. The extract obtained was dried followed by storage in steriled glass bottles (-20°C) until use. The extract was then dissolve in different solvent for testing. Extracts are store at 4°C until further use.

Experimental Animals

Strain: Albino Wistar rats
Sex: Either
Body weight: 200 ± 50 gm
Housing condition:As per CPCSEA guidelines

Methodology

(a)Physical Characteristics: The extract wastestedto check it's solubility in various polar (water, acetone, methanol, chloroform) and non polar solvents (DMSO, petroleum ether, ethylacetate).

(b) Phytochemical Screening:Phytochemical Screeningwere carried toconfirm the presence or absence of different phytoconstituents.

© Acute oral toxicity: As per OECD guidelines**,** the acute oral toxicity was performed. Initially, the acute oral toxicity studiesofTanacetum balsamitaplant extract were carried out by giving animals single doses of drug to albino wistar rats which were divided into groups. The albino rats in all the groups were kept on standard rat pelleted diet with free access to tap water ad libitum. The doses selected for the study i.e., (2000,300, 50, 5)mg/kgLastly after sample administration rats were observed for mortality in coming 72 hours.

(d) Immunomodulatory Activity

Delayed types hypersensitivities reaction (Effect on immunity)

Procedure: Initially, at day 0 the albino rats were sensitized with 0.1 ml of 10% SRBC (1×10^8 cells). Thetest sample was administered -4 to + 4 days of SRBC immunization. Furthermore, albino rats were challenged with 1×10^8 SRBC cells, intradermally into the left footpad of each animal, while PBS (pH 7.4) will inject into right hind paw on day 9 followed by measuring of increase in footpad thickness (FPT) using digital vernier calliper after 24 hr after SRBC challenge.

Analysis parameter The degree of DTH reaction was defined as the increase in the percentage in FPT over the control values.

(e) Hemagglutination Antibody Titre (Effect on Immunity)

Initially at day 0, the albino rats were injected i.p. 0.2 ml of 5×10^9 SRBC. The test sample wereinjected to albino rats on −4, −2, 0, 2, 4 days. Thevolume of vehicle received by control group were same. Furthermore,at day 7 blood samples were collected from retro-orbital plexus. In addition, two-fold dilutions of serum samples were made in 25 µl volumes of normal saline containing 0.1% BSA (BSA saline) in V bottom heamagglutination plates was added to 25 µl of 0.1% suspension of SRBC in BSA saline. Furthermore, thoroughly mixed SRBC was allowed to settle down at room temperature for 90 mins until control wells showed small button of cells (negative pattern).

Analysis parameter: Visible haemagglutination was recognised as antibody titre with highest serum dilution.

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III. RESULTS AND DISCUSSION

Plant extraction

Formula: [weight of extract / weight of powdered drug] X 100

The plant material was extracted by soxhlet apparatus and the percentage yield calculated by the following formula was found to be 17.38 %.

Solubility determination: ???

Table 1: Solubility determination of extract

S. No.	Solvent	Solubility of methanolic extract
1.	Water	Soluble
2.	Acetone	Insoluble
3.	Chloroform	Partial soluble
4.	Methanol	Soluble
5.	Petroleum ether	Partial soluble
6.	Ethylacetate	Partial soluble
7.	DMSO	Soluble

Phytochemical Analysis

The extract of plant was analysed for preliminary phytochemical screening to confirmvarious plant secondary metabolites presence in the same (summarized in Table 2).

Table 2: Phytochemical Screening of extract

S. No.	Experiment	Presence or absence of phytochemical test
1.	**Alkaloids**	
1.1	Mayer's reagent test	Present
1.2	Wagner's reagent test	Present
1.3	Hager's reagent test	Present
2.	**Carbohydrates**	
2.1	Molish's test	Present
2.2	Fehling's test	Present
2.3	Benedict's test	Present
2.4	Barfoed's test	Absent
3	**Proteins and Amino Acids**	
3.1	Biuret test	Present
4.	**Flavonoids**	
4.1	Alkaline reagent test	Present
4.2	Lead Acetate test	Present
5.	**Glycoside**	
5.1	Borntrager test	Absent
5.2	Legal's test	Absent
5.3	Killer-Killiani test	Present
6.	**Tannin and Phenolic Compounds**	
6.1	Ferric Chloride test	Present
6.2	Lead Acetate test	Present
6.3	Gelatin test	Absent
7.	**Saponin**	
7.1	Foam test	Absent
8.	**Test for Triterpenoids and Steroids**	
8.1	Salkowski's test	Absent
8.2	Libbermann-Burchard's test	Present

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Acute oral toxicity

The following four ranges of doses were used for i.e 5mg/Kg, 50 mg/Kg, 300 mg/Kg, 2000 mg/Kg. Furthermore,albino rats were observed for next 4 hrs for follow-up after dosing to check the presence of mortality during this period and 72 hours after sample administration.

Table 3: Acute oral toxicity of extract

S. No.	Dose	Lethality	Mortality
1.	5 mg/Kg	0/3	Not observed
2.	5 mg/Kg	0/3	Not observed
3.	50 mg/Kg	0/3	Not observed
4.	50 mg/Kg	0/3	Not observed
5.	300 mg/Kg	0/3	Not observed
6.	300 mg/Kg	0/3	Not observed
7.	2000 mg/Kg	0/3	Not observed
8.	2000 mg/Kg	0/3	Not observed

*0/3- zero animal dead out of three animals

Immunomodulatory effect

The aim of present research study was to analyse the immunomodulatory effect of methanolic extract of T. balsamita leaves. As the effect was ascertain on the basis of effect on cellular immunity and humoral immunity.

Delayed type hypersensitivity

Table 4 DTH readings of all four groups

S. No.	Group	Paw thickness (24 hrs.)	Paw thickness (48 hrs.)
1.	Control	0.52±0.172	0.43±0.156
2.	Standard	1.01±0.156*	0.80±0.166*
3.	Test (400mg/ml)	0.72±0.075*	0.55±0.084*
4.	Test (500mg/ml)	0.83±0.066*	0.65±0.084*

Value expressed as mean±standard deviation (SD) at no=6, one way ANNOVA, followed by bonferrony test * P< 0.05 significant compared to that of the control group.

DTH response is a type IV hypersensitivity reaction and progresses when antigen sensitizes TDTH cells. It is an expression of cell-mediated immunity. It plays an important role in various inflammatory disorders (Abid et al., 2012). These reactions are characterized by invasions of large number of non-specific inflammatory cells. These inflammatory cells are Th1 subpopulation while sometimes TC cells are also involved. The antigen presentation through appropriate APCs leads to the activation of TDTH cells that results secretion of various cytokines includes IFN-γ. These cytokines recruit and stimulate macrophages, thus promoting phagocytic activity. It has been reported that DTH reactions play a vital role in host defense against parasites and bacteria that can live and proliferate intracellularly (Gongora et al., 2000). DTH reaction to SRBC is given in table 6.4, in which data are expressed in terms of the footpad thickness. After administration of the extract (400 and 500 mg/Kg, p.o.), a significant increase in footpad thickness was observed at 500 mg/Kg after 24 and 48 h as compared with the control group, suggesting heightened infiltration of macrophages to the inflammatory site

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Haemagglutination antibody titre assay

Table :5 HAT readings of all four groups

S. No.	Group	Dilution no.
1.	Control	3.17±0.753
2.	Standard	7.67±0.816*
3.	Test (400mg/ml)	5.00±0.894*
4.	Test (500mg/ml)	5.83±1.169*

Value expressed as mean±standard deviation (SD) at no=6, one way ANNOVA, followed by bonferrony test * P< 0.05 significant compared to the control group.

The fractions active in PBMC proliferation assay were further evaluated for activity to stimulate B and T cells in relation to serum immunoglobulins IgM and IgG in presence of T dependent antigen (SRBCs). The antibody response was observed by hemagglutination titre. IgM titers were measured in mice serum of different groups, collected 7 days after immunization and treatment. The agglutinated lattice maintains the RBC's in a suspended distribution, typically viewed as a diffuse reddish solution. The formation of the lattice depends on the concentrations of the antibody and RBC's, and when the relative antibody concentration is too low, the RBC's are not constrained by the lattice and settle to the bottom of the well. The results showed significant increase titres in mice treated with extract. The augmentation of humoral immunity to T-dependent antigen (SRBC) shows the increased responsiveness of macrophages since the antibody production is closely related to the co-operation of macrophages, T and B lymphocyte response.

IV. CONCLUSION

The present investigation focused on evaluation of the immunomodulatory potential of compounds from Tanacetum balsamita (Family: Asteraceae). This plant was evaluated for their ability to stimulate both arms of the immune response, by characterizing various cellular and molecular markers in different immunologically relevant tissues.

The results presented here in show that Tanacetum balsamita was extracted. The results revealed that Tanacetum balsamita plant extract augment the immune responses to T-dependent antigen (SRBC). Our findings strongly suggested that methanolic extract induced increase in the levels of serum anti-SRBC immunoglobulins, as measured by hemagglutination and the maximum effect was observed at 500mg/Kg. Besides, treatment with methanolic extract enhanced the delay type hypersensitivity reaction, reflected by increased FT compared to the control group, compeling heightened infiltration of macrophages to the inflammatory site. The above data suggest that methanolic extract enhance both cell mediated and humoral immunity.

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