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Immunomodulatory Effect of Alcoholic Extract Of Five Faced Elaeocarpus Ganitrus Beads

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ABSTRACT:

Purpose: *To study the immunomodulatory activity of Alcoholic extracts of five faced Elaeocarpus Ganitrus beads (Elaeocarpaceae) on Male Wister rats.*

Methods: *Various parameters used for methodology were differential leukocyte count (DLC), Phagocytic activity and Znic Sulphate turbidty test.*

Results: *Oral administration of Elaeocarpus Ganitrus beads alcoholic extract (100mg/kg) was found to increase the neutrophils and lymphocytes as compared to vehicle and cyclophosphamide treated group. Elaeocarpus Ganitrus beads alcoholic extract showed linear time dependent significant phagocytic activities as compared SRBC sensitized and cyclophosphamide treated group. In Znic sulphate turbidity test Elaeocarpus Ganitrus treated rats serum showed more turbidity, which indicate the increase in the immunoglobulin level as compared to vehicle, SRBC sensitized and cyclophosphamide treated group.*

Conclusion: *The extract was found to be effective immunomodulatory agents*

Key Words*: Immunomodulatory activity, DLC, Phagocytic activity, Znic sulphate turbidity test, Elaeocarpus Ganitrus beads alcoholic extract.*

Introduction:
Elaeocarpus Ganitrus seed normally known as Rudrakasha is praised in Indian mythology for its therapeutic properties. The fruits of this plant possesses amazing medicinal property and have been used in Ayurvedic traditional medicine for the cure of mental diseases, epilepsy, asthma, hypertension, arthritis and liver diseases[1].As per Ayurvedic system of medicine, wearing *Rudraksha* beads relieves strain, insomnia, anxiety, lack of concentration, depression, palpitation, hypertension, rheumatism, infertility and asthma. It has also anti- aging effect. Different pharmacological research proves that it has activities *viz*., analgesic, antifungal, Anti-inflammatory, antimicrobial, antidiabetic, ant oxidative, antiviral, antitumor, antihypertensive, Antianxiety and antidepressant property[2-3].World Health Organization estimated that about 80% of the world population depends on traditional medicine for primary health care in which plants are the main source of medicine6. Plants are the important source of modern pharmaceutical drugs; nearly 25% of the pharmaceutically important drugs prescribed worldwide are derived from plants. The objective of the present study was to study the Immunomodulatory effect of alcoholic extract of five faced Elaeocarpus Ganitrus beads[4-5].

Material and Methods:

Plant material and Preparation of Extract: Genuine Five faced Elaeocarpus Ganitrus bead commonly known as Rudraksha were collected from online seller CHINTAN JOSHI 92/3, Bank Colony, Brahmeshwar Patna, Bhubaneswar (Orissa) Pin-751018 through EBay India in 2016 and further authenticated by X-Ray, water dipping technique in Maharana Pratap College of Pharmacy Lab. The coarse powers (400g) were extracted in Soxhlet apparatus using alcohol for 32hrs. The extract was then concentrated to dryness under reduced pressure by using rotary evaporator at 42-45^{0}C, yielded 16g of dry extract and preserved in a dessictor for further use.

Animals

Male Wister rats weighing 150-175g were collected from M.P.C.P animal house .The animals were housed under standard laboratory condition (Relative Humidity 55-65% room temperature 25.0 $\pm 2^0$C and 12h light dark cycle) and acclimatized for 7days. The animals were fed with standard diet and water. All experimental protocols were approved by the M.P.C.P College of Pharmacy animal ethics committee.

Animal grouping:
For experimental methodology, Male Wister rats were separated in following four groups containing six rats in each group.

Group-I (n=6): Negative control ,Male Wiser Rats were treated with 2ml 1% gum acacia solution in distilled water.

Group-II (n=6): Positive control ,Sensitized Male Wiser Rats (By administrating 1×10^8 SRBCs, I.P) were treated with 2ml 1% gum acacia solution orally.

Group-III (n=6), Male Wiser Rats were treated with Cyclophosphamide 100mg/kg/p.o.

Group-IV (n=6): Sensitized), Male Wiser rats treated with Elaeocarpus Ganitrus beads alcoholic extract 100 mg/Kg/p.o in the following regimens.

a) Four days prior to sensitization (-3,-2,-1,0)
b) 7 days after sensitization (days +1,+2,+3,+4,+5,+6,+7)

Methodology of Sheep Red Blood Cells (SRBC):

From healthy sheep blood was collected from local butcher house Kanpur and mixed with sterile Alsever's solution (1:1). It was assorted thoroughly by centrifugation at 3000 rpm for 5min. Supernatant was discarded; SRBC pellets were organized in phosphate buffer saline (pH7.2) couple of times. Then SRBC pellets were arranged in phosphate buffer saline (pH7.2) and total SRBC was counted using Neubauer chamber, finally 1×10^8 SRBC (0.5ml) were injected intraperitoneally for sensitization and challenging the rats[6-7].

Blood Profile for study of

Immunomodulatory Activity: Rats were divided into four groups as described as discussed .After 7days treatment, blood was collected from rats by retro-orbital plexus for study of diverse parameters.

Determination of different leukocyte count (DLC):

A drop of blood was added on the centre line of the glass slide about 1cm from one end and blood smear was organized .then smear was stained with diluted Leishman's stain for 30 min and washed with distilled water and dried at room temperature. For counting of DLC the slide was examined under microscope using Cedar wood oil. Finally total number of Neutrophils, Lymphocyte and monocytes in the 100 cells were counted and results were articulated in percentage [8-9].

In vitro Phagocytic Activity:

Preparation of Blood PMN cells

Separation of blood PMN cells was done as per the method described by .Blood sample (1ml) was collected by Retro orbital plexus in heparinised sterile tubes (20 IU Heparin/ml of blood). One part of blood was watered down with two part of sterile Tris-ammonium chloride buffer (pH7.3) and thoroughly mixed for 1-2 min and was kept for 20min at room temperature, Blood samples were centrifuged at 3000rpm for twenty minutes at room temperature . The supernatant was discarded and cell pellets were removed with 5ml sterile chilled Phosphate buffer solution (PBS) pH7.4 equal to pH of human blood. Then the solution was further centrifuged for 10 min in the same procedure twice to get the PMN cell pellet. The pellet obtained was resuspended in 1ml of sterile cold PBS[10-11].

Preparation of Microorganism

Escherichia coli (NCIM 2391) was grown and kept on slant agar media in M.P.C.P lab. Before use, the microorganism was in inoculated in 100ml of 2.5% nutrient both media for 18hrs at 37 ± 2^0C.The culture was then washed twice with sterile PBS (pH 7.2) and resuspended in 1ml gelatin HBSS (Hank's Buffer Salt Solution) to get a concentration of 1×10^7cells/ml. During each experimentation the number of viable microorganism were determined by counting colony forming units (CFU), using nutrient agar plate[12-13].

Viable PMN Cell Count:

The viable cell count were determined by Trypan blue exclusion techniques .20 µl each of cell suspension and 0.1% trypan blue were mixed and kept for 2min at room temperature .A drop of combination was loaded on haemocytometer, the viable (unstained) and dead (stained) cells were counted in WBC counting chamber

Microbiological Assay for the Phagocytosis Activity

To assess Phagocytosis and five faced Elaeocarpus Ganitrus beads alcoholic extract (100 µg/ml) in the final volume of 0.1ml were incubated with 2ml of PMNs suspension(1×10^7cells/ml) and 2ml of microorganism (1×10^7cells/ml) at 37 ± 2^0C for 1hr in 5% CO_2 atmosphere in a slanting position .1ml of the standard drug ,cyclophosphamide (100mg/ml) was incubated with fetal calf serum in the same condition .At 3o min gap up to120 min,0.5ml aliquot of the suspension was aloof and added to 1.5ml of the ice cooled gelatin –HBSS to stop Phagocytosis. The control was run using gelatin –HBSS in place of the trial compounds. These samples were centrifuged at 100g for 4four min. under this environment the non-ingested

microorganism remained in the supernatant fluid. The viable count of the microorganism was done using the colony counter. Phagocytosis was expressed as the percentage decrease in the initial number of viable extracellular bacteria [14-15]

Determination of Humoral Immunity by Znic Sulphate Turbidity Test (ZSTT):

The rats were alienated in four groups as described, six hours after the last dose blood was collected and serum was used for evaluation of immunoglobulin levels (Mullen, P.A et al1975).

Znic sulphate solution Preparation:

The triple distilled water was boiled for 15min to remove dissolved CO_2 and was used to prepare Znic sulphate solution (208mg/lit) .The $ZnSO_4$ solution was kept in an aspirate bottle to protect uptake of carbon dioxide. This was achieved by insertion of soda lime tube into stopper. A Tubing to deliver 6ml per vial was connected to the aspiration bottle.

Test Procedure:

A control vial containing 6ml distilled water and test vial containing 6ml Znic Sulphate solution were taken and added to 0.1ml serum sample. The solution was gently taken to ensure complete mixing and reading was taken by spectrophotometer at 580nm[16-17].

Statistical Analysis:

The results were expressed as mean ±S.D, statistical calculation of the data was done using students t-test and $P<0.05$ was considered as significant.

Results: Determination of Differential Leukocyte Counts:

Table 1 mention that on the zero days the neutrophils were 62.12, 63.71, 62.11 and 63.47% in the vehicle treated, SRBC sensitized, cyclophosphamide treated and *Elaeocarpus Ganitrus* alcoholic extract treated group respectively. At 14 day percentage of neutrophils in *Elaeocarpus Ganitrus* alcoholic extract group (63.43%) were almost equal to vehicle treated group (62.42). percentage of neutrophils, lymphocyte and monocytes was taken in three section o day, 7 day and 14 day as per the table-1.on 14 day the lymphocyte percentage declined very quickly in SRBC treated group as compared to *Elaeocarpus Ganitrus* alcoholic extract treated group. Similarly, monocytes percent in *Elaeocarpus Ganitrus* alcoholic extract treated group increased (4.21%) as compare to SRBC treated group (Table 1). However response in case of *Elaeocarpus Ganitrus* treated group was better than SRBC sensitized, cyclophosphamide treated group. Further, the cyclophosphamide treated group showed rapid decline in the neutrophils, lymphocytes and monocytes as compared to other groups due to its immunosuppressive action.

Table 1: Immunomodulatory effect of *Elaeocarpus Ganitrus* alcoholic extract on Differential Leukocyte Count (DLC)

0 day			
Groups	Neutrophils (%)	Lymphocyte (%)	Monocyte (%)
Vehicle	62.12±0.52	28.07±0.21	3.522±0.02
SRBC sensitized	63.71±0.68	25.06±0.19	3.08±0.03
Cyclophosphamide	62.11±0.20	27.62±0.15	3.04±0.07
E.Ganitrus	63.47±0.13*	29.52±0.16*	4.21±0.04*
7 day			
Vehicle	64.32±0.62	28.66±0.63	3.58±0.04
SRBC sensitized	61.42±0.92	23.77±0.48	2.46±0.62
Cyclophosphamide	56.55±0.53	21.51±0.41	2.72±0.34
E. Ganitrus	65.53±0.36*	28.84±0.77*	3.06±0.45*
14 day			
Vehicle	62.42±0.20	28.55±0.53	3.04±0.04
SRBC sensitized	64.78±0.68	23.55±0.23	2.06±0.07
Cyclophosphamide	52.73±0.22	17.27±0.53	1.33±0.48
E.Ganitrus	63.43±0.24*	29.52±0.42*	3.06±0.02*

*P< 0.05 when compared with SRBC sensitized and cyclophosphamide treated groups.

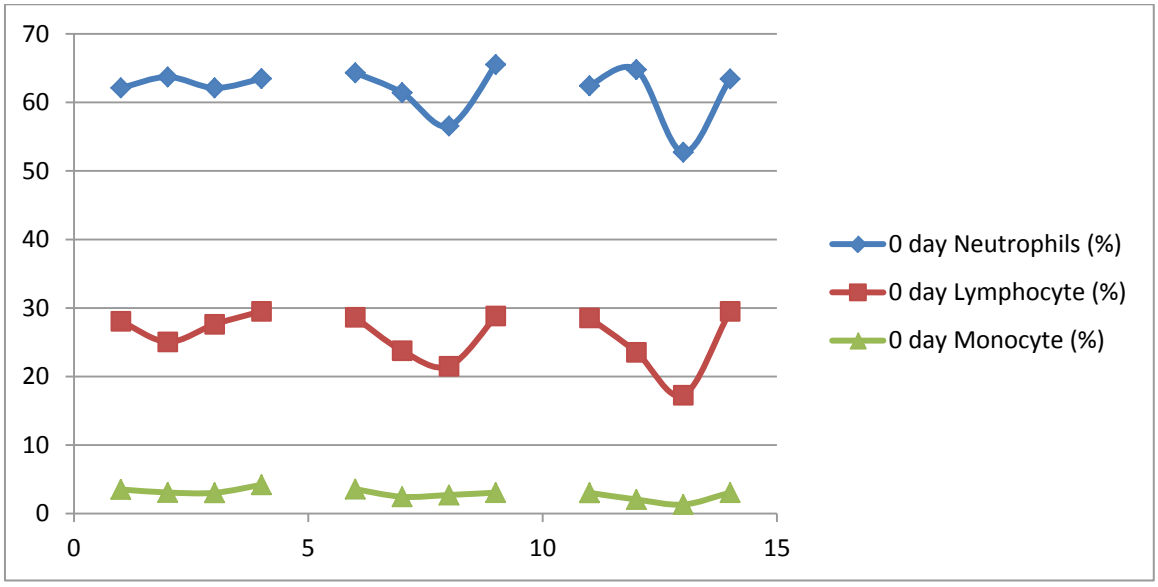

Fig 1: Immunomodulatory effect of *Elaeocarpus Ganitrus* alcoholic extract on Differential Leukocyte Count (DLC)

Phagocytic Activity Determined by Blood Polymorphonuclear Cells:

Table 2 showed that the phagocytic index significantly (P<0.05) increased after 30, 60, 90 & 120 min intervals. Maximum phagocytic index was observed in E.Ganitrus alcoholic extract group after 120 min incubation as compared to SRBC sensitizes rats (89.83%) and cyclophosphamide treated animals (75.22%) incubation The Phagocytosis index increase linearly with time.

Table 2: Phagocytic Activity Determinations

*P<0.05 when compared with control, SRBC sensitized and cyclophosphamide treated groups.

Treatment group	Dose	Phagocytosis index (%)			
		30 min	60min	90min	120min
Vehicle	2 ml of 1% gum acacia solution	60.06±1.41	71.44±1.54	82.12±1.49	90.57±1.33
SRBC sensitized	0.2ml/animal, i p.+2 ml of gum acacia solution orally	60.48±1.58	70.23±1.02	82.35±1.47	90.58±0.57
Cyclophos-phamide	100 mg/kg. orally	57.35±1.64	60.52±1.24	68.25±1.02	75.22±1.44
E.Ganitrus	100mg/kg p.o.	64.45±1.86*	72.23±1.53*	80.35±1.22*	89.83±0.21*

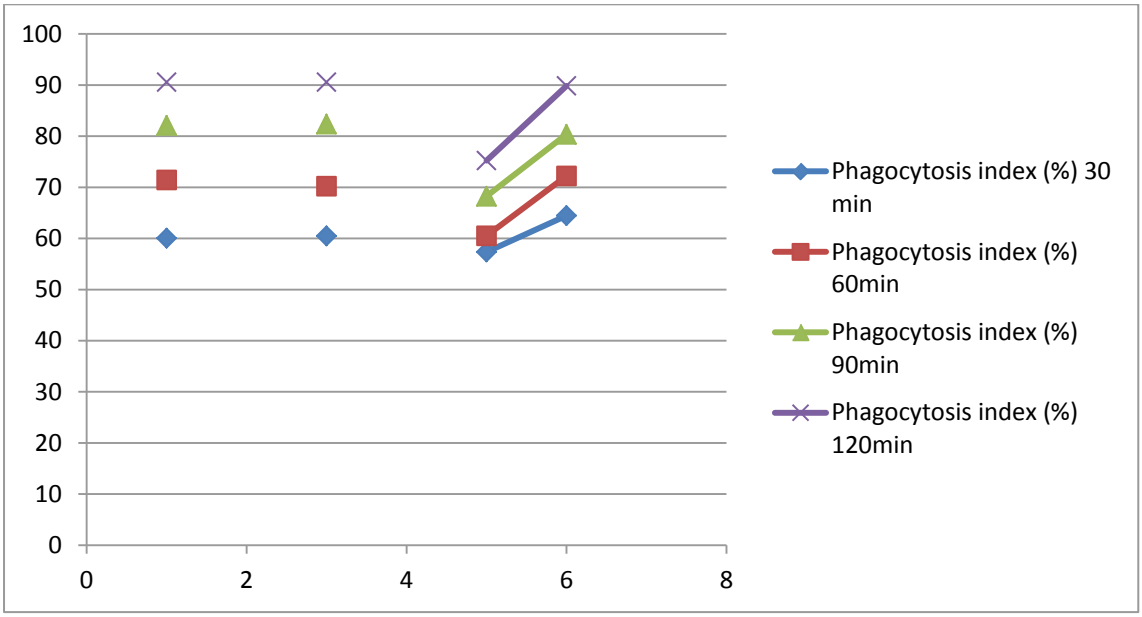

Fig. 2: Phagocytic Activity Determinations

Znic sulphate Turbidity test (ZSIT) for determination of Humoral Immunity:

Table.3 showed that significant increase in the serum immunoglobulin levels in *E.Ganitrus* alcoholic extract treated group (26.176±0.2140) but however SRBC sensitized rats did not showed any significant increase in the serum immunoglobulin levels (18.561±0.4724) as compared to vehicle (21.6723±0.7532) and cyclophosphamide treated (20.171±0.1176) rats respectively

Table 3: Zinc Sulphate Turbidity Test

Treatment group	Dose	Serum immunoglobulin level (ZST units)
Vehicle	2 ml of 1% gum acacia solution	21.6723±0.7532
SRBC sensitize	0.2ml/animal, i p.+2 ml of gum acacia solution orally	18.561±0.4724
Cyclophosphamide	100 mg/kg. orally	20.171±0.1176
E. Ganitrus	100 mg/kg p .o.	26.176±0.2140

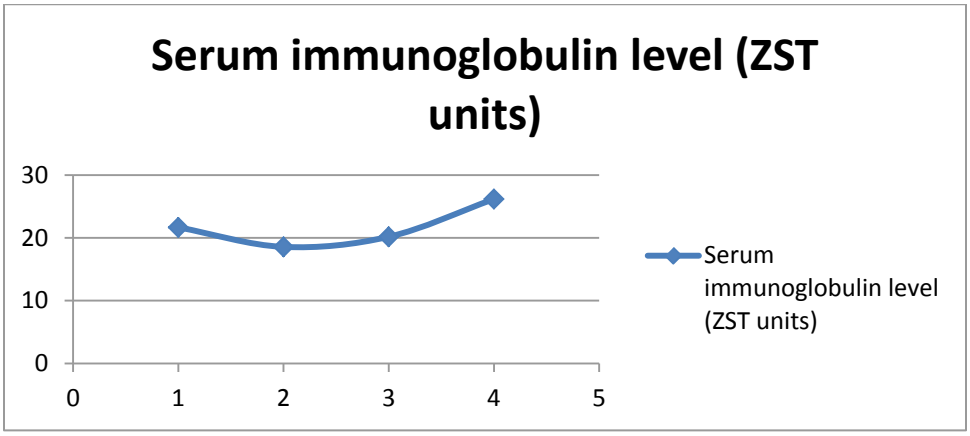

Fig.3: Zinc Sulphate Turbidity Test

Discussion:

The present study shows that *E. Ganitrus* alcoholic extract (Table-1) increases the neutrophils and lymphocytes as compared to vehicle and cyclophosphamide. Treated groups, which indicated immunostimulatory activity of. *E. Ganitrus.* The Phagocytosis and intracellular killing of microorganisms by Polymorphonuclear phagocytes was determined by the direct measurement of the microbiological killing activity[18-19]. Phagocytosis was expressed as the phagocytic index in which the percent decrease in the initial number of viable extracellular was determined microbiologically after incubation with Polymorphonuclear leukocytes. Polymorphonuclear cells engulf and destroy the foreign substances mediated immune response. Any resources by which these defense systems can be catalyzed/ enhanced will prove to boost the overall immune response and well being of all hosts .Present study showed that the *E. Ganitrus* alcoholic extract has showed the significant phagocytic activity increase in time dependent manner as compared to control, SRBC sensitized and cyclophosphamide treated group. However *E. Ganitrus* showed the more phagocytic index as compared to control, SRBC sensitized and cyclophosphamide treated group. From the obtained data, it can be concluded that *E. Ganitrus* alcoholic extract comprises of more immunostimulant activity than control and cyclophosphamide treated group. The estimation of serum immunoglobulin levels was used to evaluate the increase in serum immunoglobulin concentration after administration of drug. Immunoglobulins are antibodies that respond exclusively with SRBC antigen and formation of cloudy serum has potential immunomodulatory property. In the present study (Table 3) the *E. Ganitrus* treated rats serum showed the more turbidity (cloudy) which indicates the boost in the immunoglobulin intensity after *E. Ganitrus* alcoholic extract treatment as compared to medium, SRBC sensitized and cyclophosphamide treated assemblage[20-21]. The turbidity was articulated as ZST units which in terms indicate the amount of immunoglobulin's present in sample. This indicates the immunomodulatory property of Elaeocarpus Ganitrus beads.

Conclusion:

In conclusion, it is revealed that the alcoholic extracts of *E. Ganitrus* obtained from the dried ripe fruits possess good immunomodulatory activity. Although the ongoing research work is still under progress in order to explore the cellular changes and other pharmacological and biotechnological Investigations in male Wister rat. The experimental immunomodulatory study of alcoholic extract of Elaeocarpus Ganitrus seed extract prove and encourage worldwide scientific personnel to further research on the topic.

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