



RESEARCH ARTICLE

## Immunomodulatory Activity Of Ethyl Acetate Flavonoid Extract Of *Thespesia Populnea* Leaves In Balb/C Mice

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### ABSTRACT

The immune system is one of the most important biological systems of our body. Immunomodulation is either an increase or decrease in the immune response. To evaluate the immunomodulatory activity of the leaves of *Thespesia populnea* (Family Malvaceae) in cancer cell line induced Balb/c mice. Various extracts of the leaves of *Thespesia populnea* (TpFf) were evaluated for the flavonoid content, and the higher content of flavonoid was found to be in ethyl acetate extract and it was used to evaluate the potential immunomodulatory activity. The ethyl acetate flavonoid extract was evaluated for immunomodulatory activity *in vivo* studies, using Balb/c mice as the animal model. The extract was tested for hematological parameters, immunological parameters, weight and cellularity of lymphoid organs and Histopathological observation of spleen and thymus, using sheep red blood cells (SRBC) as the antigen. PBS served as a control in all the tests. The ethyl acetate extract exhibited a significant decrease in the WBC and increased in the RBC and Hb compared to Ehrlich's lymphoma ascites (ELA) and Dalton's lymphoma ascites (DLA) induced groups. It also resulted in a significant increase in the IL-2, IFN- $\gamma$ , weight of spleen and thymus and cellularity of lymphoid organs and malignant changes observed in the DLA and EAC tumor-induced spleen and thymus sections. The ethyl acetate extract was found to stimulate the immune responses in Balb/c mice induced ELA and DLA.

**Key words:** Immunomodulation; ethyl acetate; malvaceae; ELA; DLA.

### INTRODUCTION

Traditional and folklore medicines play an essential role in health services around the world. About three-quarters of the population in the world rely on plants and plant extracts for many healthcare. India has an extensive forest cover, enriched with so much plant diversity. Several plants have been used in folklore medicine (Premanathan *et al.*, 2000). The immune system is a remarkably sophisticated defense system that protects living things from invading agents. It is able to generate varieties of cells and molecules capable of recognizing and eliminating limitless varieties of foreign agents. Specific immunomodulators administered together with antigens known as immunological adjuvants is used to boost the immune response to the vaccine constituents (Manu and Kuttan, 2009). Plant extracts are potentially curative. Some of these extracts can improve the humoral and cell-mediated immunity against viruses, bacteria, fungi, protozoa (Jeba *et al.*, 2011). *Thespesia populnea* has been yet not scientifically reported for any of

its immunomodulatory activity. The objective of the present work is to evaluate the immunomodulatory activity of the ethyl acetate flavonoid extract of *Thespesia populnea* leaves in ELA and DLA induced Balb/c mice. The flavonoid was tested for the hematological parameters such as RBC, WBC, and Hb, immunological parameters such as IL-2 and IFN- $\gamma$ , weight, cellularity, and histopathology of lymphoid organs such as spleen and thymus. The flavonoid extract results in immunomodulation, particularly the immunostimulant activity by the coadministration of TpFf with cancer cell lines, namely ELA and DLA.

### MATERIAL AND METHODS

The *Thespesia populnea* (Tp) leaves were dried, powdered, and used to prepare the aqueous, ethanol, chloroform, and ethyl acetate extracts individually (Santhi *et al.*, 2011). Various phytochemicals were analyzed by using standard procedures. Flavonoid fractions of selected extracts were prepared by a small modification (Palanivel *et al.*, 2008) and were

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estimated by the aluminium chloride method (Chang *et al.*, 2002). The flavonoid fraction, which showed maximum flavonoid content, was selected for further studies and referred to as TpFf. *In vitro* cytotoxic studies were carried out by the trypan blue exclusion method of Salomi and Panikkar (1989). The fraction which showed a minimum concentration of flavonoid as EC50 was selected for further studies. *In vivo* studies were carried out by the intraperitoneal administration of 70 µg (EC50) of TpFf to examine their immunomodulatory role in the SRBC/ DLA, EAC tumor-induced Balb/c mice.

### Preparation of SRBC

Sheep blood was collected from a local slaughterhouses in a sterilized container in the presence of Alseiver's solution. SRBCs were obtained by centrifugation and the cells were washed three times in 0.9 per cent saline and adjusted to a concentration of 0.5x10<sup>9</sup> cells per ml for immunization and challenge.

### Grouping of animals

The mice were divided into seven groups, with 6 mice in each for each treatment period. All the

seven groups of mice received 0.5 x 10<sup>9</sup> Sheep Red Blood Cells (SRBCs) in 100µl of PBS on the 1<sup>st</sup>, 8<sup>th</sup>, and 15<sup>th</sup> day and indicated as SRBC induced mice. The Hematological parameters such as RBC count by Hayem's method, WBC count by Turke's method and Haemoglobin by Sahli's method, Immunological parameters like IL-2 and IFN-γ production (ELISA KIT Procedure), Weight and Cellularity of lymphoid organs (Haemocytometer) namely Spleen and Thymus were determined on 7<sup>th</sup> day and 15<sup>th</sup> day. Histopathological observation of Spleen and Thymus was done using the method of Culling (1974).

### Statistical Analysis

The data were expressed as the mean ± standard deviation of the means and statistical analysis was carried out employing the one-way and two-way analysis of variance (ANOVA) using Web Agri Stat Package 2.0.

## RESULTS AND DISCUSSION

Sheep Phytochemical analysis was carried out in four different extracts, namely aqueous, ethanol, ethyl acetate and chloroform.

**Table 1. Levels Of Il-2 And Ifn-γ Production In Control and Tumor Bearing Mice**

Groups	Treatments	IL-2 (pg/ml)		IFN-γ (pg/ml)	
		7 <sup>th</sup> day	15 <sup>th</sup> day	7 <sup>th</sup> day	15 <sup>th</sup> day
1	PBS	11.29 0.83	13.29 0.89	0268.35 05.79	0277.10 05.70
2	Pyro	09.04 0.67	08.03 0.72	1445.56 38.68	1531.43 20.17
3	TpFf	16.77 0.34	17.96 0.66	3603.81 40.90	2730.16 18.87
4	DLA+TpFf	12.55 0.38	15.10 0.32	2827.20 18.69	2920.00 15.49
5	EAC+TpFf	13.53 0.89	15.96 0.59	2737.35 16.31	2933.48 16.18
6	DLA	08.18 0.55	06.21 0.60	1233.86 18.57	1323.98 18.65
7	EAC	07.53 0.45	05.93 0.65	1228.70 19.08	1323.96 18.54
# CD (0.05)		0.688	0.767	31.882	24.478
## CD (0.05)			0.720		28.069

Most of the constituents are present in ethyl acetate extracts of Tp. Flavonoid was absent in chloroform extract, so that the quantification was carried out only for the three-leaf extracts, namely ethanol, ethyl acetate, and aqueous extracts. These were determined against the standard flavonoids Quercetin, Kaempferol and Myricetin. The highest flavonoid content was present in ethyl acetate extract of Tp leaves. Hence, further analysis was performed in ethyl acetate extract of Tp leaves alone and is denoted as TpFf.

### In vitro cytotoxic studies

Incubation of EAC/DLA tumor cells with TpFf showed a concentration-dependent cytotoxic effect,

which was indicated by the increase in the number of dead cells with increasing concentrations of TpFf. The extract killed 50 percent of EAC/DLA tumor cells at a concentration of 70 g of TdFf. This concentration was designated as fifty percent effective concentration (EC50) and was used in the following *in vivo* studies

### Effect of TpFf on the Haematological parameters in the blood of Balb/c mice:

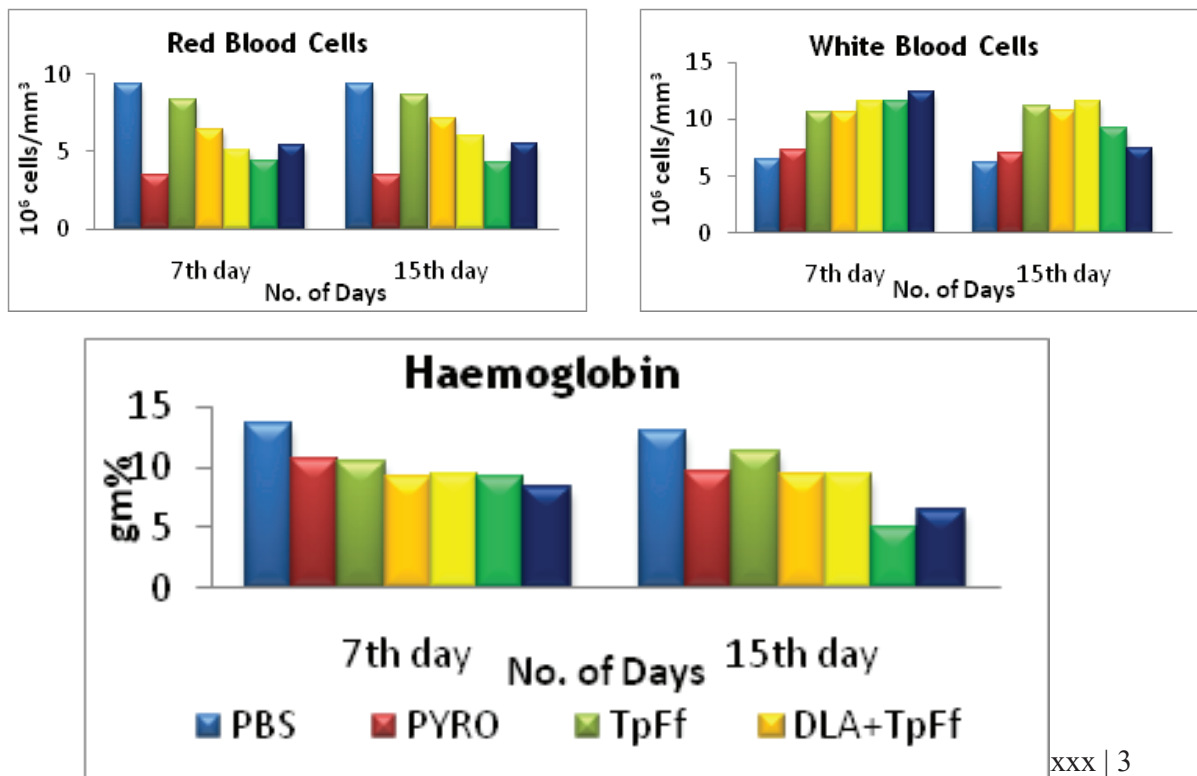
Hematological parameters in cell line induced groups of both DLA and EAC were found to be significantly altered compared to those of the normal control group are shown in Figure 1. The levels of RBC count and Haemoglobin levels were

**Table 2. Relative weight and cellularity of lymphoid organs**

Groups	Treatments	Spleen				Thymus			
		Weight (gm)		Cellularity (x 106)		Weight (gm)		Cellularity (x 106)	
		7 <sup>th</sup> day	15 <sup>th</sup> day	7 <sup>th</sup> day	15 <sup>th</sup> day	7 <sup>th</sup> day	15 <sup>th</sup> day	7 <sup>th</sup> day	15 <sup>th</sup> day
1	PBS	0.17	0.24	17.9	21.1	0.08	0.08	137.56	137.85
		0.04	0.02	0.36	0.71	0.01	0.10	1.25	0.84
2	Pyro	0.14	0.12	12.91	11.06	0.02	0.08	122.41	116.18
		0.02	0.01	0.38	0.60	0.01	0.03	2.38	0.53
3	TpFf	0.19	0.23	22.00	23.55	0.08	0.04	176.45	185.78
		0.03	0.01	0.77	0.57	0.01	0.02	1.30	0.74
4	DLA+TpFf	0.18	0.16	17.6	10.16	0.07	0.02	129.86	134.70
		0.01	0.01	0.24	0.35	0.02	0.01	0.81	1.47
5	EAC+TpFf	0.20	0.16	18.06	19.10	0.07	0.04	127.80	131.33
		0.02	0.01	0.57	0.66	0.01	0.01	1.00	0.71
6	DLA	0.12	0.15	09.55	08.76	0.03	0.02	123.51	120.85
		0.02	0.01	0.40	0.31	0.01	0.01	0.83	0.56
7	EAC	0.14	0.16	10.71	9.31	0.03	0.02	125.45	122.03
		0.03	0.01	0.38	0.36	0.01	0.02	0.72	0.52
	# CD (0.05)	0.032	0.013	0.669	0.635	0.017	0.018	1.437	1.103
	## CD (0.05)	0.024		0.644		0.035		1.265	

found to be decreased in DLA and EAC induced animals when compared to normal control, whereas WBC count were significantly increased in cell line

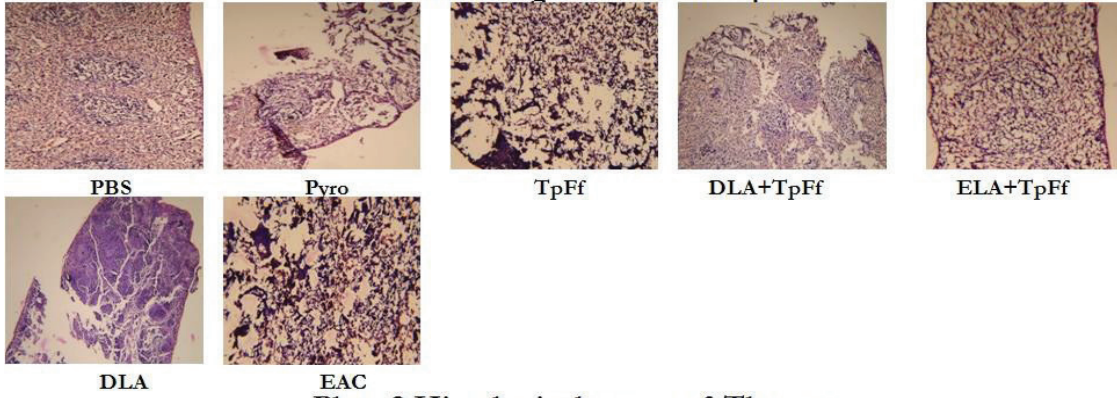
induced mice when compared to the normal control group. Treatment with TpFf showed a significant decrease in WBC count compared to cell line-



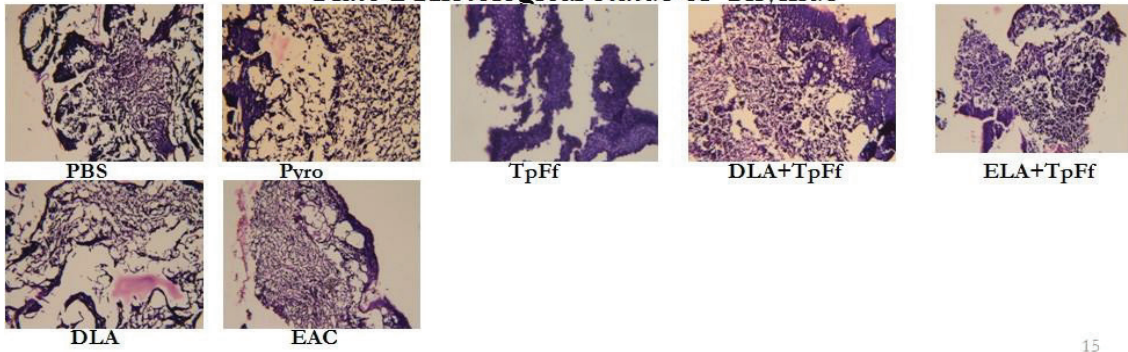
**Figure 1. Levels of RBC, WBC and Haemoglobin in controls and experimental groups**

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**Plate 1 Histological status of Spleen**

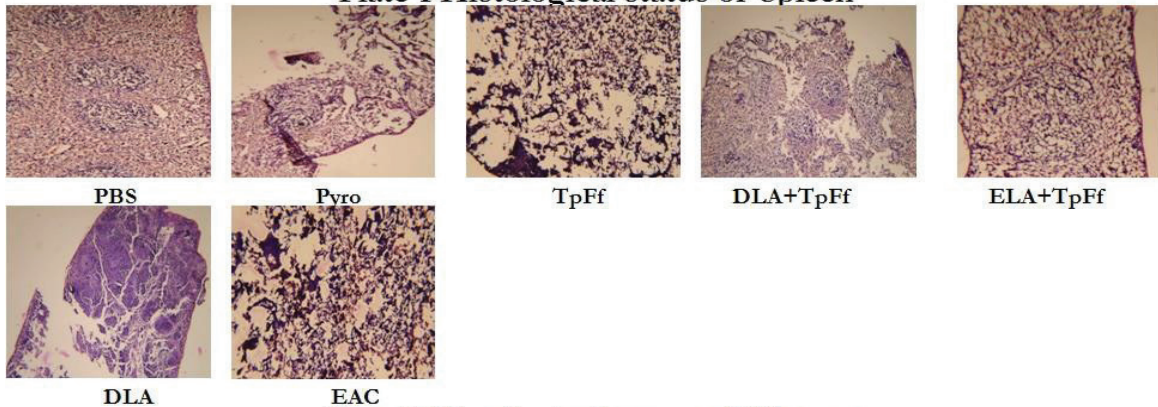


**Plate 2 Histological status of Thymus**

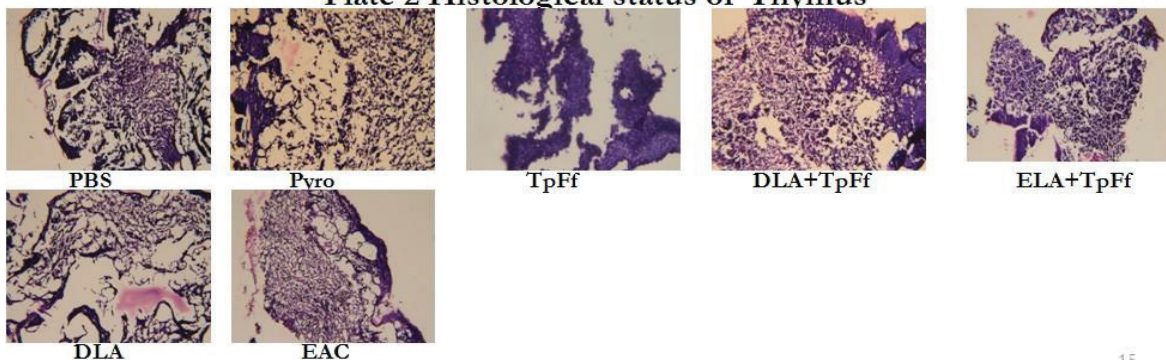


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**Plate 1 Histological status of Spleen**



**Plate 2 Histological status of Thymus**



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induced animals, whereas increased RBC count and Hb level compared to cell line-induced groups on both treatment periods. Co-administration of TpFf to DLA and EAC tumor-induced mice showed a significant decrease in WBC count compared to cell line induced animals, whereas an increase in

RBC count and Hb level when compared to cell line induced groups on both treatment periods.

**Effect of TpFf on the IL-2 and IFN- $\gamma$  production in the serum of Balb/c mice:**

IL-2 and IFN-  $\gamma$  production in cell line induced

groups of both DLA and EAC were found to be significantly altered compared to those of the normal control group are shown in Table 1. The levels of IL-2 were found to be decreased in DLA and EAC induced animals when compared to normal control; whereas IFN- $\gamma$  were significantly increased in cell line induced mice when compared to the normal control group. Treatment with TpFf showed a significant increase in IL-2 and IFN- $\gamma$  production compared to cell line induced animals on both treatment periods. Co-administration of TpFf to DLA and EAC tumor-induced mice showed a significant decrease in IL-2 and IFN- $\gamma$  compared to cell line induced animals on both treatment periods. This indicates the immunostimulation of TpFf.

#### **Effect of relative weight and Cellularity of lymphoid organs:**

Relative weight and cellularity of lymphoid organs in cell line induced groups of both DLA and EAC were found to be significantly altered compared to those of the normal control group are shown in Table 2. Weight of spleen, thymus, and cellularity of lymphoid organs were found to be decreased in DLA and EAC induced animals when compared to normal control. Treatment with TpFf showed a significant increase in all the above compared to cell line induced animals on both treatment periods. Co-administration of TpFf to DLA and EAC tumor-induced mice showed a significant increase in relative weight and cellularity of lymphoid organs compared to cell line induced animals on both treatment periods

## **CONCLUSION**

Based on all the above parameters the ethyl acetate extract of *T. populnea* was found to stimulate the immunomodulatory activity of the TpFf towards DLA and EAC tumors.

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