

Evaluate the Immunomodulatory Effects of Tanacetum Balsamita Extract in Cyclophosphamide-Induced Immunosuppressed in Mice**Manjeet Singh Chauhan¹, Rakesh Sharma², Lokesh Kumar Saini³**¹Research Scholar, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India^{2,3}Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India

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Conflict of interest: Nil

Abstract

The present study was undertaken to evaluate the immunomodulatory effects of Tanacetum balsamita extract in cyclophosphamide-induced immunosuppressed mice. The extract was subjected to preliminary phytochemical screening and quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC). The extract showed the presence of various bioactive phytoconstituents, with TPC and TFC values of 68.78 ± 0.37 mg GAE/g and 42.68 ± 0.37 mg QE/g extract, respectively. Acute oral toxicity evaluation revealed that the extract was well tolerated without mortality or significant behavioral abnormalities. Immunosuppression was induced using cyclophosphamide, and the animals were treated with T. balsamita extract at doses of 100, 200 and 400 mg/kg body weight, while levamisole (2.5 mg/kg) was used as the standard drug. Cyclophosphamide markedly reduced body weight, total WBC count, RBC count, hemoglobin, lymphocyte percentage, spleen and thymus weights, serum IgG and IgM levels, and antioxidant enzymes. Treatment with T. balsamita extract produced a dose-dependent improvement in these parameters. At 400 mg/kg, the extract increased total WBC count to $8.12 \pm 0.27 \times 10^3/\mu\text{L}$, IgG to 834.60 ± 26.12 mg/dL, IgM to 97.80 ± 3.81 mg/dL, SOD to 7.32 ± 0.31 U/mg protein, CAT to 6.02 ± 0.27 U/mg protein, and GSH to 5.02 ± 0.23 $\mu\text{mol/g}$ tissue, while reducing MDA to 2.72 ± 0.16 nmol/mg protein. Cytokine levels also improved following treatment. Histopathological examination demonstrated dose-dependent restoration of spleen and thymus architecture, with the 400 mg/kg dose showing near-normal tissue organization. Overall, the findings indicate that Tanacetum balsamita extract possesses significant immunomodulatory and antioxidant activity and may be a promising natural therapeutic agent for the management of cyclophosphamide-induced immunosuppression.

Keywords: Tanacetum Balsamita, Immunomodulatory Activity, Cyclophosphamide, Immunosuppression, Immunoglobulins, Antioxidant.

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Introduction

The immune system is a complex and highly coordinated biological defense network responsible for protecting the body against infectious microorganisms, foreign substances, and abnormal cells.

It consists of specialized organs, tissues, cells, and soluble mediators that work together to recognize

and eliminate potentially harmful agents while maintaining tolerance to the body's own tissues.

The major components of the immune system include the bone marrow, thymus, and spleen, lymph nodes, mucosa-associated lymphoid tissues, leukocytes, antibodies, complement proteins, cytokines, and other signaling molecules.

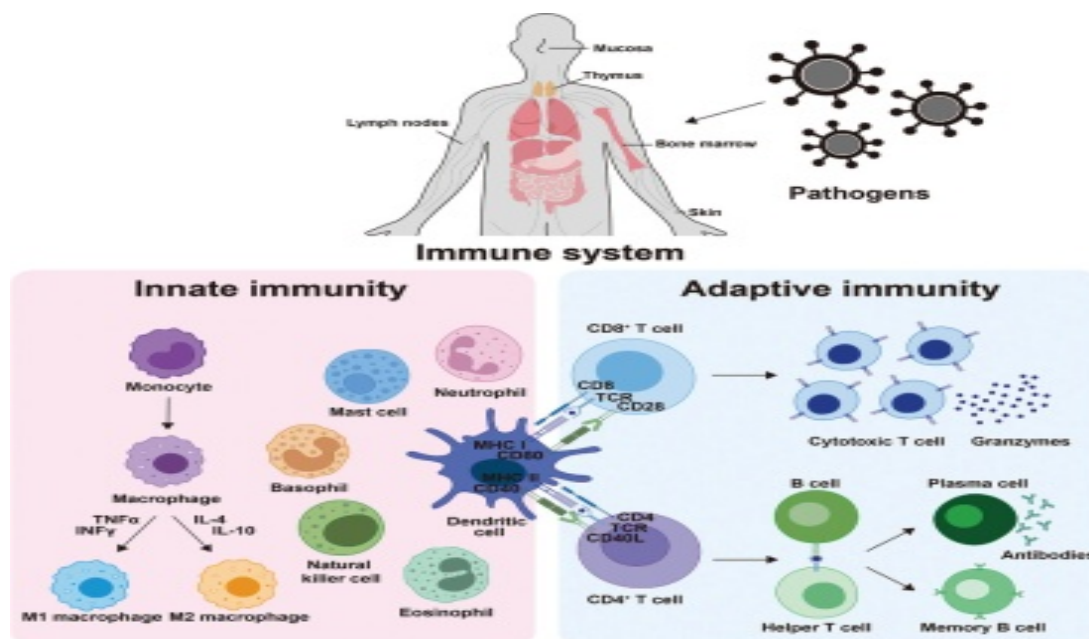


Figure 1: Image of Immune System

Plant Profile of Tanacetum Balsamita:

Tanacetum balsamita L. is an aromatic perennial herb belonging to the family Asteraceae (Compositae). It is commonly known as costmary and has a long history of traditional use as an aromatic and medicinal plant. The plant is characterized by its aromatic foliage and yellow

flower heads and has traditionally been cultivated in different regions for culinary, household, and medicinal purposes. The species is particularly notable for its aromatic essential oil and the presence of various secondary metabolites, including phenolic compounds, flavonoids, terpenoids, and volatile constituents.



Figure 2: Plant Profile of Tanacetum Balsamita

Pharmacological Uses: Tanacetum balsamita has a history of traditional use as an aromatic and medicinal herb. Its leaves have traditionally been used in culinary preparations and herbal remedies.

Reported traditional and investigated uses of Tanacetum species include:

- Digestive and carminative applications
- Aromatic preparations

- Traditional management of minor inflammatory conditions
- Antimicrobial applications
- Antioxidant activity
- Anti-inflammatory potential
- Traditional herbal preparations

However, traditional use should not be interpreted as proof of clinical efficacy. Pharmacological

activity requires confirmation through appropriate experimental and clinical studies.

Materials and Methods

Plant Material: The plant material was collected from a suitable geographical location during the appropriate season. The collected material was carefully inspected to remove foreign matter, soil, damaged portions, and other impurities. The plant material was authenticated by a qualified botanist.

Preparation of Extract: The plant material was shade-dried at room temperature and protected from direct sunlight to minimize degradation of heat- and light-sensitive phytoconstituents. The dried material was coarsely powdered using a mechanical grinder.

The powdered plant material was subjected to hydroalcoholic extraction using an appropriate proportion of ethanol and distilled water.

Preliminary Phytochemical Screening: The prepared *Tanacetum balsamita* extract was subjected to preliminary phytochemical screening to detect the major classes of secondary metabolites present in the extract. Standard qualitative chemical

tests was performed using suitable reagents and procedures.

Experimental Animals: Healthy Swiss albino mice of either sex, weighing approximately 20–30 g, was used for the present pharmacological investigation. The animals was procured from an approved laboratory animal facility and acclimatized to the experimental environment for at least 7 days before commencement of the study.

Acute Oral Toxicity Study: The study was performed according to the relevant OECD guideline for acute oral toxicity and after obtaining approval from the Institutional Animal Ethics Committee (IAEC). Healthy mice of appropriate age and body weight was selected and acclimatized to laboratory conditions before the study.

Experimental Model: Healthy mice was acclimatized to laboratory conditions before initiation of the experiment. Immunosuppression was induced by administration of cyclophosphamide at a dose and schedule selected from validated published protocols and approved by the IAEC.

Table 1: List of Animal Experimental Groups

Group	Treatment	Dose
Group I – Normal Control	Normal saline/vehicle	10 ml/kg, p.o.
Group II – Cyclophosphamide Control	Cyclophosphamide	30 mg/kg, i.p.
Group III – Standard	Levamisole + Cyclophosphamide	2.5 mg/kg, p.o. + 30 mg/kg, i.p.
Group IV – Low Dose	<i>Tanacetum balsamita</i> extract + Cyclophosphamide	Xmg/kg, p.o. + 30 mg/kg, i.p.
Group V – Medium Dose	<i>Tanacetum balsamita</i> extract + Cyclophosphamide	Ymg/kg, p.o. + 30 mg/kg, i.p.
Group VI – High Dose	<i>Tanacetum balsamita</i> extract + Cyclophosphamide	Zmg/kg, p.o. + 30 mg/kg, i.p.

Parameters Evaluation: The immunomodulatory effect of *Tanacetum balsamita* extract in cyclophosphamide-induced immunosuppressed mice was evaluated using hematological, immunological, biochemical, antioxidant, organ-index, and histopathological parameters.

Results

Preliminary Phytochemical Screening: The preliminary phytochemical screening of the prepared *Tanacetum balsamita* extract was carried out using standard qualitative chemical tests. The extract showed the presence of several important classes of phytoconstituents, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, steroids, terpenoids, carbohydrates, and proteins.

Table 2: Preliminary Phytochemical Screening of *Tanacetum balsamita* Extract

S. No.	Phytoconstituent	Test	Characteristic Observation	Result
1.	Alkaloids	Mayer's test	Cream-coloured precipitate	+++
2.	Alkaloids	Dragendorff's test	Orange-red precipitate	+++
3.	Alkaloids	Wagner's test	Reddish-brown precipitate	+++
4.	Carbohydrates	Molisch's test	Violet ring at junction	+++
5.	Reducing sugars	Fehling's test	Brick-red precipitate	+
6.	Glycosides	Legal's test	Pink-red colour	+++
7.	Cardiac glycosides	Keller–Killiani test	Brown ring at interface	+++
8.	Flavonoids	Shinoda test	Pink-red coloration	+++
9.	Flavonoids	Alkaline reagent test	Intense yellow colour	+++

10.	Phenolic compounds	Ferric chloride test	Bluish-green colour	+++
11.	Tannins	Ferric chloride test	Blue-green coloration	+++
12.	Tannins	Lead acetate test	White precipitate	+++
13.	Saponins	Froth test	Persistent foam	+++
14.	Steroids	Liebermann–Burchard test	Green coloration	+++
15.	Terpenoids	Salkowski test	Reddish-brown interface	+++
16.	Proteins	Biuret test	Violet coloration	+++
17.	Amino acids	Ninhydrin test	Bluish-violet colour	+
18.	Phytosterols	Liebermann–Burchard rxn.	Greenish coloration	+

Acute Toxicity Study: The acute oral toxicity study of Tanacetum balsamita extract was carried out according to OECD Guideline 423. The extract was administered orally at a dose of 2000 mg/kg

body weight and the animals were observed for signs of toxicity and mortality during the observation period. No mortality was observed at the tested dose of 2000 mg/kg.

Table 3: Effect of Tanacetum balsamita Extract on Acute Toxicity Study of Experimental Mice

Parameter	100mg/kg	200mg/kg	400mg/kg
Number of animals (n)	6	6	6
Mortality	0/6	0/6	0/6
Tremors	Not observed	Not observed	Not observed
Convulsions	Not observed	Not observed	Not observed
Salivation	Not observed	Not observed	Not observed
Diarrhea	Not observed	Not observed	Not observed
Lethargy	Not observed	Not observed	Not observed
Locomotor activity	Not observed	Not observed	Not observed
Behavioral changes	Not observed	Not observed	Not observed
Body-weight change	Normal	Normal	Normal
Overall toxicity	Not observed	Not observed	Not observed

Effect on Body Weight: Body weight was recorded at baseline and at regular intervals throughout the experimental period to assess the general health status of the animals and the effect of cyclophosphamide-induced immunosuppression and Tanacetum balsamita extract treatment.

The changes in body weight were compared among the normal control, cyclophosphamide control, standard-treated, and extract-treated groups.

Table 4: Effect of Tanacetum balsamita Extract on Body Weight of Experimental Mice

Group	Treatment	Initial Body Weight (g)	Final Body Weight (g)	Change (g)
I	Normal control	25.18 ± 0.42	27.06 ± 0.46	+1.88
II	Cyclophosphamide control	25.31 ± 0.40	22.84 ± 0.43	-2.47
III	Cyclophosphamide + Standard	25.27 ± 0.38	26.21 ± 0.41	+0.94
IV	Cyclophosphamide + T. balsamita 100mg	25.22 ± 0.41	24.18 ± 0.40	-1.04
V	Cyclophosphamide + T. balsamita 200mg	25.34 ± 0.39	25.43 ± 0.42	+0.09
VI	Cyclophosphamide + T. balsamita 400mg	25.29 ± 0.43	26.02 ± 0.44	+0.73

Values are expressed as Mean ± SEM; n = 6 animals/group

Hematological Parameters

Effect on Total WBC Count: The total WBC count was determined at the end of the experimental period in all experimental groups.

The values obtained from the normal control, cyclophosphamide control, standard-treated, and Tanacetum balsamita extract-treated groups were compared to evaluate the effect of treatment on leukocyte status.

Table 5: Effect of Tanacetum balsamita Extract on Total WBC Count

Group	Treatment	Total WBC Count (×10 ³ cells/μL)
I	Normal control	9.82 ± 0.31
II	Cyclophosphamide control	4.36 ± 0.24
III	Cyclophosphamide + Standard	8.47 ± 0.28
IV	Cyclophosphamide + T. balsamita 100mg	5.92 ± 0.26
V	Cyclophosphamide + T. balsamita 200mg	7.18 ± 0.29
VI	Cyclophosphamide + T. balsamita 400mg	8.12 ± 0.27

Values are expressed as Mean ± SEM; n = 6 animals/group

Effect on RBC Count: The normal control group showed an RBC count of $9.21 \pm 0.24 \times 10^6$ cells/ μ L. Cyclophosphamide administration decreased the RBC count to $6.74 \pm 0.21 \times 10^6$ cells/ μ L, indicating an adverse effect on erythrocytic status. Treatment with the standard drug improved the RBC count to $8.63 \pm 0.23 \times 10^6$ cells/ μ L. T. balsamita extract

treatment produced a dose-dependent improvement, with RBC counts of 7.32 ± 0.22 , 8.14 ± 0.20 , and $8.51 \pm 0.22 \times 10^6$ cells/ μ L in the low-, medium-, and high-dose groups, respectively.

The high-dose group showed the greatest improvement among the extract-treated groups.

Table 6: Effect of Tanacetum balsamita Extract on RBC Count

Group	Treatment	RBC Count ($\times 10^6$ cells/ μ L)
I	Normal control	9.21 ± 0.24
II	Cyclophosphamide control	6.74 ± 0.21
III	Cyclophosphamide + Standard	8.63 ± 0.23
IV	Cyclophosphamide + T. balsamita 100mg	7.32 ± 0.22
V	Cyclophosphamide + T. balsamita 200mg	8.14 ± 0.20
VI	Cyclophosphamide + T. balsamita 400mg	8.51 ± 0.22

Values are expressed as Mean $\pm$ SEM; n = 6 animals/group

Effect on Hemoglobin: The hemoglobin concentration in the normal control group was 14.26 ± 0.31 g/dL, whereas the cyclophosphamide control group showed a reduction to 10.72 ± 0.28 g/dL. Treatment with the standard drug increased the hemoglobin level to 13.48 ± 0.29 g/dL. The T. balsamita extract-treated groups showed

progressive improvement in hemoglobin concentration.

The low-, medium-, and high-dose groups exhibited values of 11.62 ± 0.27 , 12.74 ± 0.26 , and 13.21 ± 0.30 g/dL, respectively. The high-dose extract produced the most pronounced improvement among the extract-treated groups.

Table 7: Effect of Tanacetum balsamita Extract on Hemoglobin Level

Group	Treatment	Hemoglobin (g/dL)
I	Normal control	14.26 ± 0.31
II	Cyclophosphamide control	10.72 ± 0.28
III	Cyclophosphamide + Standard	13.48 ± 0.29
IV	Cyclophosphamide + T. balsamita 100mg	11.62 ± 0.27
V	Cyclophosphamide + T. balsamita 200mg	12.74 ± 0.26
VI	Cyclophosphamide + T. balsamita 400mg	13.21 ± 0.30

Values are expressed as Mean $\pm$ SEM; n = 6 animals/group

Effect on Differential Leukocyte Count: The differential leukocyte count was determined at the end of the experimental period. The percentage distribution of different leukocyte populations was

compared among the experimental groups to assess changes associated with cyclophosphamide-induced immunosuppression and subsequent treatment with Tanacetum balsamita extract.

Table 8: Effect of Tanacetum balsamita Extract on Differential Leukocyte Count

Group	Treatment	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
I	Normal control	72.40 ± 1.21	23.10 ± 1.04	2.80 ± 0.31	1.40 ± 0.18	0.30 ± 0.08
II	Cyclophosphamide control	58.20 ± 1.35	34.80 ± 1.22	4.20 ± 0.35	2.30 ± 0.21	0.50 ± 0.10
III	Cyclophosphamide + Standard	70.10 ± 1.18	24.90 ± 1.02	3.00 ± 0.28	1.60 ± 0.19	0.40 ± 0.09
IV	Cyclophosphamide + T. balsamita 100mg	61.80 ± 1.26	31.20 ± 1.10	3.90 ± 0.32	2.60 ± 0.23	0.50 ± 0.11
V	Cyclophosphamide + T. balsamita 200mg	66.40 ± 1.20	27.40 ± 1.05	3.40 ± 0.30	2.30 ± 0.20	0.50 ± 0.09
VI	Cyclophosphamide + T. balsamita 400mg	69.10 ± 1.16	25.20 ± 0.98	3.10 ± 0.27	2.10 ± 0.18	0.50 ± 0.08

Values are expressed as Mean $\pm$ SEM; n = 6 animals/group

Lymphoid Organ Parameters

Effect on Spleen Weight: The spleen weight of animals from all experimental groups was recorded after completion of the treatment period. The

values were compared to determine alterations in lymphoid-organ mass following cyclophosphamide administration and treatment with Tanacetum balsamita extract.

Table 9: Effect of Tanacetum balsamita Extract on Spleen Weight

Group	Treatment	Spleen Weight (g)
I	Normal control	0.082 ± 0.004
II	Cyclophosphamide control	0.052 ± 0.003
III	Cyclophosphamide + Standard	0.076 ± 0.004
IV	Cyclophosphamide + T. balsamita 100mg	0.060 ± 0.003
V	Cyclophosphamide + T. balsamita 200mg	0.069 ± 0.003
VI	Cyclophosphamide + T. balsamita 400mg	0.074 ± 0.004

Values are expressed as Mean ± SEM; n = 6 animals/group

The normal control group showed a spleen weight of 0.082 ± 0.004 g. Cyclophosphamide administration produced a marked reduction in spleen weight to 0.052 ± 0.003 g, indicating lymphoid-organ suppression associated with the immunosuppressive model.

Treatment with the standard drug increased the spleen weight to 0.076 ± 0.004 g. Administration of T. balsamita extract produced a progressive improvement in spleen weight, with values of 0.060 ± 0.003 g, 0.069 ± 0.003 g, and 0.074 ± 0.004 g in the low-, medium-, and high-dose groups, respectively.

The spleen index showed a similar pattern, decreasing from 0.303% in the normal control group to 0.228% in the cyclophosphamide control

group. Treatment with T. balsamita progressively restored the spleen index, with the high-dose group showing the greatest recovery (0.284%). These findings suggest that T. balsamita extract may attenuate cyclophosphamide-induced reduction in spleen mass and support recovery of lymphoid-organ function.

Effect on Thymus Weight: Thymus weight was determined at the end of the experimental period as an important indicator of immune-system status. Cyclophosphamide-induced immunosuppression can cause lymphoid organ involution, including reduction in thymus weight. The effect of Tanacetum balsamita extract was therefore evaluated by comparing thymus weight among all experimental groups.

Table 10: Effect of Tanacetum balsamita Extract on Thymus Weight

Group	Treatment	Thymus Weight (g)
I	Normal control	0.061 ± 0.003
II	Cyclophosphamide control	0.034 ± 0.002
III	Cyclophosphamide + Standard	0.056 ± 0.003
IV	Cyclophosphamide + T. balsamita 100mg	0.041 ± 0.002
V	Cyclophosphamide + T. balsamita 200mg	0.049 ± 0.003
VI	Cyclophosphamide + T. balsamita 400mg	0.054 ± 0.003

Values are expressed as Mean ± SEM; n = 6 animals/group

The normal control group showed a thymus weight of 0.061 ± 0.003 g, whereas the cyclophosphamide control group showed a marked reduction to 0.034 ± 0.002 g. This reduction indicated thymic involution associated with cyclophosphamide-induced immunosuppression. Treatment with the standard drug restored thymus weight to 0.056 ± 0.003 g. Tanacetum balsamita extract treatment produced a dose-dependent improvement, with thymus weights of 0.041 ± 0.002 g, 0.049 ± 0.003 g, and 0.054 ± 0.003 g in the low-, medium-, and high-dose groups, respectively. The thymus index showed a similar pattern, increasing from 0.149% in the cyclophosphamide control group to 0.170%, 0.193%, and 0.208% in the low-, medium-, and

high-dose extract groups, respectively. The high-dose extract produced the greatest recovery among the extract-treated groups. Overall, these findings suggest that T. balsamita extract may attenuate cyclophosphamide-induced reduction in thymus mass and may support recovery of lymphoid-organ status.

Effect on Cytokines: The levels of selected serum cytokines, including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-10 (IL-10), were determined at the end of the experimental period. The cytokine profile was compared among the normal control, cyclophosphamide control, standard-treated, and Tanacetum balsamita extract-treated groups.

Table 11: Effect of Tanacetum balsamita Extract on Serum Cytokine Levels

Group	Treatment	TNF- α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
I	Normal control	42.60 $\pm$ 2.18	31.40 $\pm$ 1.72	18.20 $\pm$ 0.94
II	Cyclophosphamide control	24.30 $\pm$ 1.64	18.70 $\pm$ 1.35	10.60 $\pm$ 0.72
III	Cyclophosphamide + Standard	38.90 $\pm$ 2.01	28.60 $\pm$ 1.58	16.80 $\pm$ 0.86
IV	Cyclophosphamide + T. balsamita 100mg	28.70 $\pm$ 1.71	22.10 $\pm$ 1.42	12.40 $\pm$ 0.78
V	Cyclophosphamide + T. balsamita 200mg	34.50 $\pm$ 1.86	26.00 $\pm$ 1.51	14.90 $\pm$ 0.82
VI	Cyclophosphamide + T. balsamita 400mg	37.80 $\pm$ 1.94	28.10 $\pm$ 1.55	16.20 $\pm$ 0.85

Values are expressed as Mean $\pm$ SEM; n = 6 animals/group

Cyclophosphamide administration produced a marked reduction in the levels of TNF- α , IL-6, and IL-10 compared with the normal control group, indicating suppression of cytokine-mediated immune responses. Treatment with T. balsamita extract progressively restored the levels of these cytokines. The high-dose extract produced the greatest improvement among the extract-treated groups and showed values approaching those observed in the normal control and standard-treated groups. The findings suggest that T. balsamita may exert an immunomodulatory effect through regulation of cytokine production in cyclophosphamide-induced immunosuppressed mice.

Histopathological Study: Histopathological examination of the spleen and thymus was performed to assess the structural alterations produced by cyclophosphamide-induced immunosuppression and the protective effects of Tanacetum balsamita extract.

Histopathology of Spleen

Group I – Normal Control: The spleen showed normal and well-preserved architecture. The white pulp and red pulp were clearly distinguishable, with normally arranged lymphoid follicles and adequate lymphocytic cellularity. No evidence of cellular degeneration, necrosis, congestion, or inflammatory changes was observed. The normal architecture of the spleen confirmed the absence of pathological changes in the normal control animals.

Group II – Cyclophosphamide Control: The spleen showed marked pathological alterations characterized by depletion of lymphoid cells, reduction in the size and cellularity of white pulp, widening of red pulp areas, and distortion of normal splenic architecture. Increased cellular degeneration and congestion were also observed.

The pronounced lymphoid depletion and architectural distortion confirmed successful

induction of immunosuppression by cyclophosphamide.

Group III – Cyclophosphamide + Standard Drug: The spleen showed considerable restoration of normal architecture.

White and red pulp were relatively well organized, with improved lymphoid follicular cellularity and reduced degenerative changes. Only mild residual structural alterations were observed.

The standard treatment substantially protected the spleen against cyclophosphamide-induced pathological changes.

Group IV – Cyclophosphamide + T. balsamita 100mg: The spleen showed partial restoration of lymphoid architecture. Mild-to-moderate lymphoid depletion and some distortion of white pulp were observed. Compared with the cyclophosphamide control group, lymphoid cellularity was improved. The low dose of T. balsamita produced a partial protective effect on splenic tissue.

Group V – Cyclophosphamide + T. balsamita 200mg: The spleen demonstrated improved white-pulp organization and increased lymphoid cellularity. Red and white pulp were better differentiated, with reduced cellular degeneration and comparatively preserved splenic architecture. The medium dose produced greater restoration of splenic architecture than the low dose, indicating an improved immunoprotective response.

Group VI – Cyclophosphamide + T. balsamita 400mg: The spleen showed almost normal architecture with well-preserved white and red pulp. Lymphoid follicles appeared clearly defined with improved cellularity. Minimal degenerative changes were observed. The high dose of T. balsamita demonstrated the greatest protective effect and produced substantial restoration of cyclophosphamide-induced splenic damage.

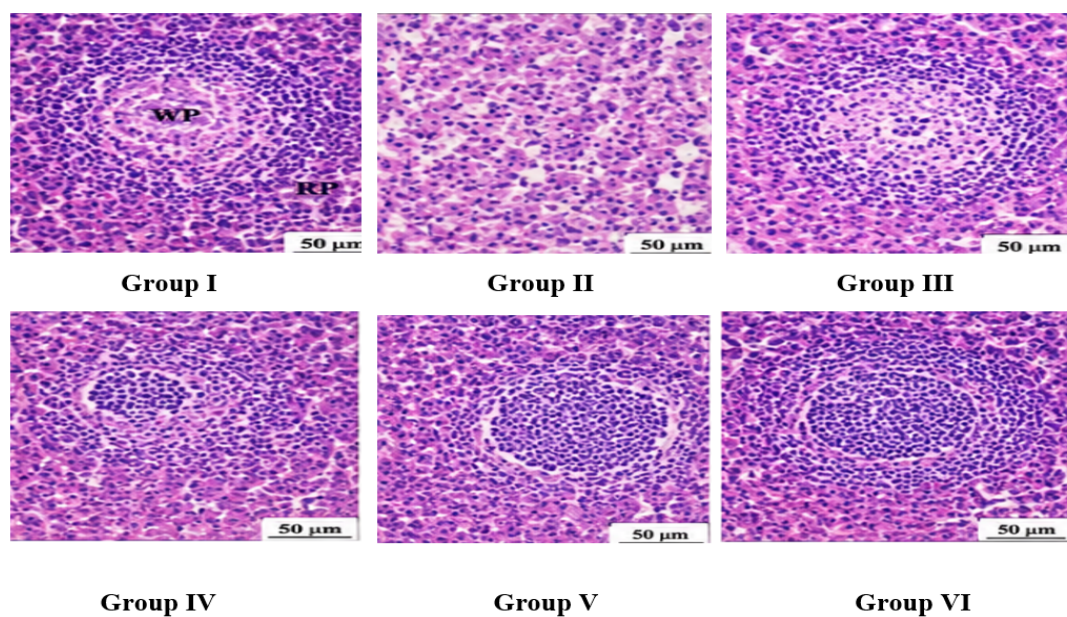


Figure 3: Microscopic Image of Spleen (Different Groups)

Histopathology of Thymus

Group I – Normal Control: The thymus showed normal architecture with a well-defined cortex and medulla. The cortical region contained densely packed thymocytes, while the medullary region showed relatively fewer cells. No pathological changes were observed. The findings indicated normal thymic morphology and preserved lymphoid tissue.

Group II – Cyclophosphamide Control: Marked thymic alterations were observed, including cortical lymphocyte depletion, reduced thymocyte density, thymic atrophy, widening of intercellular spaces, and poor definition of the corticomedullary junction. The severe reduction in thymocyte population and thymic atrophy demonstrated significant cyclophosphamide-induced immunosuppressive injury.

Group III – Cyclophosphamide + Standard Drug: The thymus showed substantial restoration of cortical and medullary organization. Thymocyte density was considerably improved, and the corticomedullary junction was more clearly defined compared with the cyclophosphamide control group. The standard drug effectively reduced cyclophosphamide-induced thymic damage and promoted restoration of thymic architecture.

Group IV – Cyclophosphamide + *T. balsamita* 100mg: The thymus exhibited partial restoration of cellularity. Mild-to-moderate reduction in cortical thymocytes was still evident, although the overall architecture was improved compared with the cyclophosphamide control group. The low dose of *T. balsamita* produced a moderate protective effect against thymic damage.

Group V – Cyclophosphamide + *T. balsamita* 200mg: The thymic architecture was considerably improved, with increased cortical cellularity and better organization of the corticomedullary regions.

Degenerative changes were reduced compared with the low-dose group. The medium dose produced a stronger protective effect than the low dose and promoted substantial restoration of thymic tissue.

Group VI – Cyclophosphamide + *T. balsamita* 400mg: The thymus showed well-preserved architecture with prominent cortical cellularity and a clearly distinguishable medulla.

The corticomedullary junction was substantially restored, and only minimal pathological alterations were observed.

The high dose of *T. balsamita* produced the greatest restoration of thymic architecture and showed a protective effect comparable to the standard-treated group.

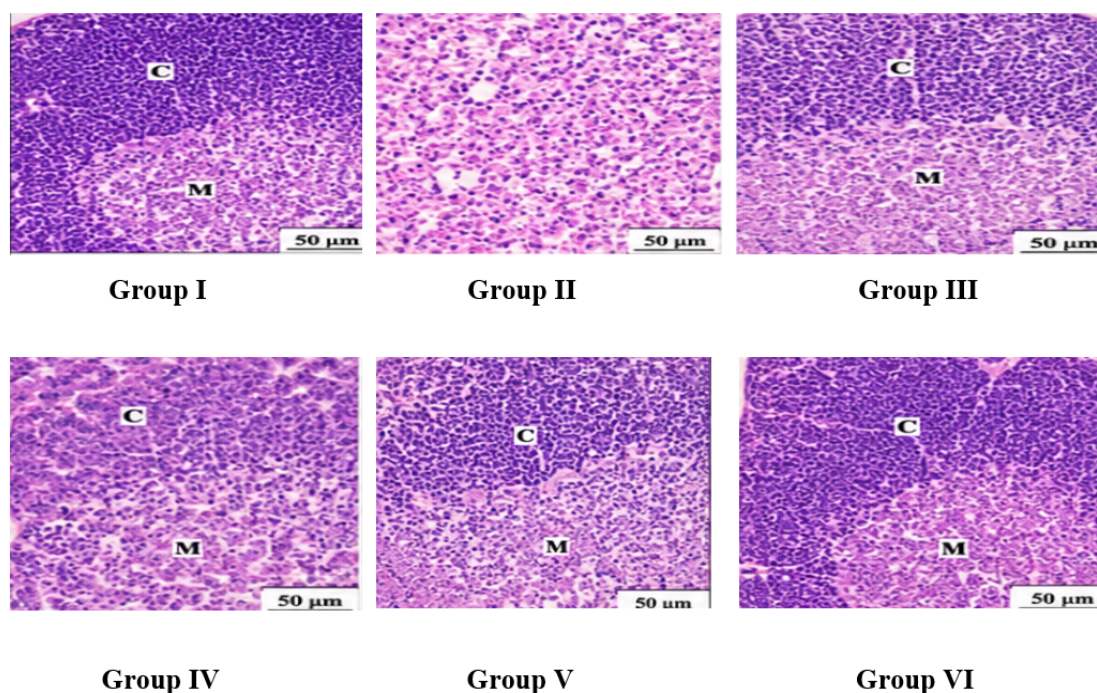


Figure 4: Microscopic Image of Thymus (Different Groups)

The protective effect was more evident with increasing dose, with the high-dose group showing the greatest restoration of spleen and thymus architecture. These morphological findings support the hematological, immunoglobulin, cytokine, lymphoid-organ weight, and antioxidant results and suggest that *T. balsamita* possesses a potential immunorestorative and tissue-protective effect against cyclophosphamide-induced immunosuppression.

Discussion

The present study demonstrated that *Tanacetum balsamita* extract significantly attenuated cyclophosphamide-induced immunosuppression in mice. Treatment with 100, 200 and 400 mg/kg improved WBC count, lymphocyte levels, spleen and thymus weights, and serum IgG and IgM levels in a dose-dependent manner.

The extract also enhanced SOD, CAT and GSH while reducing MDA, indicating improved antioxidant defense. Histopathological findings further confirmed dose-dependent restoration of spleen and thymus architecture, particularly at 400 mg/kg. These effects may be attributed to the phenolic and flavonoid constituents of the extract, supporting its potential immunomodulatory and antioxidant activity.

Conclusion

T. balsamita extract demonstrated significant, dose-dependent immunomodulatory and antioxidant effects against cyclophosphamide-induced immunosuppression in mice.

The 400 mg/kg dose showed the most pronounced improvement in hematological, immunological, antioxidant, and histopathological parameters.

Thus, *T. balsamita* may serve as a promising natural source for the development of immunoprotective agents.

Reference

1. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 10th ed. Philadelphia: Elsevier; 2021.
2. Murphy K, Weaver C. Janeway's Immunobiology. 10th ed. New York: Garland Science; 2022.
3. Kumar V, Abbas AK, Aster JC. Robbins & Cotran Pathologic Basis of Disease. 10th ed. Philadelphia: Elsevier; 2020.
4. Royal Botanic Gardens, Kew. Plants of the World Online: *Tanacetum balsamita* L. Kew: Royal Botanic Gardens, Kew.
5. Judžentienė A, Mockutė D. The essential oil composition of *Tanacetum balsamita* L. Flavour and Fragrance Journal. 2005;20:35–39.
6. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998.
7. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biology. 2020;18(7).
8. National Research Council. Biology and Diseases of Mice. National Academies/NCBI Bookshelf. Normative data for the mouse.

9. OECD. Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2002. doi:10.1787/9789264071001-en.
10. OECD. Guidance Document on Acute Oral Toxicity Testing. OECD Series on Testing and Assessment No. 24. Paris: OECD Publishing; 2002. doi:10.1787/9789264078413-en.
11. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang & Dale's Pharmacology. 9th ed. Elsevier; 2019.
12. Katzung BG, Vanderah TW. Basic & Clinical Pharmacology. 15th ed. New York: McGraw-Hill; 2021.
13. Brunton LL, Hilal-Dandan R, Knollmann BC, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 14th ed. New York: McGraw-Hill; 2023.
14. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998.