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Effects on the Selected Herbal Plant Syzygium Aromaticum Flower Buds Oil of Immunomodulatory Activity in Albino Wistar Rats

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Abstract: ***The effect of Syzygium aromaticum flower buds oil were evaluated for immunomodulatory activity in in vivo studies, using rats as the animal model. Cyclophosphamide given for the VII group showed a very weak response, because of its immunosuppressive nature. The essential oils were tested for hypersensitivity and hemagglutination reactions, using sheep red blood cells (SRBC) as the antigen while sodium carboxy methyl cellulose (SCMC) served as the control in all the tests. Orally administrated essential oils showed a significant increase of test parameters, viz., haemagglutinating antibody titre (HAT) and delayed type hypersensitivity (DTH) response. In rats immunized with sheep RBC, essential oils enhanced the humoral antibody response to the antigen and significantly potentiated the cellular immunity by facilitating the foot pad thickness response to sheep RBC in sensitized rats with these differences dose II 50-800 VI were statistically significant. Myelosuppression is a decrease in the production of blood cells. Cyclophosphamide is a most potent cytotoxic and immunosuppressive agent which act at various levels on cells involved in defense mechanism against various invaders by inhibiting both cell mediated and humoral immunity. It also causes dose dependant bone marrow suppression means it significantly decreases the Hemoglobin, Erythrocyte and Leukocyte count. Cyclophosphamide treatment for the period of 3 days showed reduction in Hemoglobin Erythrocyte and Leukocyte count there by exerted immunosuppressant effect when compared to control animals.***

Keywords: Syzygium aromaticum flower buds oil, Cyclophosphamide, Sodium Carboxy Methyl Cellulose (SCMC), Delayed type hypersensitivity(DTH), Haemagglutinating antibody titre (HAT),

1. Introduction

Traditional Indian systems of medicines like Siddha and Ayurveda have suggested means to increase the body's natural resistance to disease. A number of Indian medicinal plants and various 'Rasayanas' have been claimed to possess immunomodulatory activity (Atal *et al*.,1986, Patwardhan *et al*.,1990, Puri *et al*.,1994, Ziauddin *et al*.,1996). Herbal drugs possess immunomodulatory property and generally act by stimulating both specific and non specific immunity (Wagner and Proksh,1985). Immunology is a branch of microbiology and is defined as the study of defence mechanism of the body against harmful invading causes (Annadurai,2009).

Immunology is one of the most developing and crucial area of biomedical research. It also opens the doors of new hopes and major advances in the prevention and treatment in wide range of disorders. Arthritis, ulcerative colitis, asthma, allergy, parasitic and infectious diseases are primarily considered as immunologic disorders(Herausgegeben,1984, Samter,1971).

Severe side effects and cost of the allopathic drugs have attracted most of the researchers to find out the drugs which are without side effects especially belonging to the traditional systems of medicines. Herbal medicines have been the foundation of treatment and cure for various ailments. Natural products provide an excellent material for the discovery and development of novel immunomodulatory compounds. There is a growing interest in identifying and characterizing natural compounds with immunomodulatory activity ever since their possible use in modern medicine has been suggested (Lee *et al.,* 1995).A large number of plants and their isolated constituents have been shown to potentiate immunity (Savnur, 1950 Bhagwandash.,1978). Medicinal plants have been shown to exert anti-inflammatory, anti diabetic, anti-stress and anti-cancer effects by modulating the immune functions (Singh and Atal, 1986; Thatte, 1996). The protective effect of Syzygium aromaticum flower buds oil (Debjit Bhowmik *et al.*,2012),

2. Material and Methods

To study the immunomodulatory activity, 2.5% syzygium aromaticum flower buds oil was suspended in 1% sodium carboxy methyl cellulose (SCMC) to prepare suitable dosage forms. The control animals were given an equivalent volume of the sodium carboxy methylcellulose vehicle without essential oils. Cyclophosphamide was used as a standard immunosuppressant agent.

Antigen:
Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep Red Blood Cells (SRBCs) were washed three times in normal saline and adjusted to a concentration of 0.1 mL containing 1×10^8 cells for immunization and challenge

Humoral Antibody (HA) response
Humoral Antibody (HA) response was identified using the method described by Puri *et al.* (1994) was adopted. Rat

were divided into seven groups, each group containing six rat. Drugs were administered in various groups, i.e. Group I – Control (Sodium carboxy methyl cellulose (SCMC) 1%), Group II – VI test extracts I (5 dose levels 50 – 800 mg/kg p.o.) and Group VII- standard drug (Cyclophosphamide 50 mg/kg, p.o.).

The animals were immunized by injecting 0.1 mL of SRBCs suspension containing 1×10^8 cells intraperitoneally on day 0. Blood samples were collected from individual animal by retro-orbital puncture on day 7. The blood samples were centrifuged and serum was obtained. Antibody levels were determined by the haemagglutination technique. Briefly, equal volumes of individual serum samples of each group were pooled. To serial two fold dilutions of pooled serum samples made in 25 µL of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37°C for 1h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre.

Delayed Type Hypersensitivity (DTH)

Delayed type hypersensitivity was assessed using rat**.** On day 7, the thickness of the right hind foot pad was measured using vernier caliper. The rat were then challenged by injection of $1 \times 10^{7-8}$ SRBCs in right hind foot pad. Foot thickness was measured again 24 h after this challenge. The difference between the pre and post challenge foot thickness expressed in mm was taken as a measure of DTH. The extract was administered orally on day 0 and continued till day 7th of challenge. Cyclophosphamide was administered on day 4 to 6th day.

Effect of essential oil and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in rats – 7 days pre-treatment. Rats were divided into six groups, each group containing six rats. The Group I - was control (Sodium carboxy methyl cellulose 1%), Group II - VI essential oils I (5 dose levels 50-800 mg/kg p.o.) The pretreatment time of 15 days was based on the method described Schedule for drug administration was 7 days prior to immunization (days – 6, - 5, -4, -3, -2, -1, 0) and 7 days after immunization (days +1, +2, +3, +4, +5, +6, +7). The procedure of immunization by injecting SRBCs suspension, collection of blood sample for haemagglutination and measurement of inflammation above was followed as described.

Cyclophosphamide Induced Myelosuppression Experimental Design

Animals were divided into different groups each containing 6 animals.

The Group I - was control (Sodium carboxy methyl cellulose 1%),

Group II- IV **Syzygium aromaticum flower buds oil**,(200, 400, 800 mg/kg p.o.) respectively (1st to 13th day)

Group V- (Cyclophosphamide 30 mg/kg, p.o.). (11th, 12th, 13th day)

On 0th day, blood was withdrawn from retro-orbital plexus of animals of each group and subjected to haematological parameter determination. Drugs were fed as per the schedule from 1st to 13th day. Cyclophosphamide (30 mg/kg, p.o) was given to all animals, on 11th, 12th and 13th day, 1 hr after extracts administration except control group. On day 14th, blood was again withdrawn from retro- orbital plexus of animals of each group and subjected to haematological parameter Haemoglobin, Erythrocyte count, Leukocyte count determination and restoration of parameters were observed (Gokhale et al.,2003, Kumar *et al*.,2003, Bafna and Mishra, 2011).

3. Results and Discussion

The basic function of the immune system is to protect the individual against infectious agent and potential pathogens which puts the immune system in a vital position between a healthy and diseased sate of host. Immunomodulators and immunosuppresants. Immunoadjuvants are used to increase the efficacy of vaccines and since specific immunoadjuvants are used with specific vaccines, therefore could be considered as specific immunostimulants. Immunostimulants by definition are inherently non specific in nature as they are envisaged to enhances bodys resistance against infection. They can act through innate immune response and through adaptive immune response. Immunosuppressant could be used for control pathological immune response and are active in auto immune diseases, immediate and delayed type of hypersensitivity immune reactions and graft rejection(Allison,1997). Naturally produced medicinal plant product offer as an alternate immunomodulatory and therapeutic agents so as to overcome some of these hazards such as their non- availability in some developing countries, risk of misuse leading to drug resistance, environmental pollution and food residues and subsequently may be sustainable and environmentally acceptable. In clinical medicine both aspect of immunomodulation viz. immunostimulation and immunosupperssion are equally important. In conventional chemotherapy immunopotentiation is an ideal choice, when the host defense mechanisms are to be activated under condition of impaired immune response(Chatterjee *et al.*,1988).

In the present investigation immunomodulatory activity was studied for all the essential oils using rat model. In order to know their effectiveness on humoral immunity antibody production against SRBC was studied. In order to know the effectiveness on cell mediated immunity, delayed type hypersensitivity (DTH) was also analyzed. Sodium carboxy methyl cellulose (SCMC) is being a non immunogenic and tolerogenic substance, it has been considered as control group. Cyclophosphamide given for the VII group showed a very weak response, because of its immunosuppressive nature.

The results of immunomodulatory activity done with 7 days pretreatment are presented in (Table-1,Figure -1 and plate-1) the same experiment but done with 15 days pretreatment in (Table-2,Figure - 2 and plate-2) respectively. The result for *Syzygium aromaticum* flower buds oil as immunomodulatory agent has been analyzed using SRBC as antigen (pretreatment for 7 days) and presented in the antibody titre has been measured using haemagglutination (HA) test and it has been in increasing (8.4, 16.7,32.3, 64.5,128.6 and 256.5) proportionate to the concentration of oil dose up to 800

mg/kg. The DTH results for the same oil have also been identified.This has also been in increasing (0.26, 3.24, 3.65, 4.18, 4.78 and 4.34 mm) proportionate to the concentration of oil dose up to 800 mg/kg. Results of the batches of 7 days pretreatment have almost been comparable to 15 days pretreatment.

Cyclophosphamide Induced Myelosuppression

Myelosuppression is a decrease in the production of blood cells. Cyclophosphamide is a most potent cytotoxic and immunosuppressive agent which act at various levels on cells involved in defense mechanism against various invaders by inhibiting both cell mediated and humoral immunity. It also causes dose dependant bone marrow suppression means it significantly decreases the Hb, RBC, and WBC counts (Doerge *et al.*,1982, Lippincott *et al.*,2005). Cyclophosphamide treatment for the period of 3 days showed reduction in Hb and WBC count and thereby exerted immunosuppressant effect when compared to control animals.

Estimation of haemoglobin sahlis methods in (Table -3, Figure-3) the clinical significance cyclophosphamide induced rats, 0 days 12.58 gm% compared to 14th days 6.8 gms% decrease level in haemoglobin concentration in anemia. Then cyclophosphamide induced treated essential oil in syzygium aromaticum flower buds different concentration oil 200, 400 and 800 mg/kg on the days essential oil treated in rats improve on the normal level of the haemoglobin compared to control rats, effective on the essential oil in haemoglobin activity. Estimation of total RBC count (Direct methods) in (Table - 4, Figure – 4) the clinical significance cyclophosphamide induced rats 0 days 5.8 million cells/cu mm compared to 14 days 4.25 million cell/ cu mm decrease level in the number of circulating erythrocytes indicate erythropenia. Then another group cyclophosphamide induced treated with essential oil in syzygium aromaticum flower buds different concentration oil 200, 400 and 800 mg/kg on the days treated in rats erythropenia level improve on the normal level of the erythrocytes compared to control rats, effective on the essential oil in erythrocytes activity.

Estimation of total WBC count (Direct methods) in (Table - 5, Figure-5) the clinical significance cyclophosphamide induced rats 0 days 8.75 cell/cu mm compared to 14th days 3.24 cell/cu mm decrease in total WBC count indicate Leucopenia. Then another group cyclophosphamide induced treated with essential oil in syzygium aromaticum flower buds different concentration oil 200, 400 and 800 mg/kg on the days treated in rats Leucopenia level to improve normal level of the total WBC count compared to control rats effective on the essential oil in leukocyte activity.

The immunomodulatory effect of clove, Syzygium aromaticum (Family: Myrtaceae) essential oils was evaluated by studying humor- and cellmediated immune responses. Essential oils were administered to mice (once a day, orally, for a week) previously immunized with sheep red blood cells (SRBCs). Clove essential oil increased the total white blood cell (WBC) count and enhanced the delayed-type hypersensitivity (DTH) response in mice. Moreover, it restored cellular and humoral immune responses in cyclophosphamide - immunosuppressed mice in a dose-dependent manner. The findings were established that the immunostimulatory activity found in mice treated with clove essential oil is due to improvement in humor- and cellmediated immune response mechanisms (Carrasco *et al.*,2009).

4. Conclusion

The results have clearly indicate that the effective medicine selected plant possess syzygium aromaticum flower buds oil Immunomodulatory activity in the experimental groups of rats.

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Table 1: Effect of Syzygium aromaticum flower buds oil and Cyclophosphamide on HA titer and DTH response using SRBC as an antigen in rat 7 days pretreatment

Syzygium aromaticum flower buds oil Group/Dose Mg/Kg	Haem agglutination titer	DTH response mean paw edema in mm
I Control	8.4±0.44	0.26±0.04
II 50	16.7±0.34	3.24±0.58
III 100	32.3±0.38	3.65±0.53
IV 200	64.5±0.60	4.18±0.57
V 400	128.6±1.86	4.78±0.46
VI 800	256.5±3.65	4.34±0.52
VII 50	4.00±0.25	0.54±0.54

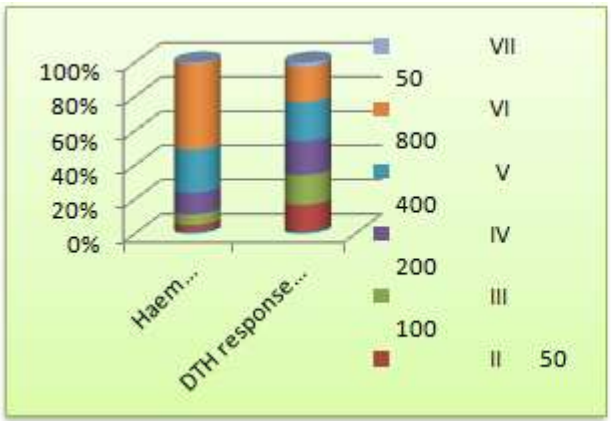
Different superscripts in the same column are significantly different at P<0.05 level (Least Significance Difference) mean followed by ± S.D.

Table 2: Effect of Syzygium aromaticum flower buds oil and Cyclophosphamide on HA titer and DTH response using SRBC as an antigen in rat 15 days pretreatment

Syzygium aromaticum flower buds oil Group / Dose Mg /Kg	Haem agglutination titer	DTH response mean paw edema in mm
I Control	16.3±0.64	0.29±0.04
II 50	32.5±1.12	3.12±0.24
III 100	64.4±1.54	3.86±0.35
IV 200	128.6±2.26	4.24±0.58
V 400	256.7±2.85	4.72±0.74
VI 800	512.5±3.21	4.46±0.46

Different superscripts in the same column are significantly different at P<0.05 level (Least Significance Difference) mean followed by ± S.D.

Immunomodulation study- Delayed type hypersensitivity (DTH) response in paw of rats (A-Strong response B-Normal)



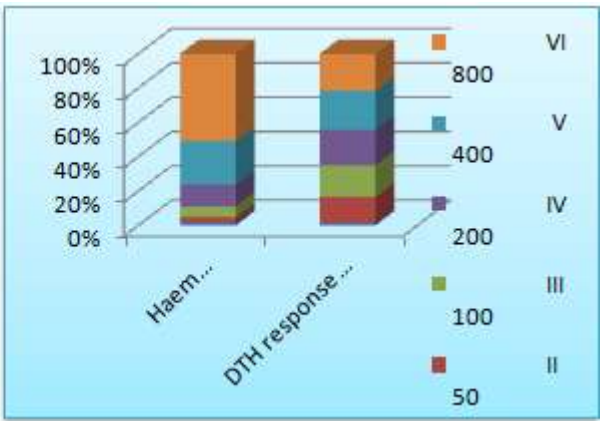


Plate 1 & 2: Effect of Syzygium aromaticum flower buds oil and Cyclophosphamide on HA titer and DTH response using SRBC as an antigen in rat 7 & 15 days pretreatment

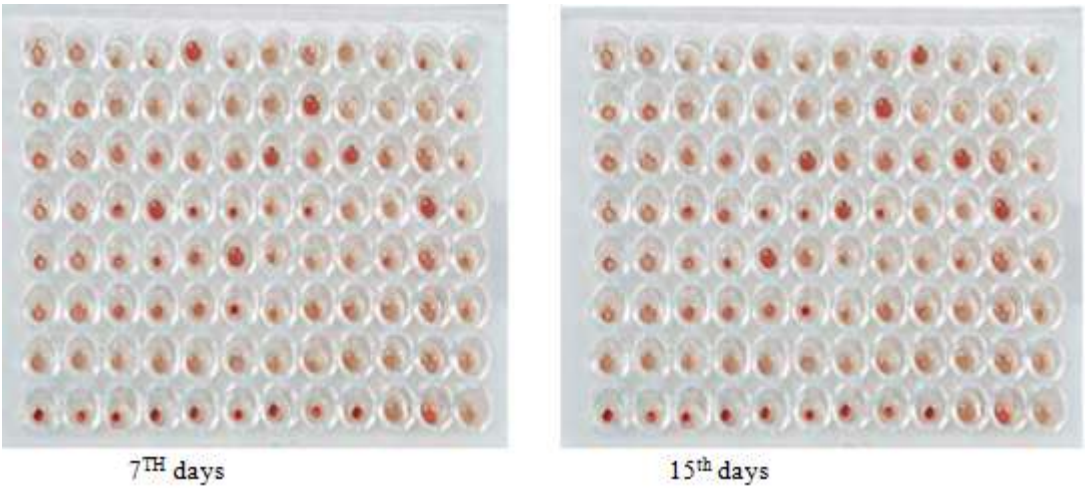


Table 3: Effect of Syzygium aromaticum flower buds oil on cyclophosphamide induced haemoglobin in rats

Syzygium aromaticum flower buds oil and Drug dose Mg/kg	HB (g/dl)	
	0 Day	14 Day
Control	12.18±0.28	12.0±0.51
Cyclophosphamide 50	12.58±0.75	6.8±0.56
SA+Cp 200	12.25±0.24	12.54±0.36
SA+Cp 400	12.56±0.28	12.85±0.54
SA+Cp 800	13.46±0.36	13.76±0.58

Different superscripts in the same column are significantly different at P<0.05 level (Least Significance Difference) mean followed by ± S.D.

Table 4: Effect of Syzygium aromaticum flower buds oil on cyclophosphamide induced RBC in rats

Syzygium aromaticum flower buds oil and Drug dose Mg/kg	RBC cells/cu mm	
	0 Day	14 Day
Control	5.27±0.06	6.24±0.21
Cyclophosphamide 50	5.83±0.24	4.25±0.16
SA+Cp 200	5.53±0.18	6.43±0.28
SA+Cp 400	5.58±0.20	6.00±0.26
SA+Cp 800	5.72±0.16	6.48±0.17

Different superscripts in the same column are significantly different at P<0.05 level (Least Significance Difference) mean followed by ± S.D.

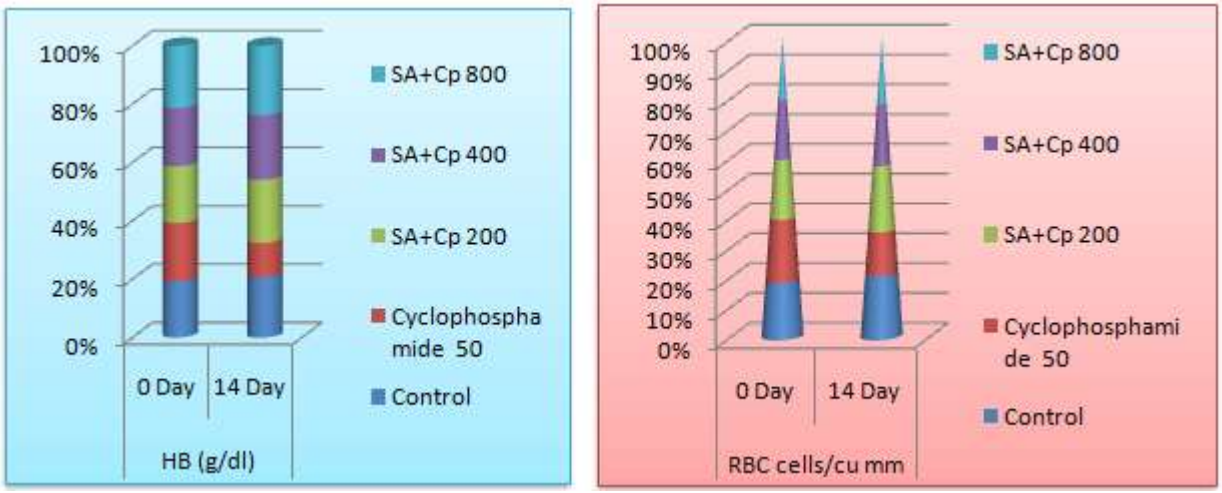


Figure 3 & 4: Effect of Syzygium aromaticum flower buds oil on cyclophosphamide induced haemoglobin and RBC in rats

Table 5: Effect of Syzygium aromaticum flower buds oil on cyclophosphamide induced WBC in rats

Syzygium aromaticum flower buds oil and Drug dose Mg/kg	WBC cells/cu mm	
	0 Day	14 Day
Control	8.55±0.74	9.3±0.46
Cyclophosphamide 50	8.75±0.27	3.24±0.48
SA+Cp 200	6.32±0.18	7.24±0.19
SA+Cp 400	6.81±0.25	7.65±0.23
SA+Cp 800	6.67±0.34	7.94±0.73

Different superscripts in the same column are significantly different at P<0.05 level (Least Significance Difference) mean followed by ± S.D.

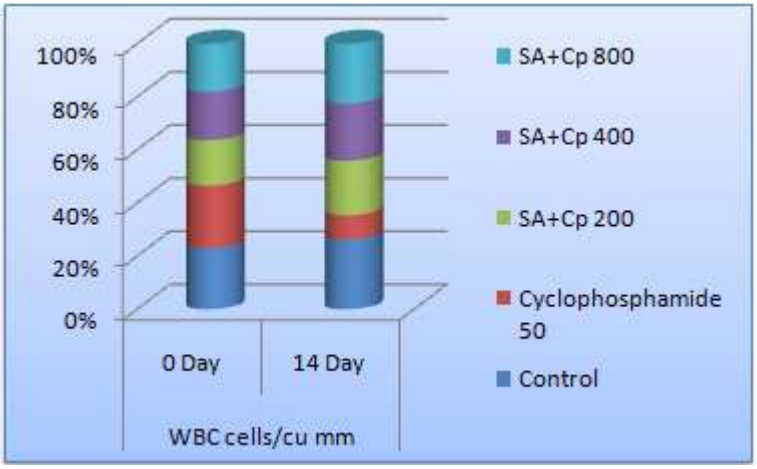


Figure 5: Effect of Syzygium aromaticum flower buds oil on cyclophosphamide induced WBC in rats