RESEARCH ARTICLE

IMMUNOMODULATORY ACTIVITY OF ETHYL ACETATE FLAVONOID EXTRACT OF THESPESIA POPULNEA LEAVES IN BALB/C MICE

Dr. Santhi Ramalingam1 and Dr. Annapurani. S2
Department Of Biochemistry, Biotechnology And Bioinformatics,Avinashilingam Institute For Home Science And Higher Education For Women, Coimbatore - 43

ABSTRACT

The immune system is one of the most important biological systems of our body. Immunomodulation is either increase or decrease in the immune response. Objective: To evaluate the immunomodulatory activity of the leaves of Thespesia populnea (Family Malvaceae) in cancer cell line induced Balb/c mice. Methods: 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 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. Findings: The ethyl acetate extract exhibited a significant decrease in the WBC and increase in the RBC and Hb when 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-γ, 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. Conclusion: The ethyl acetate extract was found to stimulate the immune responses in Balb/c mice induced ELA and DLA.

Keywords: immunomodulation, ethyl acetate, malvaceae, ELA, DLA

INTRODUCTION

Traditional and folklore medicines play an important role in health services around the world. About three quarters of the population in the world relies on plants and plant extracts for many healthcare. India has an extensive forest cover, enriched with so many plant diversity. Several plants have been used in folklore medicine (Premanathan et al., 2000). Immune system is remarkably sophisticated defence systems that protect 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 virsuses, 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-γ, weight, Cellularity and histopathology of lymphoid organs such as spleen and thymus. The flavonoid extract results in the immunomodulation particularly the immunostimulant activity by the coadministration of TpFf with cancer cell lines namely ELA and DLA.

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 analysed by using standard procedure. Flavonoid fractions of selected extracts were prepared by a small modification (Palanivel *et al.,* 2008) and were estimated by Aluminium chloride method (Chang *et al*., 2002). The flavonoid fraction which showed maximum flavonoid content was selected for the further studies and referred as TpFf. *In vitro* cytotoxic studies were carried out by trypan blue exclusion method of Salomi and Panikkar, (1989). The fraction which showed minimum concentration of flavonoid as EC_{50} was selected for the further studies. *In vivo* studies were carried out by the intraperitoneal administration of 70 µg (EC_{50}) 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 slaughter houses in 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 concentration of 0.5x109 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 periods. All the seven groups of mice received 0.5 x 109 Sheep Red Blood Cells (SRBCs) in 100µl of PBS on the 1st, 8th and 15th 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 7th day and 15th 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 one-way and two-way analysis of variance (ANOVA) using Web Agri Stat Package 2.0.

RESULTS AND DISCUSSION

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

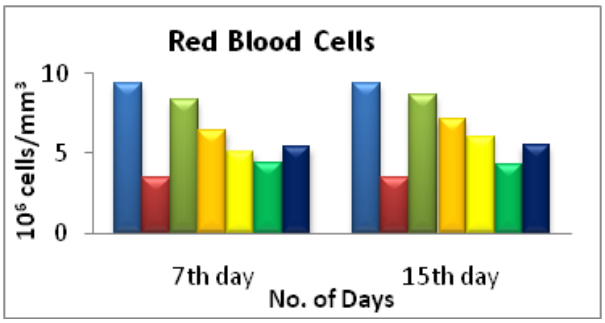
In vitro cytotoxic studies

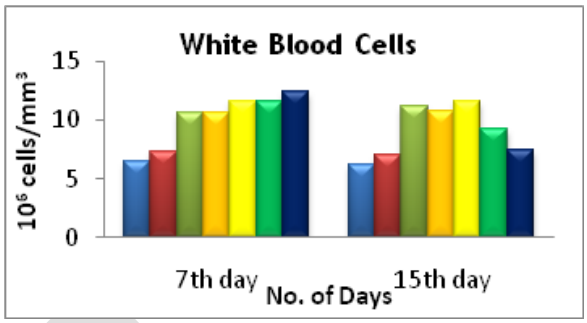
Incubation of EAC/DLA tumor cells with TpFf showed a concentration dependent cytotoxic effect which was indicated by the increase in number of dead cells with increasing concentrations of TpFf. The extract killed 50 percent of EAC/DLA tumor cells at a concentration of 70 □g of TdFf. This concentration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

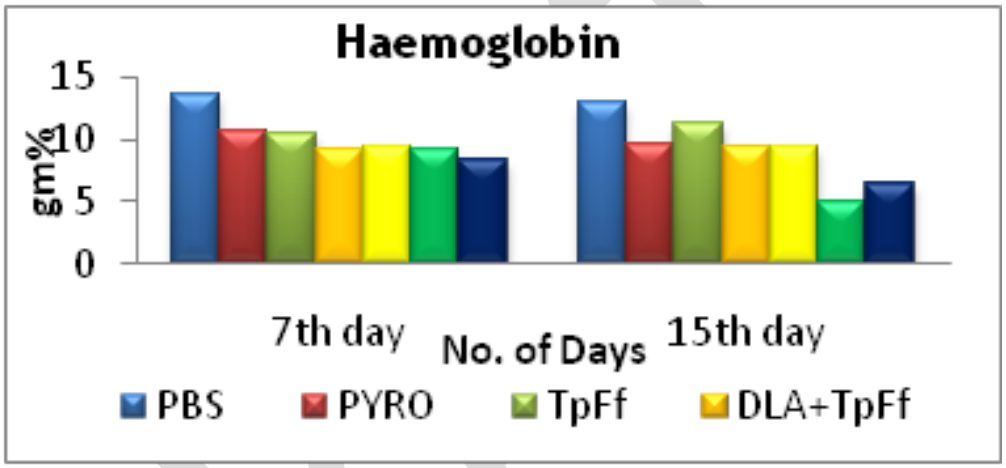
Effect of TpFf on the Haematological parameters in the blood of Balb/c mice:

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

Figure 1
Levels Of Rbc, Wbc And Haemoglobin In COntrols And Experimental Groups







Effect of TpFf on the IL-2 and IFN-γ production in the serum of Balb/c mice:

IL-2 and IFN- γ 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- γ were significantly increased in cell line induced mice when compared to the normal control group. Treatment with TpFf showed significant increase in IL-2 and IFN- γ production compared to cell line induced animals on both treatment periods. Co administration of TpFf to DLA and EAC tumor induced mice showed significant decrease in IL-2 and IFN- γ compared to cell line induced animals on both treatment periods. This indicates the immunostimulation of TpFf.

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)	
		7th day	15th day	7th day	15th 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	

One way ANOVA, ## Two way ANOVA
The values are the mean ± SD of six animals

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 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 significant increase in relative weight and cellularity of lymphoid organs compared to cell line induced animals on both treatment periods.

TABLE 2
RELATIVE WEIGHT AND CELLULARITY OF LYMPHOID ORGANS

Groups	Treatments	Spleen				Thymus			
		Weight (gm)		Cellularity (x 106)		Weight (gm)		Cellularity (x 106)	
		7th day	15th day	7th day	15th day	7th day	15th day	7th day	15th day
1	PBS	0.17 ± 0.04	0.24 ± 0.02	17.9 ±0.36	21.1 ± 0.71	0.08 ± 0.01	0.08 ± 0.10	137.56 ± 1.25	137.85 ± 0.84

2	Pyro	0.14 ± 0.02	0.12 ± 0.01	12.91 ± 0.38	11.06 ± 0.60	0.02 ± 0.01	0.08 ± 0.03	122.41 ± 2.38	116.18 ± 0.53
3	TpFf	0.19 ± 0.03	0.23 ± 0.01	22.00 ±0.77	23.55 ± 0.57	0.08 ± 0.01	0.04 ± 0.02	176.45 ± 1.30	185.78 ± 0.74
4	DLA+TpFf	0.18 ± 0.01	0.16 ±0.01	17.6 ± 0.24	10.16 ± 0.35	0.07 ± 0.02	0.02 ± 0.01	129.86 ± 0.81	134.70 ± 1.47
5	EAC+TpFf	0.20 ± 0.02	0.16 ± 0.01	18.06 ± 0.57	19.10 ± 0.66	0.07 ± 0.01	0.04 ± 0.01	127.80 ± 1.00	131.33 ± 0.71
6	DLA	0.12 ± 0.02	0.15 ± 0.01	09.55 ± 0.40	08.76 ± 0.31	0.03 ± 0.01	0.02 ± 0.01	123.51 ± 0.83	120.85 ± 0.56
7	EAC	0.14 ± 0.03	0.16 ± 0.01	10.71 0.38	9.31 ±0.36	0.03 ± 0.01	0.02 ± 0.02	125.45 ± 0.72	122.03 ± 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	

One way ANOVA, ## Two way ANOVA The values are the mean ± SD of six animals

Effect of TpFf on the histological status of Spleen and Thymus in DLA and EAC challenged mice:

Plate 1 Histological status of Spleen

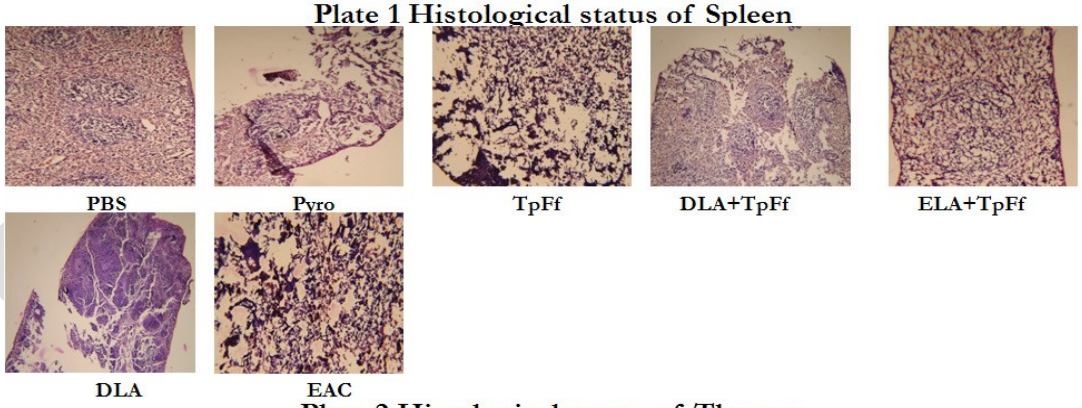
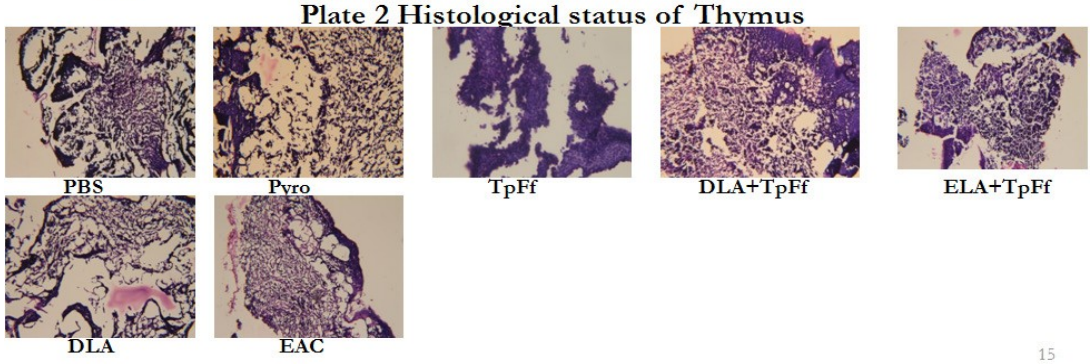


Plate 2 Histological status of Thymus



Histological examination of tissues indicated the malignant changes observed in the DLA and EAC tumor induced spleen and thymus sections as given in Plate 1 and 2.

CONCLUSION

All the above parameters conforms the immunomodulatory activity of the TpFf towards DLA and EAC tumors.

REFERENCES

1. Chang C, Yang M, Wen H and Chern J. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods (2002), J Food & Drug Analysis, 10(3): 178-182.
2. Culling CFA. Handbook of histophathology and histochemistry techniques (1974), III Edition, Butterworths and Co (Publishers) Ltd., London, 115-117.
3. Jeba RC, Vaidyanathan R and Rameshkumar G. Efficacy of *Ocimum basilicum* for immunomodulatory activity in wistar albino rat (2011), IJPPS, 3: 199-203.
4. Manu KA and Kuttan G. Immunomodulatory activities of Punarnavine, an alkaloid from *Boerhaavia diffusa* (2009), Immunopharmacology and Immunotoxicology, 2: 377–387.
5. *Palanivel MG, Rajkapoor B, Kumar RS, Einstein JK, Kumar EP, Kumar MP, Kavitha K, Kumar MP and Jayakar B. Hepatoprotective and antioxidant effect* Pisonia aculeata *L. against* CCl_4 *induced hepatic damage in rats (2008), Scientia Pharmaceutica,76: 203-215.*
6. Premanathan M, Rajendran S, Ramanathan T, Kathiresan K, Nakashima H and Yamamoto N. A survey of some Indian medicinal plants for anti-human immunodeficiency virus (HIV) activity (2000), Indian J Med Res., 112: 73-7.
7. Salomi MJ and Panikkar KR. Cytotoxic action of *Nigella sativa* seeds (1989), Research Bulletin, 11: 60-63.
8. Santhi R, Lakshmi G, Priyadarshini AM and Anandaraj L. Phytochemical screening of *Nerium oleander* Linn. leaves and *Momordica charantia* leaves (2011), International research journal of pharmacy, 2(1): 131-