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IMMUNOMODULATORY EFFECT OF *ANDROGRAPHIS PANICULATA* NEES ON HUMAN PERIPHERAL BLOOD MONOCYTES

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ABSTRACT

The assessment of immunomodulatory effect of aerial parts of *Andrographis paniculata* extracted with various organic solvents on stimulated human peripheral blood Monocytes revealed the most significant inhibitory effect only with aqueous extract. Further, it was also proved that the effect is exclusively due to the drug and not because of cytotoxicity.

Keywords: *Andrographis paniculata* Nees, immunomodulatory effect, human, peripheral blood monocytes.

INTRODUCTION

Andrographis paniculata Nees a potent immunostimulator exhibits both antigen specific and non specific immune responses and hence effective against a variety of infectious and ongogenic agents (Puri *et al*., 1993). In a recent study, Xu *et al*. (2007) have explored that the gavages of mice immunized with an inactivated *Salmonella typhimurium* vaccine with *A. paniculata* extract / andrographolide (AD), the most bitter compound and medicinally active phytochemical (Sharma *et al*., 1992), specifically rated very high for its therapeutic action (Siddhartha *et al*., 2007), resulted in an enhancement of *Salmonella* - specific antibody response and induction of cell mediated response against salmonellosis. Shen *et al*. (2002) have reported that the AD inhibits inflammatory responses by rat neutrophils. An ethanol extract of aerial parts (500 mg / kg body weight) of *A. paniculata* on intragastric administration decreased yeast – induced pyrexia in rat (Vedavathy and Rao, 1991). Andrographolide also exhibits antiinflammatory property by modulating macrophage and neutrophil activity (Chiou *et al*., 1998; 2000; Shen *et al*., 2000; 2002; Sheeja *et al*., 2006). Thus the extracts of aerial parts of *A. paniculata* / andrographolide have been proved to modulate various biological / immunological processes / responses. Hence, the present study not only attempts to screen the drugs prepared from the extracts of aerial parts of *A. paniculata* for their immunomodulatory effect on human peripheral blood monocyte (PBMC) / lymphocyte proliferation, but also for their cytotoxic effect on PBMC under *in vitro* condition.

MATERIALS AND METHODS

Collection of Plant Material

Andrographis paniculata Nees was collected from Marunduval Mallai Hills, Kanyakumari District, Tamil Nadu, India , known for its rich collection of medicinal plants.

Sequential Extraction

The fine powder prepared from the dried aerial parts of the plants was subjected to sequential extraction (Manjula *et al*., 2005) with various organic solvents (Hexane, Dichloromethane, Ethyl acetate, Methanol and Water), ranging from non polar to polar (polarity index 0, 2.5, 6 and 8) in 1: 5 ratio (w/v). Each of these extracts was concentrated in a Rota evaporator.

Drug Stock Solution

The drug was prepared by the addition of 20 mg dried extracts of various solvents with 250 ml DMSO.

Drug Dilution

The drug was further diluted to obtain the final concentration of 100, 50, 20, 10 and 1 µg / ml with respective solvents (Manjula *et al.,* 2005).

Table 1. Effect of *A. paniculata* drug on induced human PBMC / lymphocyte Proliferation.

S.No.	Conc. (µg / ml)	No of cells and % of inhibition with various drugs									
		Hexane	% of inhibition	DCM	% of inhibition	Ethyl acetate	% of inhibition	Methanol	% of inhibition	Aqueous	% of inhibition
1.	Control	2156		2156		2156		2156		2156	
2.	LPA	22436		22436		22436		22436		22436	
3.	100	16114 ± 0.33	28	17116 ± 0.67	23	15588 ± 0.33	30	10588 ± 0.67	52	6808 ± 0.67	69
4.	50	19002 ± 0.82	15	20434 ± 0.67	8	19984 ± 0.67	10	12857 ± 0.67	42	8808 ± 1.00	60
5.	20	20248 ± 0.58	10	21257 ± 0.67	5	21151 ± 0.67	5	17117 ± 0.58	23	10552 ± 0.67	52
6.	10	21751 ± 0.82	3	21687 ± 0.19	3	21551 ± 0.33	3	19596 ± 0.67	12	10857 ± 1.00	51
7.	1	22433 ± 0.05	1	21967 ± 0.33	2	21669 ± 0.58	3	20434 ± 1.00	8	11550 ± 0.67	48

Table. 2. Cytotoxicity effects of Aqueous extract of *A. paniculata* on human PBMC / lymphocytes by MTT assay.

S.No.	Conc. (µg / ml)	% Cytotoxicity
1.	100	9
2.	50	8
3.	20	5
4.	10	2
5.	1	2
6.	Control	85

Peripheral Blood Monocyte (PBMC) Assay

Monocytes isolated from the peripheral blood of a normal man diluted to 10^6 cells / ml were stimulated with (20 µg / ml) phytohaemagglutinin (PHA) and then cultured in RPMI 1640 medium, supplemented with penicillin G (200 µ ml), gentamycin (10 µg ml) and L.glutamine (0.3 mg ml). The PBMC suspension (100 µl) was then added to microtitre wells treated with 10 µl drug prepared in various solvents to a final concentration of 100, 50, 20, 10 and 1

µg / ml and incubated in a CO_2 Incubator for 18 hrs with 5 % CO_2. After an incubation period, 25 µl (H^3) TDR was added to the cells. Using a cell harvester the cells were then harvested over a filter paper followed with the addition of scintillation fluid. The cells were then counted on a scintillation counter. The experiments were carried out in triplicate.

MTT Assay

Cell viability was determined by MTT Assay. 100 µl MTT stock solution (5µg / ml) was added to 1 ml culture and incubated at 37 ^{o} C for 3 hrs. To which 1 ml 0.1 N HCl in absolute isopropanol was added and the absorbance of converted dye was measured at 570 nm (Stanilova *et al*., 2003).

RESULTS AND DISCUSSION

In the present study the yield of the extract of the aerial parts of *A.paniculata* was 50 % whereas Amirghofran *et al.* (2000) have obtained only 33.33 % yield from the flower heads of *Echium amoenum.*

PHA has been reported to be associated with unmodified production of T cell derived cytokines. Screening the drugs for their efficacy on stimulated PBMC / lymphocytes proliferation which in turn would elicit crucial immunological responses revealed the respective inhibition of 28, 15, 10, 3 and 1 % with hexane extract . Whereas the extract of dichloromethane exhibited 23, 8, 5, 3, and 2 % inhibition. Ethyl acetate extract had shown 30, 10, 5, 3 and 3 % inhibition. While the drug extracted with methanol exhibited 52, 42, 23, 12 and 8 % inhibition, the aqueous extract had shown the highest inhibition of 69, 60, 52, 51, and 48 % with respective concentrations (100, 50, 20, 10, 1, µg / ml) (Table 1). Thus the most significant inhibitory effect was recorded with the crude aqueous extract / drug of *A. paniculata*. The similar results have been reported with aqueous extracts of *Azadirachta indica* (Van *et al*., 1987) , *Mumromia pumila* (Labadie *et al*., 1989), *Wia azederach* and *Cedrela tubitora* Bost (Benencia *et al*., 1994) . The aqueous extract / drug of *A .paniculata* was then subjected to cytotoxicity assay. In general, the drug is said to be toxic to cells when the cytotoxicity is more than 20 % (Payne , 2003). Where as in the present study the aqueous extract / drug showed only 9 % inhibition which confirmed that the effect is exclusively due to the drug and not because of the cytotoxicity (Table 2). Interestingly, the aqueous extract of *A. paniculata* has been widely used for the isolation of AD in many pharmacological preparations / investigations.

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