

**STUDIES ON IMMUNOMODULATORY ACTIVITY OF FLAVONOID FRACTIONS OF TERMINALIA CATAPPA IN SWISS ALBINO MICE**

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**ABSTRACT**

To investigate the immunomodulatory activity of flavonoid fractions of *Terminalia catappa* in Swiss albino mice. The flavonoid fraction of *T.catappa* was administered intraperitoneally at a dose of ED<sub>50</sub> to healthy albino mice. Assessment of immunomodulatory activity was carried out by assessment of neutrophil adhesion and phagocytic index. Intraperitoneally administered Tcff showed a significant increase in neutrophil adhesion and phagocytic index. This study demonstrates that the Tcff shows immunomodulatory effect.

## KEY WORDS

Phagocytic index, neutrophil adhesion, *T.catappa*, flavonoid fraction

## INTRODUCTION

Immune system plays an important role in biological adaptation, contributing to the maintenance of homeostasis. The plant products have long been used as immunomodulators by the traditional healers<sup>1</sup>. Immune activation is an effective and protective approach against emerging infectious diseases<sup>2</sup> and alternative medicine is becoming more popular for the treatment of these illnesses. Medicinal Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases<sup>3</sup>. Thus the present investigation was aimed at evaluating the immunomodulatory role flavonoid fractions of *Terminalia catappa* in Swiss albino mice.

*Terminalia catappa* belongs to the family of Combretaceae also known as badam. It is well known herb in Ayurvedic system of medicine. Juices of young leaves are employed in preparation of ointment for leprosy, scabies and head ache. It is also known as Indian almond, Malabar almond and Tropical almond. The aqueous and cold extracts of leaves of the *Terminalia catappa* have been reported to be antioxidant, hepatoprotective, and anti-diabetic<sup>4</sup>. The leaves extract of *T.catappa* inhibit Lewis lung carcinoma cells that contribute to lung cancer<sup>5</sup>. The plant is very well known for its therapeutic values since long and has proved by many researchers to be useful as an anticancer<sup>6</sup>, antihepatotoxic<sup>7</sup>, antimicrobial<sup>8</sup>, insecticidal and molluscicidal activities<sup>9</sup>. Previous studies from our research group reported that *Terminalia catappa* leaf protein possess antioxidant and antitumorogenic effect<sup>10</sup> against Ehrlich's Lymphoma Ascites in Swiss albino mice.

## MATERIALS AND METHODS

### PLANT MATERIAL

Fresh leaves of *Terminalia catappa* was collected in area free of pesticides and other contaminants from the area surrounding Coimbatore, Tamilnadu. The collected leaves were washed thoroughly and blotted dry with filter paper and used for the flavonoid fraction preparation.

### EXTRACTION OF FLAVONOID FRACTIONS OF TERMINALIA CATAPPA

Fresh leaves of *Terminalia catappa* were shade dried and pulverized. The powder was treated with petroleum ether for dewaxing and removal of chlorophyll. In the preliminary screening, the direct methanol extract of *Terminalia catappa* showed a characteristic orange to magenta colour in Shinoda test (powdered magnesium + conc. HCl) which indicated the presence of flavonoids. The colour is due to the reductive conversion of the flavone into the corresponding anthocyanin pigment<sup>11</sup>.

Knowing the presence of flavonoid in methanol extract, the extraction was undertaken with 20 g of powdered plant material and 200ml. of light petroleum ether (b.p. 40<sup>o</sup> – 60<sup>o</sup> C) in a Soxhlet apparatus for 18 hours to remove the chlorophyll, non flavonoid components and lipid dewaxing<sup>12</sup>. The treated material was dried and extracted with methanol using Soxhlet apparatus<sup>13</sup>. This fraction is referred as Tcff.

### ANIMALS.

Seven to eight weeks old Swiss albino male mice weighing about 25-30 g were brought from small animals breeding station, Perundurai Medical College, Perundurai, Tamilnadu. The animals were acclimatized for 15 days under standard

laboratory conditions and fed with standard diet with water ad libitum. All animal experiments were carried out according to the guidelines prescribed by Animal Welfare Board and with the approval of Animal Ethic Committee (Register no: 623/02/b/CPCSEA).

**ANTIGEN:**

Sheep blood was collected from a local slaughter house in sterilized container in the presence of Alsever’s solution. SRBC were obtained by centrifugation and the cells were washed three times in 0.9% saline and adjusted to concentration of  $5 \times 10^9$  cells per ml for immunization and challenge.

**TREATMENT**

**ASSESSMENT OF NEUTROPHIL INDEX AND NEUTROPHIL ADHESION IN MICE**

The animals were divided into four groups with 6 mice each.

**Group- I**

SRBC induced mice received Pyrogallol (50mg per g body weight) in 100µl of PBS from 2<sup>nd</sup> to 14<sup>th</sup> day intraperitoneally.

**Group-II**

SRBC induced mice received 100µl of DMSO from 2<sup>nd</sup> to 14<sup>th</sup> day intraperitoneally

**Group-III**

SRBC induced mice received Tcff (75 µg /100 µl) in 100µl of DMSO from 2<sup>nd</sup> to 14<sup>th</sup> day intraperitoneally

**NEUTROPHIL ADHESION TEST:**

On the 14<sup>th</sup> day of drug treatment, blood samples were collected (before challenge) by puncturing the heart and analyzed for TLC (Total Leukocyte Count) and DLC (Differential Leukocyte Count) by fixing blood smears and staining with field stain 1 and Leishmans stain. After initial counts, blood samples were incubated with 80mg/ml of nylon fiber for 15 minutes at 38°C. The incubated blood samples were analyzed for TLC and DLC. The product of TLC and % Neutrophil gives Neutrophil index of blood samples<sup>14</sup> Percent of Neutrophil adhesion was calculated as shown below.

$$\text{Neutrophil adhesion (\%)} = \frac{NI_U - NI_T}{NI_U} \times 100$$

Where,  $NI_U$ . Neutrophil index of untreated blood sample

$NI_T$ . Neutrophil index of treated blood sample.

**ASSESSMENT OF PHAGOCYTTIC INDEX OF Tcff**

The mice were divided into two groups with 6 mice in each. The phagocytic index was followed by carbon clearance test using the serum of two groups of mice on the 7<sup>th</sup> day.

**Group 1** received 100µl of DMSO (i.p) for 5 days.

**Group 2** received 75µg of Tcff in 100µl of DMSO (i.p) for 5 days.

For all the above groups, Carbon ink (10µl/g body weight) was administered on the 7<sup>th</sup> day and the blood samples were drawn from the retro orbital vein at 0<sup>th</sup> and 15<sup>th</sup> minutes.

Group I served as control and was given 100 µl of DMSO for 5 days intraperitoneally. Group II served as test which was administered with the Tcff for 5 days. After 48 hours of administration of the last dose of the extract, mice were injected 0.1ml of Carbon ink via the tail vein. Blood samples were withdrawn at 0 and 15 minutes after injection. 25 µl of blood samples were mixed with 2 ml of 0.1 per cent sodium carbonate solution and the absorbance of this solution was determined at 660 nm (Jayathirtha and Mishra, 2004). The Phagocytic index K was calculated using the following equation:

$$K = \frac{(\text{Log}_e OD_1 - \text{Log}_e OD_2)}{15}$$

Where,  $OD_1$  and  $OD_2$  are the optical densities at 0 and 15 minutes respectively

**STATISTICAL ANALYSIS**

The results were expressed as mean ± standard deviation of 6 animals.

**RESULTS AND DISCUSSION**

Immunomodulatory agents obtained from plant and animal origin generally enhances the immune responsiveness of an organism against a pathogen by activating the system. Pyrogallol can be used to induce

immunosuppression while screening the immunomodulatory activity of any agent<sup>15</sup>.The effect of Tcff on neutrophil adhesion in Swiss albino mice was given in Table1.The neutrophil index decreased in group III in treated and untreated blood when compared to controls and Tcff.In the present study Tcff when

administered intraperitoneally, significantly increased the adhesion of neutrophils to nylon fibers when compared to untreated control, which correlates to the process of migration of cells in blood vessels, indicating possible immune stimulant effect

**Table.1.**  
**Effect of flavonoid fraction of Terminalia catappa on neutrophil adhesion in Swiss albino mice**

Groups	Neutrophil index		Neutrophil Adhesion (%)
	UB	FTB	
PBS	273.10±8.24	249.00±10.48	11.33±0.65
DMSO	277.16±8.84	252.50±8.26	11.53±0.73
Pyrogallol	248.00±14.79	223.83±12.30	9.83±0.62
Tcff	295.50±9.52	272.00±9.27	17.76±1.35

The values are the mean ± SD of six animals

**EFFECT OF Tcff ON PHAGOCYtic INDEX**

The non-specific immune mechanisms are particularly important early in infection, as the antigen-specific response takes several days to develop, but the non-specific mechanisms continue to play a role in the immune response right through to resolution of the infection and healing of tissue damage. The phagocytosis is a type of non-specific immune response which helps to remove the foreign particles from the living system

Rate of carbon clearance is the measure of competency of the reticuloendothelial system and its granulopoetic activity, the faster removal of carbon particles has been correlated with the enhanced phagocytic activity. The results obtained for phagocytic index in carbon ink induced swiss albino mice are shown in Table

2.In the present study, a significant increase in phagocytic index was observed in flavonoid fraction treated groups when compared to control, indicating that the rate of elimination of carbon particles is more in flavonoid fraction treated group than that observed in the control group .When reticuloendothelial system is stimulated,there is increase in number of phagocytic cells,which engulf the antigen,indicating increase in immunity.<sup>16</sup> Thus the Tcff potentiate the phagocytosis of foreign particles by the reticuloendothelial system and acts as a potent stimulator of non specific immune response in mice. Dashputre and Naikwade<sup>17</sup> and Kalpesh Gaur *et al*<sup>18</sup> found that ethanolic and aqueous extract of leaves of *Abutilon indicum* and the hydro-alcoholic extract of *Hibiscus rosasinensis* Linn increases phagocytic index.

**Table.2.**  
**Phagocytic index in mice administered with and without Tcff**

Groups	Phagocytic index (k)
DMSO	0.0793 ± 0.0042
Tcff	0.3030 ± 0.0175

The values are the mean ± SD of six animals

In conclusion, the results obtained in the present study suggests that the flavonoid fraction of *T.catappa* stimulate neutrophil

adhesion,neutrophil index and phagocytic index which confirmed that flavonoid fraction of *T. catappa* possess immunomodulatory

property. Eventhough, this is only preliminary study of the immunomodulatory potential of flavonoid fraction of *T.catappa*.Further studies using *in vivo* models of immunomodulation are

needed to confirm the immunomodulatory activity of flavonoid fraction of *T.catappa* leaves and its mechanism of action.

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