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Effect of different immunomodulator drugs in combination for treatment of HIV-AIDS

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ABTRACT

The plant use as a immunomodulator namely Andrographis paniculata, Momardica charantia, Phyllanthus niruri, Terminallia chebula, Glycyrrhiza glabra, Punica granatum. It has good impact in the treatment and management of HIV-AIDS because these plant not only treats disease but also enhance the body vitality and immunity. The humoral and cell-mediated immune response was observed through Delayed Type Hypersensitivity (DTH) model. This formulation has studied on swiss albino mice in normal and suppressed immune system by Cyclophosphamide (CP) in formulation (F1) and (F2) doses it shown maximum decrease in foot paw oedema from 2.15mm to 0.35 mm, similarly maximum increase in the WBC and Platelet count upto 10.4±0.07 thousand / mm^3 and 96142 ±142 in (F1) respectively. It shown signi ficant statistical analysis P<0.001.

Keywords: HIV-AIDS, immunity, immune response, Humoral and cell mediated immunity.

INTRODUCTION

HIV virus attacks and impairs the body's natural defense system against disease and infection. The underlying problem identified as a severe depression of immune system that is cause by the nearly complete lack of one class of T-lymphocyte, which are called helper cell that are needed for initiating and maintaining many immune responses.[1, 2.]

The world health organization estimated that, in the year 2007, the cumulative total infected were 33.2 millions. Numbers of people newly infected in each year are 2.5 million and 2.1 million people dying by AIDS in each year. Every day over 6800 persons become infected with HIV and over 5700 persons dying from AIDS.[3] So there is a need to control the death of people and this

formulation have negligible side effect and have potential to strengthen immune system and suppressed the virus so it cannot detect in the blood and it prolong the life of human beings.

MATERIALS AND METHODS

Selection of the crude drugs have been done after the extensive review of the literature taking into consideration the specific activity of active constituent present in the medicinal plant. Some of the crude drugs have also been added for their immunostimulant or tonic effect, or simply as a bioavailability enhancer[4,5,6]. The medicinal plants selected are given in table 1.

Table 1 : The medicinal plants used in Herbal Formulation

Sr. No	Name of plant	Plant Part Used	Active constituents	Activity
1	*Andrographis paniculata*	Aerial part	Andrographolide, bicyclic diterpenoid lactone and kalmeghin	Immunostimulant Inhibite syncytium formation, Anti HIV activity.
2	*Momardica charantia*	Seeds	MAP 30 (Momardica Anti HIV protein), Alpha momarcharin, MRK 29 (RIP), momardicosides	RT activity, inhibits HIV-1 infection, syncytium formation, herpes simplex virus, HIV-1 integrase, Beta glucosidase inhibitory.
3	*Phyllanthus niruri*	Whole plant	Phyllanthin, Hypophyllanthin, Rapendusinic acid A monosodium salt (RA)	HIV-1 inhibitor, Inhibite HIV-1 RT ,
4	*Terminallia chebula*	Fruit	Gallic acid, Ellagic acid,Chebulic acid, Galloyl glucoses	HIV-1 Integrase, Inhibitor of HIV-1 Protease
5	*Glycyrrhiza glabra*	Roots	Glycyrrhizin	Anti HIV activity, Inhibite HIV induce plaque formation.
6	*Punica granatum*	Bark	Punicalin and Punicoretin	HIV-1 RT activity.

Preparation of Herbal Formulation

The quantity of extracts required for formulating herbal drug formulation (Table 2) are calculated on the basis of human dose of powder form and percentage practical yield of respective crude drugs. Three formulations are prepared using 2% w/v gum tragacanth as suspending agent and considered as Lower dose, Average dose and Higher dose formulation[7].

Table 2 : Quantity of plant extracts used for preparing herbal formulations F1, F2, F3

Sr. No.	Extract Name	Quantity of Extract mg/kg(F1)	Quantity of Extract mg/kg (F2)
1	*Andrographis paniculata*	155	312
2	*Momardica charantia*	130	392
3	*Phyllanthus niruri*	550	1100
4	*Terminallia chebula*	425	851
5	*Glycyrrhiza glabra*	524	1049
6	*Punica granatum*	513	1026

Animal

The experimental protocol was submitted and approved by Institutional Ethical Committee (IAEC No. 648/02/C/CPCSEA), J. L. C. College pharmacy, Nagpur.

Albino mice (Swiss) of either sex weighing between 20-25 g were employed in this investigation. They were housed under standard conditions of temperature 22^0C (± 3^0C) humidity 35 % to 60 %, and light (12:12 hr light/dark cycle) in polypropylene mice cage.

Cyclophosphamide (High-media.) was used as standard immunosuppressant. Carbon ink suspension- Pelikan 4001, Germany black ink was diluted eight times in a dose of 10 µl/gm body weight of mice (Bafana, A., 2004). Antigenic material - The sheep RBCs (SRBCs) were used as antigenic material. The sheep blood was withdrawn from external jugular vein of sheep (Government Vetneiry college, Nagpur). It was mixed in 1:1 proportion in Alsever's solution & stored at 2^0 to 8^0C in refrigerator.

Procedure

Swiss albino mice (HA strain) weighing between 20-30gm were brought. All mice were marked with picric acid and randomly divided into eight groups, each group comprising of six animals. Weight of individual mice was taken on electrical signal pan balance and numbering was done to each mice.

Preparation of SRBC suspension

Sheep blood was collected from Veterinary College in Alsevar's solution (1:1) and centrifuged at 2500-3000 rpm for 10 minutes. Supernatant was removed with Pasture pipette, and packed SRBCs were washed thrice with sterile Alsevar's solution. The resulting SRBCs were suspended in sterile Alsevar's solution to obtain a cell density of 10^6 SRBCs/mm^3, using improved Neubaur chamber[8].

Preparation of suspension of dose of herbal formulation

Three formulations were prepared considering Lower, Average, and Higher in distilled water using 2% w/v gum tragacanth as a suspending agent.

Preparation of solution of Cyclophosphamide

30 mg / kg solution of cyclophosphamide was prepared in sterile normal saline[8].

Pharmacological Study

Toxicity Study

Toxicity studies of Herbal drug formulations were carried out in swiss albino mice according to OECD guideline 423. Dose ranging between 500, 1000, 2000, 4000 and 5000 mg/kg of body wt. of formulation were administered stepwise to the mice according to their weights. The mice were observed individually after at least once during the first 30 minutes, periodically during the first 24 hrs, there was no mortality found till dose of 5000 mg/kg body weight in formulation F1 and F2 [8].

Delayed Type Hypersensitivity Model

Procedure

On day zero Cyclophosphamide (CP) 30 mg/kg was administrated IP to the animal of group iv, v, vi 2 hrs before sensitizing with 1x 10^6 SRBCs, as antigen, through IP route .

All the group were treated as per the table 3 for next five days, i.e. day 1 to day 5. All the animals were maintained on same diet and environment throughout the duration of the experiment. Administration of extract was done by oral route using animal feeding needle and 1 ml syringe.

On day 5 the left hind paw thickness was measured for all the animals. The animals were then challenged with the same antigen SRBCs, 1x10^6 in 0.1 ml, in the left hind paw by SC route. The Paw thickness measurement was again done at interval of 24, 48, 72 and 96 hrs from the challenge.

On the day 10 all mice were anaesthetized by putting in an anesthetic ether chamber. Blood was collected from the retro obituary vein for WBC and Total Platelet Count[8].

Table 3: Procedure for Treatment in DTH model

Day 0	Group IV- VI	30 mg/kg of CP by IP route, 2 hrs before Sensitization
	Group I-VI	Sensitization with 1x10^6 SRBCs by IP route
Day 1 To Day 5	Group I and IV	2% w/v gum tragacanth
	Group II and V	F1: Lower dose
	Group III and VI	F2: Average dose
Day 5	Group I- VI	Measurement of Paw thickness
		Challenge with 1x10^6 SRBCs by SC route
Day 6 to Day 9	Group I- VI	Measurement of Paw thickness
Day 10	Group I- VI	Collection of Blood for WBC and Total Platelet Count

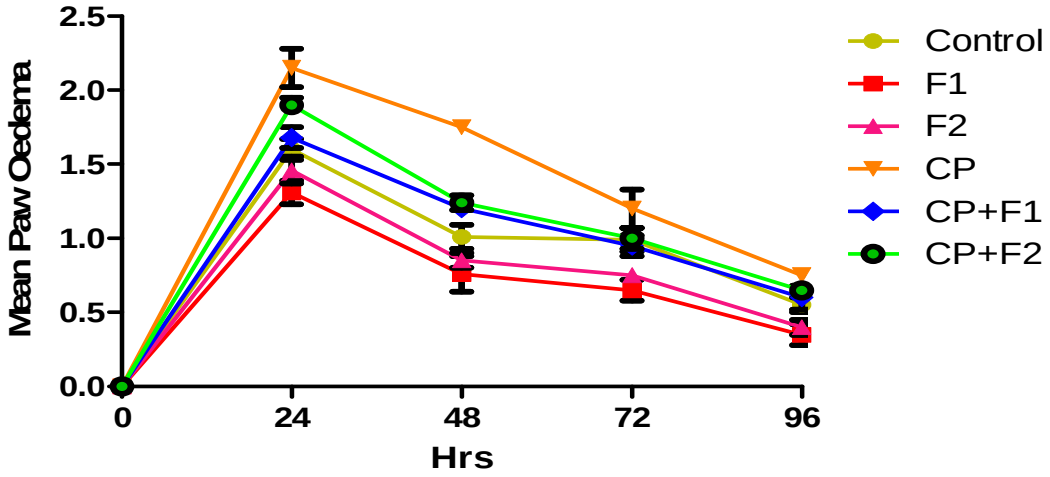

Fig1: Effect of Prepared Formulation on Foot Paw Oedema.

Table 5: Effect of Prepared Formulation on Leukocyte and Platelet Count in DTH model

Sr. No.	Groups	Haematological Parameters	
		Leukocyte Count (Thousand/mm^3)	Platelet Count (Thousand/mm^3)
I.	Control	9.0 ± 0.1*	77180 ± 320*
II.	F1	11.23 ± 0.4*	99182 ± 242*
III.	F2	9.9 ± 0.2	92122 ± 270
V.	CP	5.2 ± 0.27*	52329 ± 410*
VI.	CP+F1	8.25 ± 0.27*	76201 ± 350*
VII.	CP+F2	7.07± 0.32	64038 ± 375

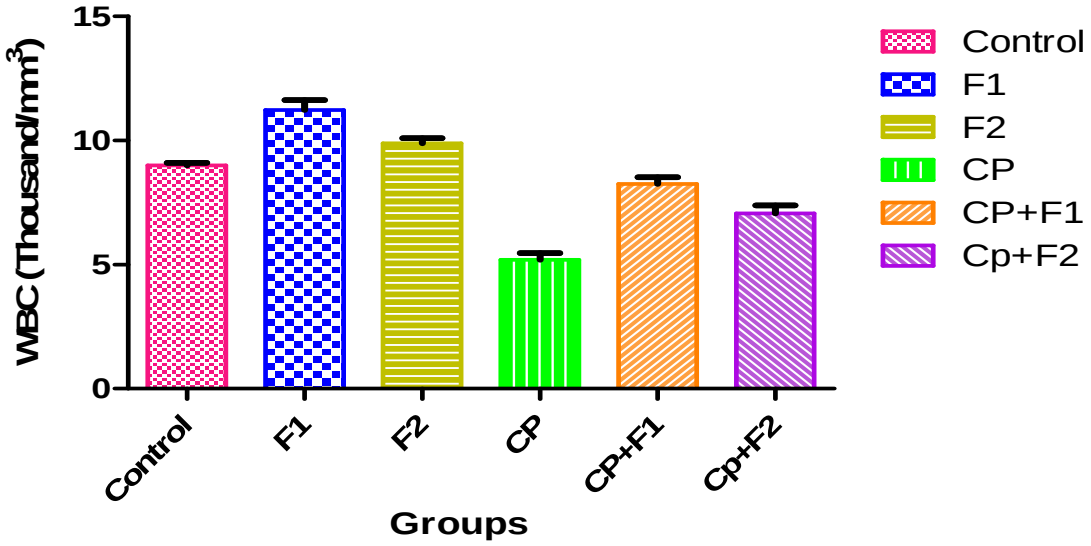

Fig 2: Effect of Prepared Formulation on WBC Count.

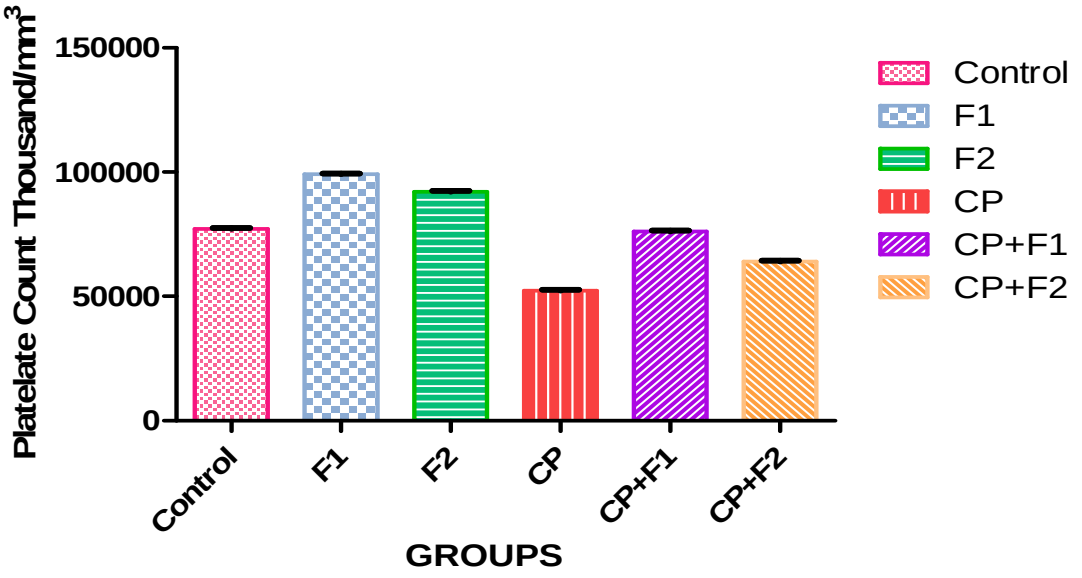

Fig 3: Effect of Prepared Formulation on Platelate Count.

Table 4: Effect of Prepared Herbal Formulations on Mean Foot Pad Oedema in DTH model

Gp. No.	Gp. Description	Mean Foot Paw Oedema			
		24 hrs	48hrs	72 hrs	96hrs
I.	Control	1.6 ± 0.07*	1.01 ± 0.08*	0.99 ± 0.08 *	0.55 ± 0.5*
II.	F1	1.31 ± 0.08*	0.76 ± 0.12*	0.65 ± 0.07*	0.35 ± 0.07*
III.	F2	1.46 ± 0.09	0.85 ± 0.05	0.75 ± 0.03	0.40 ± 0.05
IV.	CP	2.15 ± 0.13*	1.75 ± 0.02*	1.20 ± 0.13*	0.75 ± 0.04*
V.	CP+F1	1.68 ± 0.07*	1.20 ± 0.02*	0.95 ± 0.07*	0.60 ± 0.08*
VI.	CP+F2	1.90 ± 0.05	1.24 ± 0.05	1.0 ± 0.07	0.65 ± 0.04

RESULTS AND DISCUSSION

The formulations have shown immunostimulant activity in DTH model, when used alone and also a significant immunostimulant activity in the animals whose immunity was suppressed using cyclophosphamide.

The crude drugs viz. namely *Andrographis paniculata, Momardica charantia, Phyllanthus niruri, Terminallia chebula, Glycyrrhiza glabra, Punica granatum* which have been collected and authenticated.

The crude drugs were shredded and powder to a coarse powder consistency, which was subjected to extraction by using suitable solvents. The solvent and method of extraction was selected such that the resultant extract contains the active constituents. The extract obtained were concentrated and dried in desiccator or by sun draying process.

All the individual dried extracts were checked for their active ingredients by proximate chemical analysis. It showed that all extract contains required active constituent.

All the extracts were subjected to chromatographic evaluation to check possible number of component in respective extracts using thin layer chromatography technique. The extracts of *Andrographis paniculata* was compared with standard samples of *A. paniculata* by their R_f values. For the design and preparation of an effective immunostimulant herbal formulation, all the individual dried extracts were mixed in a requisite amount and 2% w/v Gum tragacanth was added to the formulation as a suspending agent. The quantities of extracts were calculated on the basis of human dose of powder form of drugs and their respective percentage yield.

The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 5000 mg/kg of body wt./mice with no toxic reaction being observed.

The immunostimulant activity of the prepared herbal formulations was studied using delayed type hypersensitivity (DTH) model and carbon clearance test in mice. The results obtained from the DTH model have indicated significant decrease in the mean foot paw oedema, after challenging with 10^6 SRBC, when plotted against time. The CP receiving group has shown maximum oedema of 2.15 ± 0.13 mm after 24 hrs, of challenge, deceasing to 0.75 ± 0.04 mm after 96 hrs. The group receiving F_1 have shown significantly lower values of mean foot paw oedema as compared with the controlled group, indicating a strong immunostimulant effect of formulation F1 than that F2. The group receiving CP + F1 has also shown marked decreased in

the mean foot paw oedema as compared with the CP receiving group confirming the immunostimulant effect of the formulations in the suppressed immune system. Results of WBC and Platelet counts for the animals receiving F1 and F2 have also shown significant increasing in count as compared to the control group animals. The results of CP + F1 and CP + F2 groups have shown a marked increase in the Platelet counts respectively, as compared with the CP treated group. This clearly indicates that the formulation has a strong immunostimulant activity on the suppressed immune system. The result obtained during the present investigation showed that there is significant antibody production in response to SRBCs in formulation F1.

CONCLUSION

The herbal formulation designed and developed as potential immunostimulant has been found to be safe up to a very high dose of 5000 mg/kg/day/mice during toxicity studies. The formulations have shown immunostimulant activity in DTH model, when used alone and also a significant immunostimulant activity in the animals whose immunity was suppressed using cyclophosphamide. Thus the formulations under study have the ability to recover the suppressed immune system. There it can be used for the treatment and management of AIDS and AIDS related complex in which the immune system is suppressed or deranged by the HIV-infection.

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