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ASSESSMENT OF IMMUNOMODULATORY ACTIVITY IN SWISS ALBINO RATS UTILIZING A POLY-HERBAL FORMULATION: A COMPREHENSIVE STUDY ON IMMUNOLOGICAL RESPONSE MODULATION

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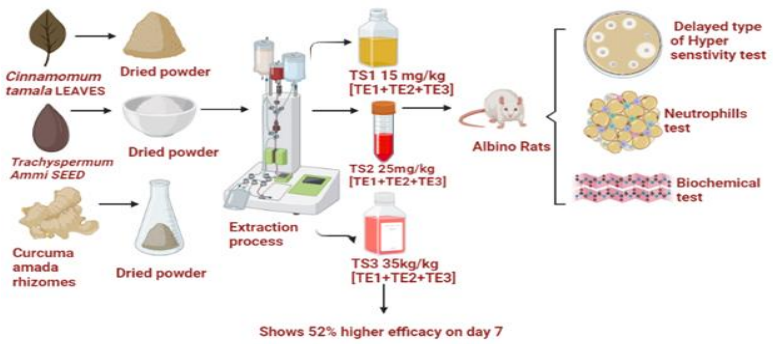
Abstract

Aim: Poly-herbal formulation to evaluate immunomodulatory activity on Swiss albino rats. **Material and method:** Cinnamomum Tamala, Trachyspermum ammi, Curcuma amada. We are estimating the delayed type of hypersensitivity (footpad swelling), neutrophils adhesion test, and biochemical parameters. Experimental rats are divided into three negative and one positive control group. Negative control groups are given the test formulations TS1, TS2, and TS3, and the positive control group is given S1, a standard drug. The formulation parameters, like organoleptic property, pH, and solubility and in-vivo parameters, delayed-type hypersensitivity, neutrophil adhesion test, the biochemical study primarily evaluates the test formulations. **Result:** The group treated with TS3 solution showed a better immunomodulatory effect as compared to a control group receiving TS1, TS2, and Levamisole. All formulations show immunomodulatory effects in Delayed-type hypersensitivity (DTH) response, Neutrophil adhesion test, and biochemical study. TS3 showed 80% immunomodulatory effect on day-7, TS2 showed 52%, then an immunomodulatory effect, and TS1 showed 49% immunomodulatory effect on day -7. It has a higher degree of treating delayed types of hypersensitivity reactions and Neutrophil adhesion value.

Keywords: Immunity, Cinnamomum Tamala, Trachyspermum Ammi, Curcuma Amada, Delayed Type of Hypersensitivity, Neutrophil Adhesion Study, Biochemical Study.

Graphical Abstract:



1. INTRODUCTION

The immune system is the body's primary defense against germs and other pathogens. The immune system performs various bodily tasks and has several defenses against outside invaders. Numerous immunomodulatory elements are available in the environment, yet each has unique effects and purposes. Without the use of artificial medications, the immune system defends against certain hazardous chemicals. Self-treatment for noxious chemical acute conditions using immunological responses. [1] [2]

Currently, immunomodulatory therapy is most often utilized since it decreases illness and increases bodily activity. Immunomodulatory chemicals are described as being both naturally occurring and safe for ingestion. [3] They are capable of fighting and reacting in resistance. The immune system can fight against dangerous organisms. [4] [5] The resistance effect is a safe gauge, preventing the pathogen from directly infecting you. In an infection state, inflammation is produced as a defense mechanism at the injection site. Cytokines are released when bacteria and other microorganisms enter the body too much, aiding in the defense of a particular organ. A cytokine is a crucial component of the immune system in humans. [6] [7]

1.1 Immunity

The resilient structure has traditionally been divided into integral and adaptable components. The flexible portion is organized around two distinct kinds of cells: T and B cells. Since each cell displays a single type of fundamentally unique receptor, the population of lymphocytes has a vast and diverse array of antigen receptors. [8]

1.1.1 Types of immunity

The main two types of immunity

(i) Innate Immunity or Natural or Non-specific Immunity.

(ii) Acquired Immunity or Adaptive Immunity. [9]

1.2 Innate Immunity:

Innate immunity may be thought of as consisting of four different kinds of protective barriers: endocytic and phagocytic, inflammatory, physiologic (temperature, low pH, and chemical mediators), and anatomic (skin and mucous membrane). [10] Numerous studies have been conducted on the cells and mechanisms necessary for successful innate defense against viruses that bypass anatomical barriers. Innate immunity to infections depends on pattern recognition receptors (PRRs), enabling a subset of immune cells to quickly identify and react to pathogens with similar molecular structures. (PAMPs). Lipopolysaccharides (LPS), a component of bacterial cell walls, and double-stranded RNA (RNA) generated during viral infection are two examples of these. [11] [12]

1.3 Adaptive immunity:

The adaptable immune system, also known as the acquired safe system, is a component of the safe system made up of individual, essential cells and cycles that eliminates pathogens or stops their growth. [13] One of vertebrates' two primary resistance mechanisms is the acquired invulnerable framework. (The other being the inborn invulnerable framework). [14] The adaptable defensive framework includes humor- and resistance-resistant components that enter cells and eliminate invading

germs as part of its internal structure. [15] When a person becomes ill, they develop what is known as active immunity, a normal invulnerability. Take, for instance, a person who is diagnosed with chicken pox as an example. The body will build immunity to the illness when exposed to it for the first time. Those who have had chicken pox are protected against the disease for a very long time after that due to the characteristic dynamic susceptibility that comes with the disease. [16]

1.4 Immune cells

A cell of the immune system that supports the body's defaces against disease and infections. When immune cells develop, several types of white blood cells are produced from stem cells in the bone marrow. [17] They include natural killer (B and T cells) lymphocytes, mast cells, eosinophils, basophils, neutrophils, macrophages, and neutrophils. [18]

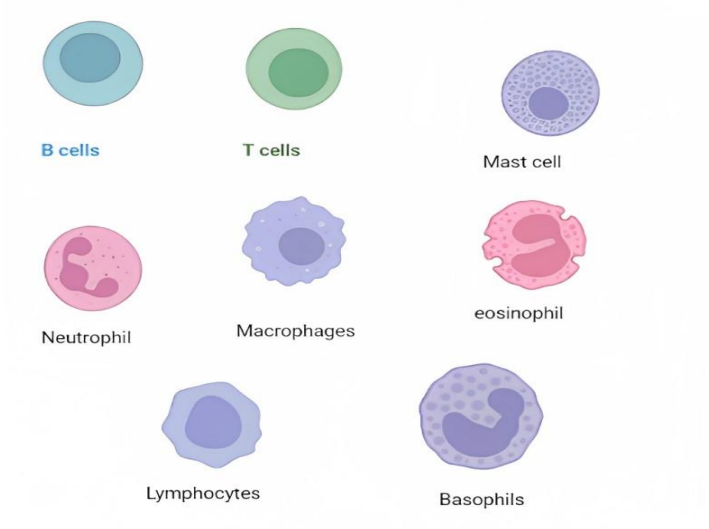


Fig.no 1.1: Showing Different types of Immune Cells

2. MATERIALS AND METHODS

Three distinct herbal extracts are utilized in this experimental immunomodulatory technique at various doses to alter the immune response of Swiss albino rats to Ovalbumin. Three herbal extracts have been utilized:

a) Cinnamomum tamala (bay leaf)

b) *Trachyspermum ammi* (carom seed),

c) *Curcuma amada* (mango ginger).

2.1 *Cinnamomum tamala*

- The tiny evergreen tree Cinnamomum tamala-Nees and Eberm. It is a member of the *Lauraceae* family. Indian cassia is a popular name for this spice.
- The tree's maximum height is 7.5 m, and its diameter can reach 1.4 m.
- The Himalayas area (3000–7000 feet) and the Sylhet and Khasia highlands in northeastern India. [19]
- The primary geographic origins, though they can be found all over India (3000–4000 ft). [20]

- The tree's bark is a rugged dark brown or blackish color shown in fig.no:2. [21]
- A 1.3-centimeter blaze can be rosy or reddish brown with white lines on the surface. [20]

Fig.no 2.1: Showing dried *Cinnamomum tamala*

2.2 *Trachyspermum ammi*

- Trachyspermum ammi (L.) Sprague, more commonly known by its Arabic name, Ajwain, is a perennial grass plant in the *Apiaceae* family. The plant is said to be cultivated extensively in dry and semiarid areas with a high sodium concentration in the earth.
- Ajwain's height ranges from 30 to 90 centimeters, and its upright, striate stalk is either hairless or finely pubescent.
- They have a sharp, bitter flavor and a pale green to brown color, Showing in Fig.no:2.1. [22]
- Egyptian origin despite Ajwain's widespread cultivation and distribution in countries like Iran, Pakistan, Afghanistan, India, and Europe.[23]–[25]

Fig.no:2.2: Showing dried *Trachyspermum ammi*

2.3 Curcuma amada

- Mango ginger, also known as Curcuma amada, is a member of the *Zingiberaceae* family and the genus Curcuma. It is often utilized in the food sector and complementary therapies. [26]
- **Geographic source:** The Curcuma genus has 60 to 100 species, which are distributed around the globe in a variety of locations, including southern China, the Indian Subcontinent, New Guinea, Florida, tropical Africa, Southeast Asia, and northern Australia. [27]

Fig.no 2.3: Showing rhizomes and dried powder of *Curcuma amada*

2.2 Collection and identification of herbal plants

All test drugs were collected from the Herbal Garden of IIMT University, Meerut, and were then identified by Dr. Vijay Malik, professor at DEPARTMENT of BOTANY, Chaudhary Charan Singh University, Meerut.

2.3 Extraction of test drug plants

2.3.1 Extraction of *Cinnamomum tamala* (TE1): The extraction process follows *Cinnamomum tamala* leaves by [28] [29]

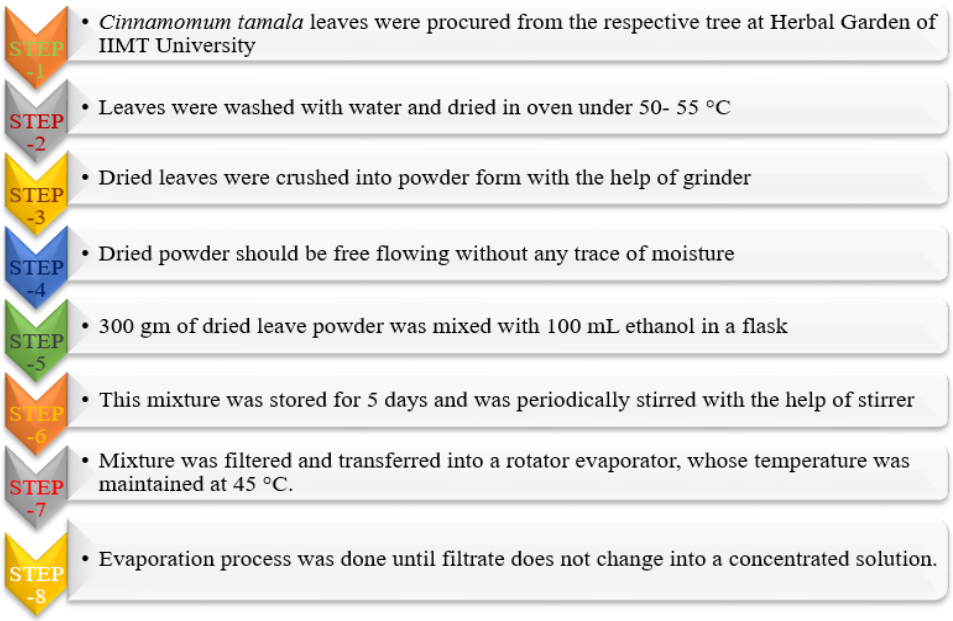


Fig.no 2.4: Extraction procedure of *Cinnamomum tamala* (TE1)

2.3.2 Extraction procedure of *Trachyspermum Ammi* (TE2):

The extraction process of *Trachyspermum ammi* seeds is shown in Fig: 6 [30] [31]

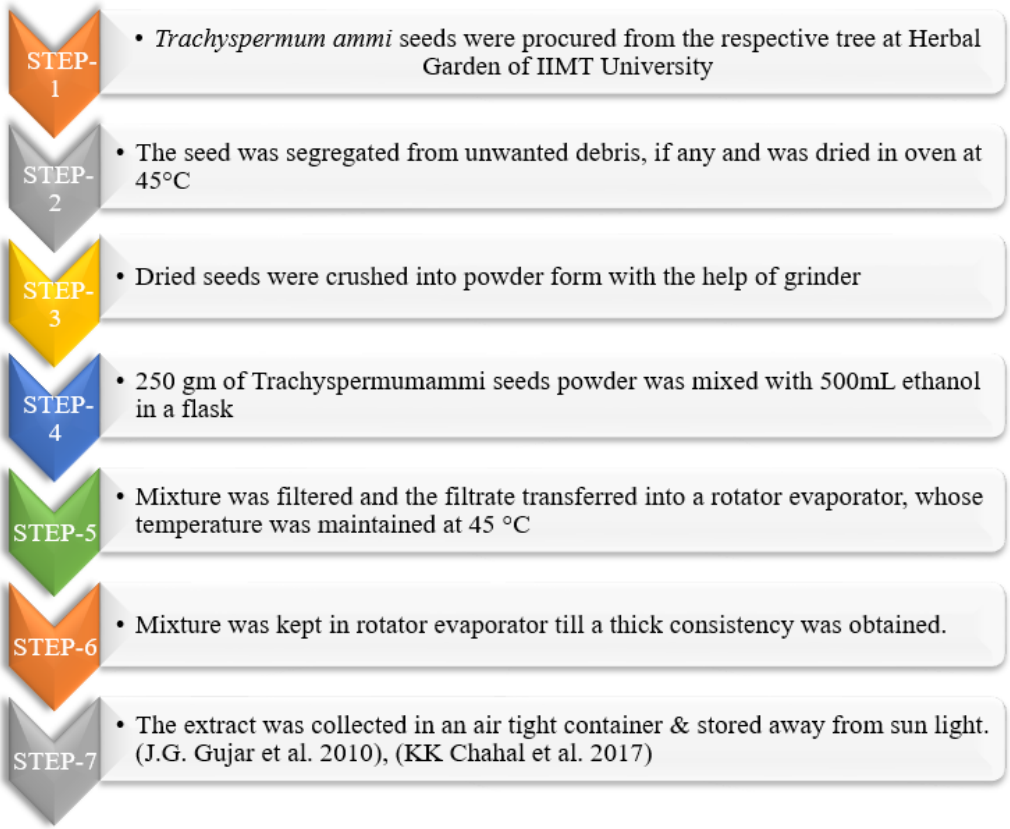


Fig.no 2.5: The extraction process of *Trachyspermumammi* seeds

2.3.3 Extraction procedure of Curcuma amada (TE3): Extraction process of Curcuma amada follow by [32] [33]

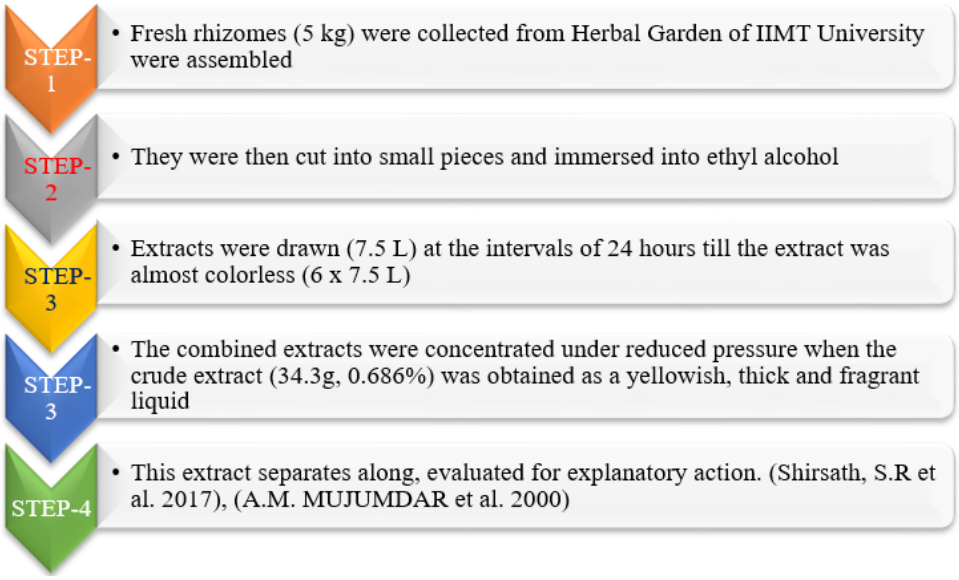


Fig.no 2.6: Extraction procedure of Curcuma amada (TE3)

2.4 Characterization of test herbal solution

2.4.1 Organoleptic characterization:

Organoleptic evaluation. To evaluate changes in appearance, color, fragrance, and spread ability over time, the samples' organoleptic characteristics were tested under the same temperature, lighting, and packing circumstances. [34] [35]

2.4.2 Analytical evaluation of herbal test solution

2.4.2.1 pH: A mechanical pH meter was used to evaluate the pH of a prepared homemade salve. The balm mixture was prepared using 100ml of distilled water and then allowed to set for two hours. The primary area for pH measurement is that the herbal extract solution does not irritate the test medication. The physiological pH range is between 7 and 7.5. That implies the herbal extract solution's pH valve should be between 7.2 and 7.5.[36] [37]

2.4.2.2 Experimental animals

In this experiment, Swiss albino rats were employed as the test animals. The CPCSEA has authorized the authenticity of experimental Swiss albino rats. Animals used in experiments lived in cages constructed of plastic. The cages were organized serially and maintained clean. Each cage contained a decent water bottle and bedding made of maize. All experimental cages were kept in the dark for 12 hours before being exposed to light for a further 12 hours. The noise level surrounding the cages was kept lower. The food was always available in the cages, and the best conditions for the tests were chosen based on trial tests that were approved using six rats in each group. The experimental rat's weight should fall between 150 and 35 mg. There are six rats in each group. All animals should follow the CPSCEA and the 1960 Prevention of Cruelty to Animals Act. [38] [39]

2.5 IN VIVO PHARMACODYNAMICS STUDIES

The delayed kind of hypersensitivity will be used for the pharmaceutical inquiry. Six groups of six rats each were created from the rats. A marker is used to indicate each group's whole population of rats. Grouping occurs before starting the research as the initial phase. The following six groups, each with six rats, were formed:

Group-1, also called the untreated or control group

Group 2, also called the negative control group, in which the paw edema was induced by antigen

Group 3, was administered herbal extract solution TS1

Group 4, treated with herbal extract solution TS2

Group 5, treated with herbal extract solution TS3

Group 6, or the standard group, was treated with the standard drug, levamisole

For 14 days, the formulation was taken orally. Levamisole is the standard medication, and the dosage is 25 mg/kg; this standard group is (Group-6). Group 3 is then given TS1 (Test Solution-1) at a 15 mg/kg dosage. The next test solution, TS2, is given to Group 4 at a dosage of 25 mg/kg, and the last test solution, TS3, is given to Group 5 at a dose of 35 mg/kg. These estimates are for test solution dosage for immunomodulatory action. The negative group in this group is (Group 2), which

received subcutaneous injections of 0.1 ml ovalbumin to make the paws of Swiss albino rats swell. Ovalbumin brings on Swiss albino rats' edema. [5] , [40]

Table.no 2.1: Description and dosage of an In-vivo study

Group's	Description	Dose	Animal per group
Group 1	Normal Saline	NS (oral)	6
Group 2	Antigen ovalbumin(1% solution of ovalbumin in DW)	NS+0.1 ml antigen (I.P.)	6
Group 3	Extract (TS1) *(Cinnamomumtamala, Trachyspermumammi,Curcuma amada)*	15mg/kg (oral)	6
Group 4	Extract *(Cinnamomum tamala, Trachyspermumammi,Curcuma amada)*	25mg/kg (oral)	6
Group 5	Extract *(Cinnamomum tamala, Trachyspermumammi,Curcuma amada)*	35mg/kg (oral)	6
Group 6	Standard (Levamisole)	25mg/kg (oral)	6

Table: 2.2 shows the experimental group with a dose

S.no.	Formulation	Dose
1	**Extract (TS1)***(Cinnamomum tamala*TE1*, Trachyspermumammi*TE2*,Curcuma amada* TE3*)*	**15 mg/kg**
2	**Extract (TS2)***(Cinnamomum tamala*TE1*, Trachyspermumammi*TE2*,Curcuma amada* TE3*)*	**25mg/kg**
3	**Extract (TS3)***(Cinnamomum tamala*TE1*, Trachyspermumammi*TE2*,Curcuma amada* TE3*)*	**35kg/kg**

2.5.1 Delayed-type Hypersensitivity (DTH) response (Paw edema)

Delayed-type hypersensitivity (DTH) may activate T-cell-mediated immune responses in living things. Hypersensitivity, induration, and erythema—all indicators of a positive reaction to an antigen—appear 48 to 72 hours after injection. Research using experimental animal models led to the discovery of the granulomatous response's macrophage, monocytic cell, and T lymphocyte components. Additionally, contact sensitivity to damage, nickel, dinitrochlorobenzene (DNCB), and picryl chloride has been linked to DTH-like reactions. All three of these drugs—sulfonamides, phenytoin, and carbamazepine—display delayed non-IgE (non-IgE)-induced drug affectability. Antigen-presenting cells (APCs) introduce specific antigens into the skin, resulting in a DTH response. (i.e., Langerhans dendrite cells). Convergence of monocytes, lymphocytes, and macrophages happens in response to the antigens. Mononuclear cells and CD4+ T cells are the two main cell types that makeup DTH. Are these cells' subsequent production of IFN and tumor necrosis factor-alpha (TNF-a) responsible for their existence? Serotonin and histamine supply rise early in the reaction, increasing Vaso permeability, and cement factors are up-directed to stimulate additional cell components to move towards the area of antigen presentation adjacent to them. Delayed-type hypersensitivity (DTH) may promote T-cell-mediated immune responses in living things. Hypersensitivity, induration, and erythema—all indicators of a positive reaction to an antigen—appear 48 to 72 hours after injection. Research using experimental animal models led to the discovery of the granulomatous response's macrophage, monocytic cell, and T lymphocyte components.[41] Additionally, contact

sensitivity to damage, nickel, dinitrochlorobenzene (DNCB), and picryl chloride has been linked to DTH-like reactions. Drugs with delayed non-IgE-induced drug affectability include sulfonamides, phenytoin, and carbamazepine. Antigen-introducing cells (APCs) introduce specific antigens into the skin, which results in a DTH reaction. (i.e., Langerhans dendrite cells). Monocytes, lymphocytes, and macrophages congregate in the area in response to the antigen. Mononuclear cells and CD4+ T lymphocytes are the main cell types in DTH. In the subsequent stages, do these cells create interferon and tumor necrosis factor-alpha (TNF-a)? Serotonin and histamine production rises early in the reaction, which enhances vaso-permeability. Cement factors are up-directed, causing extra cell components to flow toward the surrounding antigen presentation site. [42],[43], [44]

Include n=6 distinct species for each set of six animal classifications. They sought to confirm that all of the animals in each group had access to food and water in line with the standards of the CPCSEA before they began their assessment. Group 1, the control group, had delayed-type hypersensitivity since they were not exposed to any antigen. (DTH). In the absence of this, each group of animals received a subcutaneous injection of 0.1 milliliters of Ovalbumin, which caused Swiss albino rats' right hind paws to swell. All animal groups that were administered TS1, TS2, and TS3 were given medication orally twice a day after receiving an injection of 0.1 milliliters of Ovalbumin.[41] The response from Ovalbumin will come after a day. The quantity of paw edema after treatment up to day 14 in this experiment shows that TS1, TS2, and TS3 successfully elicited a response. [45] [46]

2.5.2 Neutrophil adhesion test

This experimental investigation lasts 14 days; however, blood samples are taken before and after the animal study for the neutrophil adhesion study. The blood is drawn on days 0 and 14—the retro-orbital plexus accumulation of ocular blood. Blood is drawn via glass capillaries and put into vials that have been heparinized. [47] The vials holding the blood samples need to be correctly labeled and kept at the right temperature. The neutrophil adhesion test, which fixes blood clots and uses I- and II-Leishman's stain for staining, aids in the determination of total leukocyte counts (TLC). After that, the blood was gestated with 70 mg of nylon fiber per milliliter for 12 to 15 minutes at 370°C. This incubation aids in the determination of TLC. The neutrophil index is calculated using the neutrophil percentage algorithm. [48] [49].

$$\text{Neutrophil Adhesion (\%)} = \frac{NI_U - NI_T}{NI_U} X100$$

Where,

NIu= untreated blood sample of a neutrophil index,

Nit= Treated blood sample of neutrophil index

2.5.3 Biochemical study:

All rats were randomly divided into groups of 1-6, with (n=6) in each group. All groups are isolated as directed. TE1, TE2, and TE3 test extracts and the reference medication were administered twice daily for 14 days. Every day, the animal was discovered. Blood was drawn independently on days 0 and 14 for pre-hematological analysis and post-hematological inquiry. Blood was taken from the test creature's eyes, such as the orbital sinus. [41] A thin glass was used to collect the blood. The collected blood was stored in tubes made of EDTA (K2) and (Gel + BCA).[49],[50]

2.5.4 Statistical analysis:

The standard mean (SEM) represented the experimental data (n = 6). Graph Pad Prism version 8.4.3 was used to analyze experimental data's statics and perform one-mode variance analysis (ANOVA) tracked t-tests. It is statistically quantified by the P < 0.05

3. RESULT

3.1 Pre formulation studies

3.1.1 Organoleptic evaluation:

Table 3.1: showing the color and odor of TE1, TE2, and TE3.

S.NO.	TEST DRUG SOLUTION	ODOUR	COLOR
1	**Extract (TS1)** *(Cinnamomum tamala, Trachyspermumammi,Curcuma amada)*	Aromatic	Greenish in color
2	**Extract (TS2)** *(Cinnamomum tamala, Trachyspermumammi,Curcuma amada*	Aromatic	Greenish in color
3	**Extract (TS3)** *(Cinnamomum tamala, Trachyspermumammi,Curcuma amada*	Aromatic	Greenish in color

3.1.2 P^H EVALUATION:

Table 3.2: Showing p^H of test extract solution (TSI, TS2, TS3)

S.NO	TEST DRUG SOLUTION	P^H
1	TS1 (TE1+TE2+TE3)	7.2
2	TS2(TE1+TE2+TE3)	7.5
3	TS3(TE1+TE2+TE3)	6.7

The evaluation of the herbal extract test solution showed favorable pH for oral administration for the rats. Because of the oral cavity, the acceptable pH is (5.8-7.4pH value). According to this study's evaluation parameter, the experimental research is satisfactory. It does not irritate the animal's mouth and intestine.

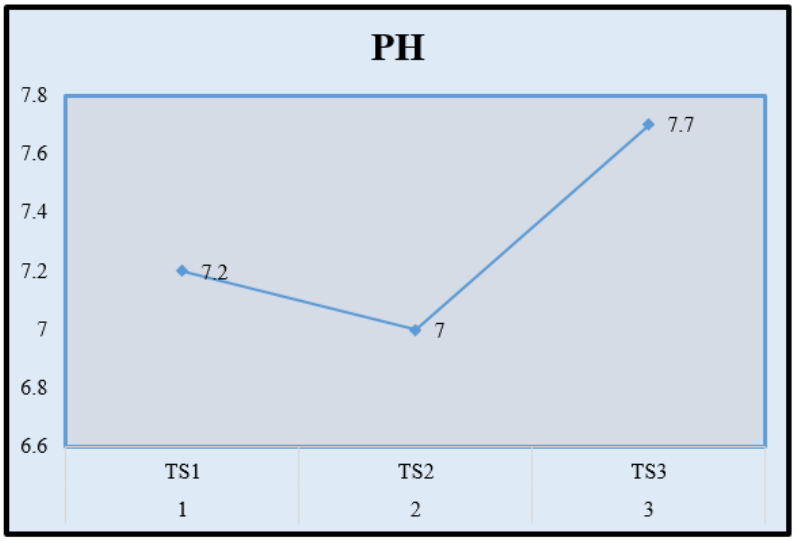


Fig 3.1: Showing pH of all test solution

3.2 Analytıcal Evaluatıon Of Herbal Extract Solutıon

3.2.1 Weight variation

Table 3.3 : showing weight variation of the experimental animal during the study

GROUP	DAY 0	DAY 3	DAY 7	DA7 14
GROUP 1(normal control)	152	158	172	184
GROUP 2(negative control)	154	149	145	143
GROUP-3(TS1)	151	157	163	174
GROUP-4(TS2)	154	175	187	192
GROUP-5(TS3)	152	168	179	198
GROUP-6(Standard control)	154	158	160	178

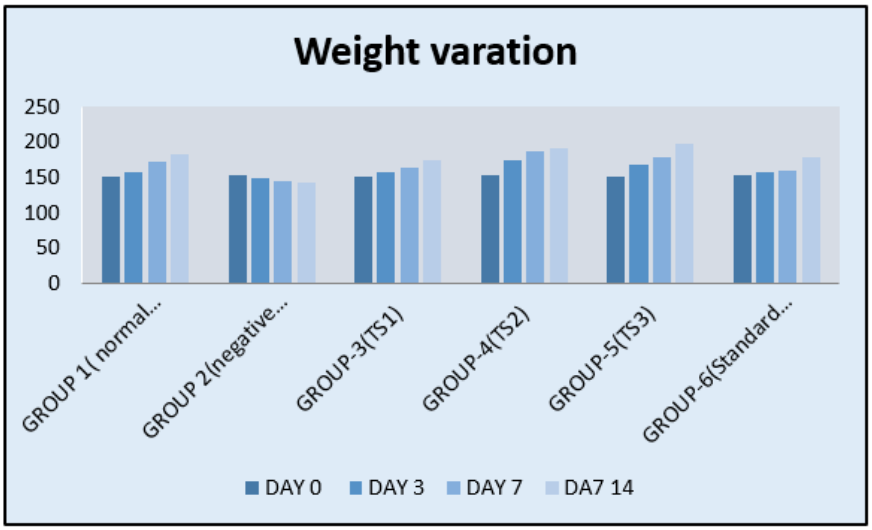


Fig 3.2: showing weight variation of different group of animal

In this weight variation study showing result, the experimental animal's weight increased in all animal groups. Still, group 2 (negative control group) showed decreasing weight during the experimental study. According to this study, the weight variation of the group is in ascending order. Group 5 (treated with TS3) shows a higher weight variation rate, and this group condition is the healthiest. In group-4 (treated with TS2), weight increases in all animals. In group 6 (treated with standard drug-Levamisole).

3.2.2 Safety and efficacy test

Table 3.4: showing the efficacy of test and standard drug in (%)

GROUP	Day-3	Day-7	Day-14
GROUP 2(negative control)	10%	25%	50%
GROUP-3(TS1)	20%	49%	75%
GROUP-4(TS2)	25%	52%	80%
GROUP-5(TS3)	50%	80%	100%
GROUP-6(Standard control)	38%	50%	85%

3.2.3 Neutrophil adhesion test:

Table 3.5: showing % of neutrophil adhesion

GROUP	DAY 0	DAY 3	DAY-7	DA7 14
GROUP 1(normal control)	10.21	11.11	11.14	12.31
GROUP 2(negative control)	5.61	5.55	6.56	6.68
GROUP 3(TS1)	16.23	17.56	18.67	20.12
GROUP 4(TS2)	20.43	20.46	20.58	21.45
GROUP 5(TS3)	22.21	22.58	23.23	23.67
GROUP 6(Standard control)	16.32	16.78	17.31	17.48

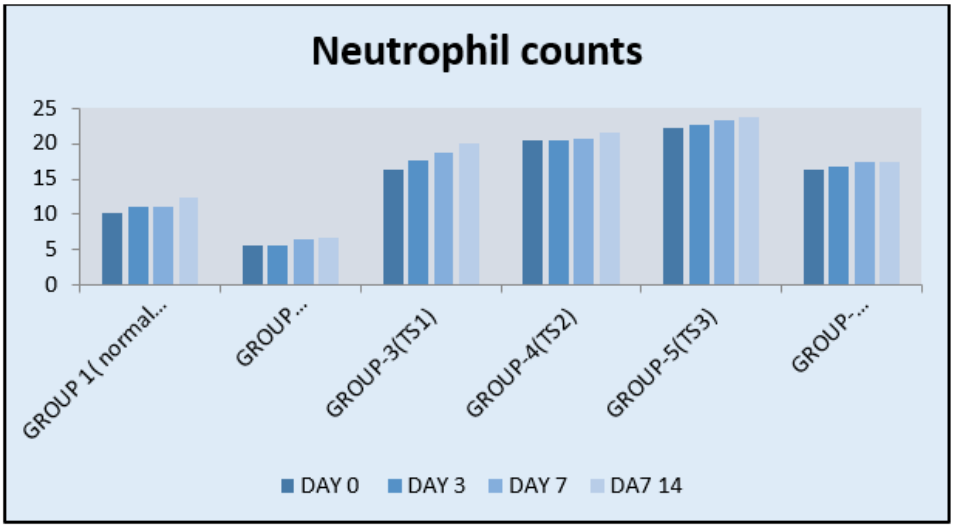


Fig 3.3: showing % of neutrophil counts during the study

In the neutrophil adhesion test, group-5 shows an increased % of neutrophil adhesion. That means the combination of extract solution on 35mg/kg shows a higher % ($p < 0.05$) of neutrophil adhesion. Show. According to the study, the ascending order of neutrophil adhesion, group-2 < group-1 < group-6 < group-3 < group-4 < group-5.

3.2.4 Delayed-type hypersensitivity (DTH) response (Paw edema size in albino wister rat)

Table 3.6: showing the size of edema in different groups of animals

GROUP	DAY-0	DAY-3	DAY-7	DAY-14
GROUP 1(normal control)	0.31	0.31	0.32	0.31
GROUP 2(negative control)	0.52	0.56	0.64	0.54
GROUP-3(TS1)	0.49	0.45	0.41	0.39
GROUP-4(TS2)	0.52	0.46	0.38	0.34
GROUP-5(TS3)	0.53	0.42	0.32	0.31
GROUP-6(Standard control)	0.52	0.45	0.39	0.31

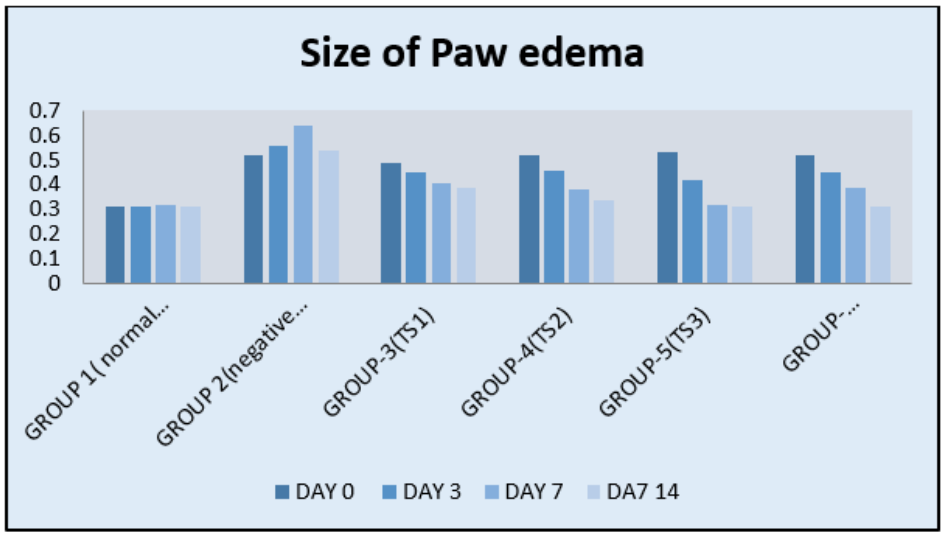


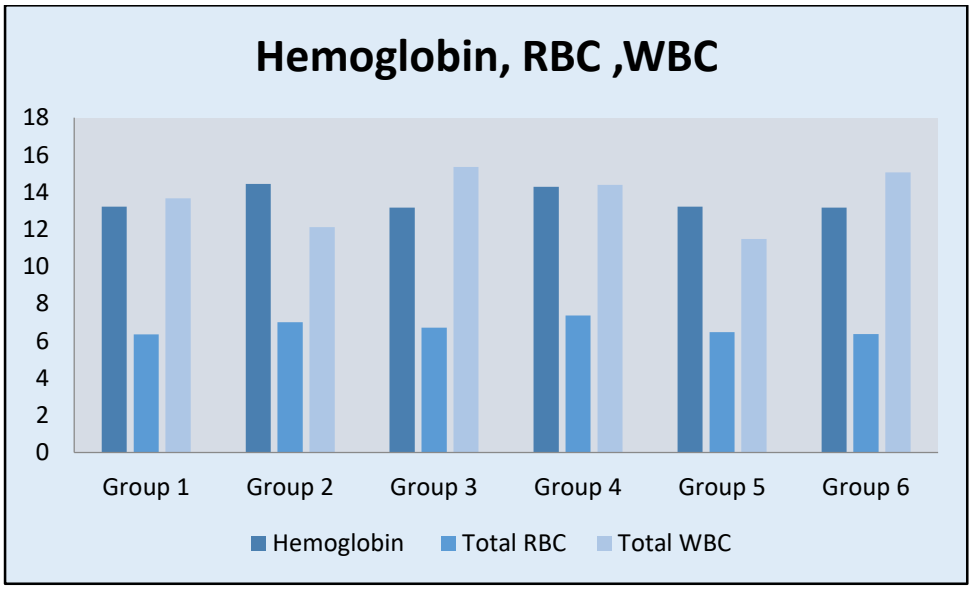
Fig 3.4: showing size of paw edema in different groups of animal

3.2.5 Biochemical study:

3.2.5.1 Pre-Biochemical study:

Table. no.3.7: showing pre-biochemical evaluation

	Hemoglobin	Total RBC	Total WBC	SGOT	SGPT	Platelet Counts
Group 1	13.22	6.35	13.66	238	82	7.8
Group 2	14.43	7.01	12.11	240.16	69.5	7.3
Group 3	13.16	6.72	15.35	203.5	75.33	7.34
Group 4	14.28	7.37	14.38	230	82.5	7.11
Group 5	13.22	6.47	11.48	230	83.21	7.2
Group 6	13.17	6.37	15.05	188.66	68.16	6.9



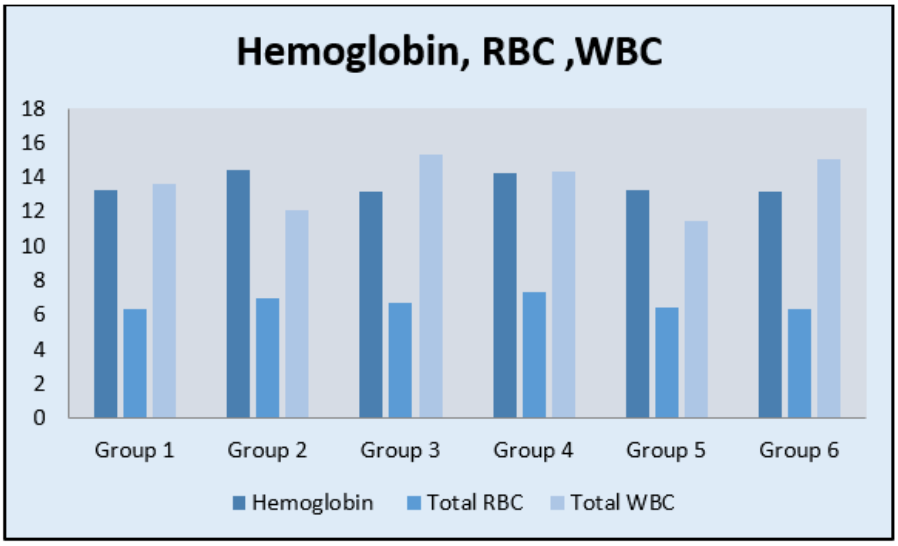


Fig 3.5: showing graph of hemoglobin, RBC, and WBC in pre-biochemical study

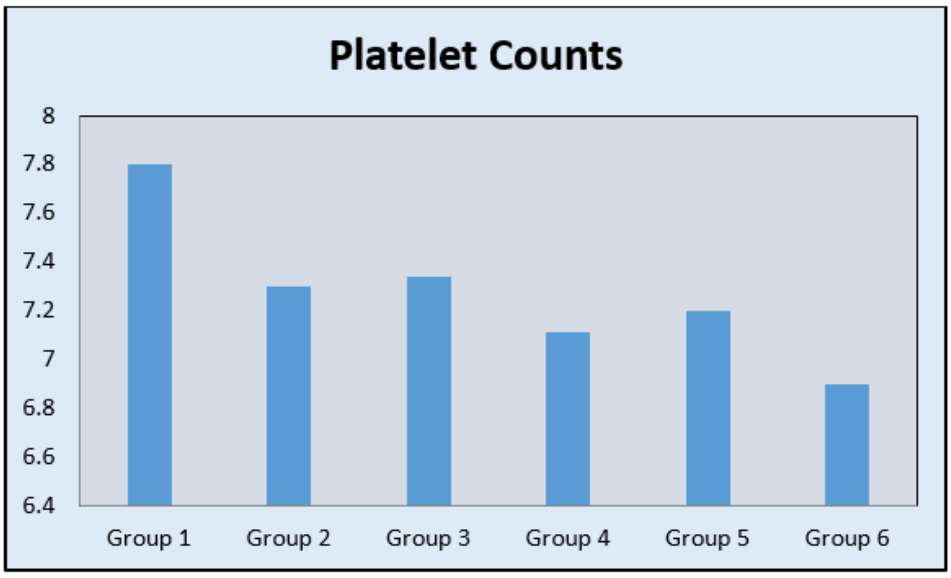


Fig 3.6: showing graph of total platelet count in the pre-biochemical study

3.2.5.2 Post-Biochemical Study:

Table 3.8: showing post-biochemical study

	Hemoglobin	Total RBC	Total WBC	SGOT	SGPT	Platelet Counts
Group-1	14.42	7.05	33.92	256.83	85.83	8.6
Group-2	13.12	7.12	20.63	230.66	57.33	6.2
Group-3	14.21	7.21	35.16	246	89.66	7.8
Group-4	14.16	7.68	19	255	90	7.92
Group-5	15.16	8.29	39.83	267.33	101.5	8.86
Group-6	14.18	7.11	38.83	250.16	78.5	7.4

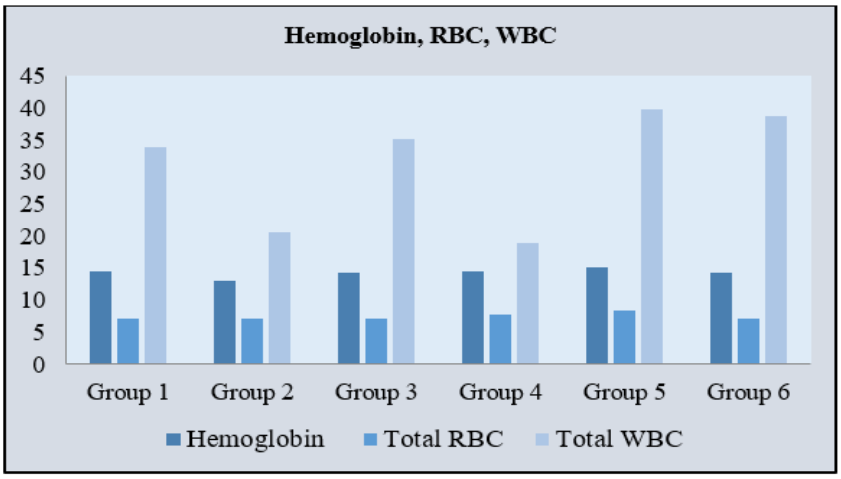


Fig 3.7: showing graph of total hemoglobin, RBC, WBC in post-biochemical study

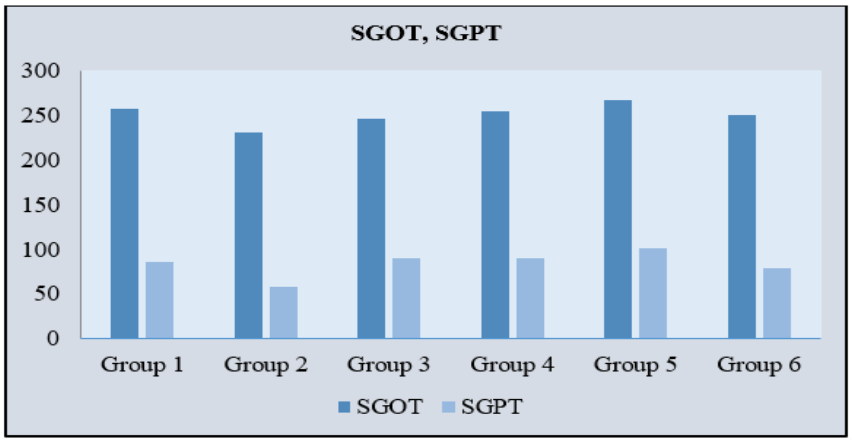


Fig 3.8: showing SGOT and SGPT count

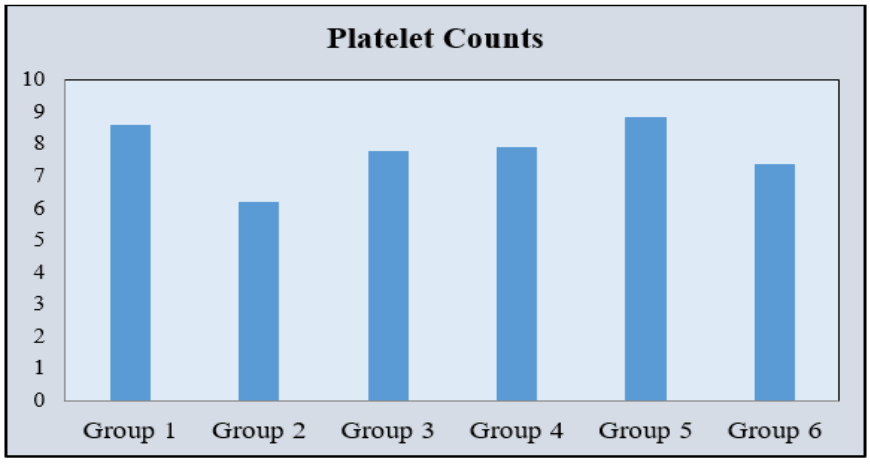


Fig 3.9: showing graph of total platelet count in the post-biochemical study

In the biochemical study, evaluate the value of biochemical in blood increases and decreases during the experimental study. According to this study, the hemoglobin is increased after administration of TS1, TS2, and TS3 and with the standard drug, but the higher hemoglobin value is found in group 5. The value of RBC and WBC count increases after the administration of TS1, TS2, and TS3 but the RBC and WBC higher

count increases in group 5. The value of SGOT and SGPT increases after administering the test drug shown in the table. 3.6. In pre- and post-biochemical studies, the platelet counts in an experimental animal increase after administering TS1, TS2, and TS3, as also shown in table no. 3.7.

3.3 Statistical analysis

This experimental data was analyzed with the help of the data evaluator method (ANOVA). The (ANOVA) analyzer uses a one-way method analysis; a student t-test follows this. P value.

Test formulations prepared during the experimental study showed a substantial increase in weight after administration of test formulation; these formulations also showed decreases in the paw edema size after administration of the drug. The test formulation also showed neutrophil adhesion ($p < 0.05$) at 35mg/kg in group 5 during the experimental study. In the delayed type of hypersensitivity test drug increases the immunity against Ovalbumin (1%), as shown in table no.3.5.With doses of 15 and 30 mg kg1 day1 immunity, 0.53 untreated groups of animals, but after the administration of the test drug, the delayed type of hypersensitivity is an increase the immunity 8.86.

4. DISCUSSION

During CMI reactions, when tested by the antigen, sharpened T-lymphocytes are changed to lymphoblasts and discharge lymphocytes, drawing in more forager cells to the response site. The competent cells are, in this way, immobilized to advance protective (provocative) responses. In our investigations, foot volume was improved after TS3 treatment recommending cell-interceded resistant upgrade. An increase in the DTH reaction shows that TS3 has a stimulatory impact on lymphocytes and embellishment cell types needed for the outflow of the response. Based on the outcomes obtained in the current investigation, it is presumed that TS3 can invigorate cell-interceded invulnerability and might be a possible remedial applicant in a few immunosuppressed clinical conditions. Even so, more thorough work should be performed to validate the case.

5. CONCLUSION

Cinnamomum tamala, Trachyspermum ammi, and Curcuma amada show immunomodulatory effects in different doses. So, this experimental study shows the impact of the drug in other dose graphs. This exploratory study includes different test formulations and various in-vivo experimental studies to determine the drug. In this study, we prepared different test formulations in different quantities. In this test, groups apply various quantities tests for the determination of the chemical properties of the test drug. After all, the test used in test drug formulation these formulations give different names: TSI, TS2, and TS3. Then test the drug ready for in-vivo study. We already mention the up-word on to manuscript. In this study, we apply different in-vivo studies such as Immunomodulatory Effect, Neutrophil Adhesion Study, and Biochemical study. After the result, the test formulation shows the effects on experimental animals. The group treated with TS3 solution showed better immunomodulatory effects than the control group and S1. All formulations showed immunomodulatory effects, but TS1 showed significant improvement in Delayed-type hypersensitivity (DTH) response, Neutrophil adhesion test, and biochemical study. TS3 showed an 86% immunomodulatory effect on day 10, and TS2 showed more than

an immunomodulatory effect to TS1 on day -7. It has a higher degree to entreat delayed types of hypersensitivity reactions and Neutrophil adhesion value**.**

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