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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 administrated intraperitonealy at a dose of ED_{50} to healthy albino mice. Assessment of immunomodulatory activity was carried out by assessment of neutrophil adhesion and phagocytic index. Intraperitonealy administered Tcff showed a significant increase in neutrophil adhesion and phagocytic index.This study demonstrates that the Tcff shows immunomodulatory effect.

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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[1].Immune activation is an effective and protective approach against emerging infectious diseases[2]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[3]. 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 [4]. The leaves extract of *T.catappa* inhibit Lewis lung carcinoma cells that contribute to lung cancer[5] The plant is very well known for its therapeutic values since long and has proved by many researchers to be useful as an anticancer[6] antihepatotoxic[7]antimicrobial[8] insecticidal and molluscicidal activities[9].Previous studies from our research group reported that *Terminalia catappa* leaf protein possess antioxidant and antitumorogenic effect[10] 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[11].

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. $40^0 - 60^0$ C) in a Soxhlet apparatus for 18 hours to remove the chlorophyll, non flavonoid components and lipid dewaxing[12].The treated material was dried and extracted with methanol using Soxhlet apparatus[13] 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, Perudurai,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 5×10^9 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) in100µl of PBS from 2nd to 14th day intraperitonially.

Group-II

SRBC induced mice received 100µl of DMSO from 2nd to 14th day intraperitonially

Group-III

SRBC induced mice received Tcff (75 µg /100 µl) in 100µl of DMSO from 2nd to 14thday intraperitonially

NEUTROPHIL ADHESION TEST:

On the 14th 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 15minutes 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[14] 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 PHAGOCYTIC 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 7th 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 7th day and the blood samples were drawn from the retro orbital vein at 0th and 15th 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 0.1per 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:

$$K = \frac{(Log_e\ OD_1 - Log_e\ OD_2)}{15}$$

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[15].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

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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.[16] 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 [17]and Kalpesh Gaur *et al*[18]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 premilinary 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.

REFERENCES

1. Tan B and Vanitha J .Immuno modulatory and antimicrobial effects of some traditional Chinese medicinal herbs.A Review Med.Chem, 11:1423-1430,(2004).
2. Hackett C. J. Innate immune activation as a broad-spectrum biodefense strategy: prospects and research challenges, J. Allergy. Clin. Immunol, 112: 686-694,(2003).
3. Perianayagam JB, Sharma SK, Joseph A and Christiana A J M. Evaluation of Antidiarrheal Potential of *Emblica officinalis*.J Ethnopharmacol, 95(1): 85-87, (2004).
4 .Ahmed SM, SwamyB.M.V, Dhanapal PGR and ChandrashekaraVM. Anti-Diabetic activity of *Terminalia catappa Linn.* Leaf extracts in alloxan induced diabetic rats. IJPT, 4(1): 36-39, (2005).
5. Chu SC, Yang SF, Liu SJ, Kuo WH, ChangYZ and Hsieh YS. *In vitro* and *in vivo* antimetastatic effects of *Terminalia catappa* L. leaves on lung cancer cells.Food Chem. Toxicol 45(7): 1194-1201,(2007).
6 .Kandil FE, Soliman AM, Skodack SR and Mabry TJ. A new anticancer tannin and known tannins from *Terminalia catappa.* Asian J Chem, 11(3):1001-1004,(1999).
7 .Lin CC, Hsu YF and Lin TC.Antioxidant and free radical scavenging effects of the tannins of *Terminalia catappa* L. Anticancer Res, 21(1A): 237-243,(2001).
8. Nair R and Chanda S. Antimicrobial activity of *Terminalia catappa, Manilkara zapota* and *Piper betel* leaf extract. Indian J Pharm Sci 70(3): 390-393,(2008).
9. Jayasinghe ULB, Wannigama GP and Fujimoto Y.Chemistry and bioactivity of saponins from some Sri Lankan plants.Proc Phytochem Soc Eur, 45:113-119,(2000).
10 .Santhi R and Annapoorani S.Antioxidantive role of *Terminalia catappa* leaf protein against ELA induced mice.Int.J.Drug Dev.& Res 1: 81-8,(2009).
11. Krishnaswamy NR.Chemistry of natural products, Universities Press, India,197,(1999).
12. Palanivel Muthu Gounder, Balasubramanian rajkapoor ,Raju senthil kumar , John Wilking Einstein ,Ekambaram Prem Kumar ,Mani Rupesh Kumar ,Kunchu Kavitha,Mohanraj Pradeep Kumar and Balasundaram Jayakar.Hepatoprotective and Antioxidant Effect of *Pisonia aculeata* L. against CCl4-Induced Hepatic Damage in Rats. Sci Pharm, 76: 203–215,(2008).
13. Ansari WH, Rahman W, Barraclough D, Maynard R and Scheinman F.S. Chem. Soc.2: 1458, (1976).
14. Brahmvarchas K,Ayurveda Ka Pran;Vanaushdi Vigyan.3rd Edition,Ved,Mata Gayatri Trust,188,(2003).
15. Jayathirtha MG, and Mishra SH. Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asiatica.* Phytomed, 11(4): 361-365,(2004).
16. Ghule BV, MuruganathanG, Nakhat PD and Yeole PG.Immunostimulatory effects of *Capparis zeylanica.* J Ethnopharmacol,108:311-315,(2006).
17. Dashputre NL and Naikwade NS.Immunomodulatory Activity of *Abutilon Indicum* linn on Albino Mice. IJPSR.1(3):178-184,(2010).
18 .KalpeshGaur, KoriMLand Nema RK.Comparative Screening of Immunomodulatory Activity of Hydro-alcoholic Extract of *Hibiscus rosa sinensis* Linn and Ethanolic Extract of *Cleome gynandra* Linn. Global Journal of Pharmacology, 3 (2): 85-89,(2009).