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IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF *ANACARDIUM OCCIDENTALE* LINN

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ABSTRACT

The aim of present study was to investigate the phytochemical analysis and immunomodulatory activities of ethanolic extract of nuts of *Anacardium occidentale* Linn in experimental animal of three different dose viz.150, 300 and 450 mg/kg. Nut extracts of *Anacardium occidentale* Linn revealed that presence of several biologically active component that include flavonoids, alkaloids, glycosides, phenols, tannins, vit C and quercetin. The immunomodulatory activities were determined by phagocytic activity, delayed type hypersensitivity (DTH), neutrophil adhesion test, haemagglutination antibody (HA) titer response and T cell population Assay. The response produced by oral administration of ethanolic nut extracts of *Anacardium occidentale* Linn showed a significant dose dependent increase in Phagocytic activity, DTH response, neutrophil adhesion response, augmentation of humoral immune response to sheep red blood cells and increase in T cell population response. This study demonstrates immunomodulatory activities and therapeutic potential of phytochemical components extracted from *Anacardium occidentale* Linn to combat against immunological disorders.

Keywords: *Anacardium occidentale* Linn, immunomodulatory activity, phytochemical analysis

INTRODUCTION:

Nutraceuticals are the products, which other than nutritional importance are also used as medicine. A nutraceuticals product may be defined as the substance, which has physiological benefit on health or provides protection against chronic disease. They may be used to improve health, boost immune system delay the aging process, prevent chronic diseases, increase life expectancy, or support the structure or function of human body. Nowadays, nutraceuticals have received considerable interest due to potential nutritional, safety and therapeutic effects. It also having positive impact on immune system. Herbs could be the better options to formulate as Nutraceuticals **[1]**. However the prevention is better than cure strategies such as vaccination, use of immunomodultors might have beneficial effect to controlling pathological immune response to the viruse.so recently researchers might have thinking more to word the way of immunomodultors as a future of treatment **[2]**.

Anacardium occidentale Linn. is a tree native to Brazil, which is rich in phenolic lipids also contain abundant amount of nutritive component like vitamin flavonoid, alkaloid and it is better potion as nutraceuticals. Nowadays, the cashew bark (Cashew Nut Shell Liquid) has received great attention in the pharmaceutical industry, due to its economy, abundance and important chemical compounds **[3]**. In the modern era, these substances have been used as anti-diabetic, anticancer, antimicrobial, and gastro-protective agents; and therefore, these herbs could be better options to be formulated as nutraceuticals which are helpful for the prevention and treatment of particular diseases

The purpose of the study to find out immunopharmacological activity of *Anacardium occidentale* Linn (cashew nut, kaju) as a nutraceuticals which has suggested in the Indian system of medicine for to cure a number of diseases.

MATERIALS AND METHODS:

- **Collection of nutraceuticals:**

  The selected nutraceuticals *i.e*. *Anacardium occidentale* Linn was collected from local market of Ratnagiri, Maharashtra. After collection plant where authenticated from botanical serve of India Pune, Maharashtra. (BSI/WRC/IDEN.CER/2020 /95 dated 01/10/2020)

- **Sample preparation:**

The nuts of *Anacardium occidentale* Linn was thoroughly clean. They were cut into small pieces, grinded into powder from and store in airtight container.

- **Extraction of sample:**

  The dried powder samples of nuts of *Anacardium occidentale* Linn were undergone soxhlet extraction by using ethanol solvent. The concentrated extract was collected and stored in airtight container for further use **[4, 5]**.

- **Preliminary Phytochemical investigation:**

  Phytochemical investigation were performed to identify the presence of various chemical constituents in the extracts as per Dr. Khandelwal K R **[6]**.

- **Animals used:**

  Wistar albino rats (Approx 150 to 200 g) were taken from animal house of Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra. The experimental protocol was approved by the institutional animal ethics committee (IAEC/ABCP/16/2020-21) and the care of laboratory animal was taken as per the guideline of CPCSEA.

- **Acute toxicity studies:**

  ethanolic extract of nuts of *Anacardium occidentale* Linn was tested for acute toxicity studies as per procedure given in OECD guidelines 425. Doses selected 150,300 and 450 mg/kg body weight of animals and were observed for any sign of toxicity and mortality **[7, 8]**.

- **Antigenic material:**

  - **Preparation of sheep RBCs**: Sheep blood was collected in sterile Alsever's solution in 1:1 proportion, Alsever's solution (freshly prepared) blood was kept in the refrigerator and processed for the preparation of SRBCs batch, by centifugating at 2000 rpm for 10 min and washing with physiological saline 4-5 times and then suspending into buffered saline for further use **[9]**.
  - **Preparation of carbon ink suspension:** Camlin ink was diluted eight times with saline and used for carbon clearance test in a dose of 10 µl/gm. body weight of rat **[9]**.

- ***In vivo* immunopharmacological activity**

  a) Carbon clearance test.
  b) Delayed type hypersensitivity test
  c) Neutrophil adhesion test.
  d) *In vivo* antibody (HA) titer response to SRBCs.
  e) T-cell population assay.

- **Grouping and treatment Schedule:**

Table: 1.1. Grouping and Treatment Schedule

Sr. No	Groups	Test Substance	Dose (P.O)
1	Group I	Control	10 ml/Kg
2	Group II	Levamisole (Std-1)	50 mg/Kg
3	Group III	Cyclophosphamide (Std-2)	50 mg/Kg
4	Group IV	Ethanolic extract of nuts of *Anacardium occidentale* Linn	150 mg/Kg
5	Group V	Ethanolic extract of nuts of *Anacardium occidentale* Linn	300 mg/Kg
6	Group VI	Ethanolic extract of nuts of *Anacardium occidentale* Linn	450 mg/Kg

- **Carbon clearance test.**

Phagocytic activity of reticuloendothelial system was assay by carbon clearance test; phagocytic index is used to calculate as a rate of carbon elimination by reticuloendothelial system.

Procedure:

1. In this test Animals were divided into six groups comprising 6 animals in each. As per grouping schedule mention on table in 1.1
2. Carbon ink suspension was injected via tail vein to each rat 48 hours, after the seven days of treatment.
3. Blood sample (25 µl) were then withdrawn from the retro-orbital plexus under mild ether anesthesia at 5 and 15 min after injection of colloidal carbon ink lysed in 0.1% sodium carbonate solution (3 ml).
4. The optical density was measured spectrophotometrically at 660 nm.
5. The phagocytic activity was calculated using the following formula **[10-13]**

$$K = \text{Log } OD1 - \text{Log } OD2 / t2 - t1$$

Where ODI and OD2 are the optical densities at time t1 & t2, respectively

- **Delayed type hypersensitivity test**

Delayed hypersensitivity reaction is a reaction of cell mediated immunity and become visible only after 16-24 hrs.

Procedure:

1. In this test Animals were divided into six different groups comprising 6 animals in each. As per grouping schedule mention in **Table 1.1**.
2. Immunized Rat with 0.1ml of 20% SRBCs in normal saline intraperitonially on 14th day of the study.
3. On day 21st, animals from all groups get challenge with 0.03ml of 1% SRBCs in sub plantar region of right hind paw. Foot pad reaction was assessed after 4 h, 8 h and 24 h *i.e*. on 22nd day. Increase in footpad oedema was measured with the help of vernier caliper **[10-13]**.

- **Neutrophil adhesion test.**

  Increase the recruitment of neutrophils adhesion to nylon fibers which correlates to the process of margination of cells in blood vessels.

Procedure:

1. In this test Animals were divided into six groups comprising 6 animals in each. As per grouping schedule mention in **Table 1.1**.
2. On 16th day of the treatment, blood sample from all the group were collected by puncturing retro-orbital plexus under mild anesthesia.
3. Blood was collected in vials pre-treated by disodium EDTA and analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC).
4. After initial count blood sample were collected with nylon fiber (80 mg/ml, previously sterilized by 95% alcohol) for 15 min at 37^0C. The incubated blood samples were analyzed for TLC and DLC.
5. The product of TLC and % neutrophils adhesion was calculated as follows **[10-13]**

$$\textbf{Neutrophil adhesion } (\%) = \frac{NIU - NIT}{NIU} \times 100$$

Where,

NIU: Neutrophil index before incubation with nylon fibers.

NIT: Neutrophil index after incubation with nylon fibers.

- ***In vivo* antibody (HA) titer response to SRBCs:**

  The highest dilution of a sample at which clumping is seen is considered as haemagglutination.

Procedure:

1. The animals were immunized by injecting 0.1 ml of SRBCs suspension containing 0.5×10^9 cells intra-peritoneally on day 0.
2. Blood samples were collected in micro centrifuge tubes from individual animal by retro-orbital puncture on day 11.
3. The blood samples were centrifuged and serum was obtained.
4. Antibody levels were determined by the haemagglutination technique. Equal volumes of individual serum samples of each group were pooled. Two-fold serial dilutions of pooled serum samples made in 25 µl volume of normal saline in micro-titration plates were added to 25 µl of 1% suspension of SRBCs in saline.
5. After mixing, the plates were incubated at 37°C for 1 h and

examined for haemagglutination under a microscope.

6. The reciprocal of the highest dilution of the test serum agglutination was taken as the antibody titre **[14]**.

- **T cell population assay:**

  Humoral immune response and cell mediated response can be assayed by T cell population assay.

  Procedure:

1. Antigen Challenge: On 0^{th} day, all groups were sensitized with 0.1 ml of SRBCs containing 1×10^8 cells, i.p. On 11^{th} day, blood was collected from the retro-orbital plexus and anticoagulated with Alsever's solution in separate test tubes.
2. Test tubes containing blood were kept in sloping position (45°) at 37°C for 1 h. RBCs were allowed to settle at bottom and supernatant was collected from each test tube by using micropipette which contains lymphocytes.
3. 50 µl of lymphocyte suspension & 50 µl SRBCs were mixed in test tube and incubated. Resultant suspension was centrifuged at 200 rpm for 5 min and kept in a refrigerator at 4^0C for 2 h.
4. The supernatant fluid was removed and one drop of cell suspension was placed on a glass slide. Total lymphocytes were counted and a lymphocyte binding with three or more erythrocytes was considered as rosette and number of rosettes was counted **[12, 13, 15]**.

Statistical Analysis: Mean Value ± SEM was used to express the test outcome. The one way analysis of variance (ANOVA) technique was used to estimate the variation in a collection of data. The individual comparison of group mean value were done by Dunnet's Test. Statistics were considered significant when the *p* value< 0.05.

RESULT:

The phytochemical screening of ethanolic extract of nuts of *Anacardium Occidentale* Linn were done by qualitative chemical test and results obtained were plotted in table **[Table 1.2]**.

Table: 1.2. Phytochemical investigation of ethanolic extract of nuts of *Anacardium occidentale* Linn

Sr. No	Test	Result
1	Test for Carbohydrate	
	➢ Molish's test (General test)	++
	➢ Benedict's test	-
2	Test for protein and Amino acid	
	➢ Biuret test (General test)	-
	➢ Millon's test	-
	➢ Ninhydrin test	-
3	Test for Glycoside	
	1. For cardiac Glycoside	
	➢ Baljets test	++
	➢ Legal test	-
	➢ Keller-Killiani test	-
	2. For Anthraquinone Glycoside	
	➢ Borntargers test	-
	➢ Modified Borntargers	-
	3. For Saponin Glycoside	
	➢ Foam test	-
4	Test for Flavonoid	
	➢ Sulphuric acid test	+++
	➢ Shinoda test	+++
	➢ Lead acetate solution test	-
5	Test for Alkaloid	
	➢ Dragendroff's test	++
	➢ Mayer's test	+++
	➢ Hager's test	+++
	➢ Wagnar's test	++
6	Test for Tannin and Phenolic compound	
	➢ 5% Fecl3	-
	➢ Lead Acetate solution	+++
	➢ Bromine Water	+++
	➢ Nitric acid	-
7	Test for Steroids	
	➢ Salkowaski test	++
	➢ LibermannBurchard test	++
8	Test for Vitamin C	+++

Note: - (-): Absent, (+): Present, (++): Moderate Present, (+++): Strongly Present

1. Carbon Clearance Test: (Table 1.3, Figure 1)

Table: 1.3. Result of carbon clearance test

Sr. No	Group	Treatments	Dose and route of administration	Phagocytic index (Mean±SEM)
1	I	Control	10 ml/kg (P.O.)	0.0122±0.0014
2	II	Levamisole (Std-1)	50 mg/kg (P.O.)	0.0729±0.0038****
3	III	Cyclophosphamide (Std-2)	50 mg/kg (P.O.)	0.0067 ± 0.00005^{ns}
4	IV	EAO	150 mg/kg (P.O.)	0.0230±0.001807****
5	V	EAO	300 mg/kg (P.O.)	0.0365±0.0014****
6	VI	EAO	450 mg/kg (P.O.)	0.0489±0.0030****

Value are expressed as (Mean ±SEM) n=6 ****p<0.0001 statistically significant when compared with control group by ANOVA followed by Dennett test.

Where, EAO= Ethanolic extract of nut of *Anacardium occidentale* Linn.

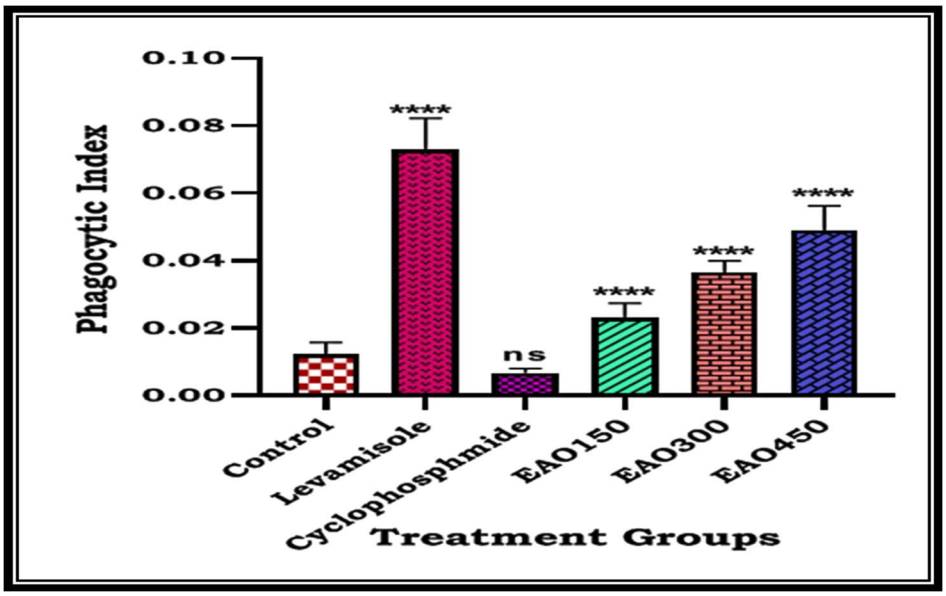

Figure 1: Graphical representation of carbon clearance test

2) Delayed type hypersensitivity test: (Table 1.4, Figure 2, 3, 4)

Table: 1.4. Result of delayed type hypersensitivity test

Sr. No	Group	Treatments	Dose and route of administration	% Increment in foot pad thickness		
				4 h	8 h	24 h
1	I	Control	10 ml/kg (P.O.)	7.20± 0.5510	14.67± 0.4261	20.39± 0.4597
2	II	Levamisole (Std-1)	50 mg/kg (P.O.)	26.875± 0.57698****	34.94± 0.4822****	44.50± 0.4191****
3	III	Cyclophosphamide (Std-2)	50 mg/kg (P.O.)	5.83± 0.4890^{ns}	9.382± 0.5684****	11.01± 0.7774****
4	IV	EAO	150 mg/kg (P.O.)	8.94± 0.0435****	15.95± 0.1407^{ns}	23.63± 0.1476^{ns}
5	V	EAO	300 mg/kg (P.O.)	11.73± 0.01334****	17.15± 0.1459***	24.00± 0.1104****
6	VI	EAO	450 mg/kg (P.O.)	14.29± 0.1414****	20.38± 0.1001****	26.22± 0.1070****

Values are expressed as (Mean ±SEM) n=6 ****p<0.0001 statistically significant when compared with control group by ANOVA followed by Dunnett test

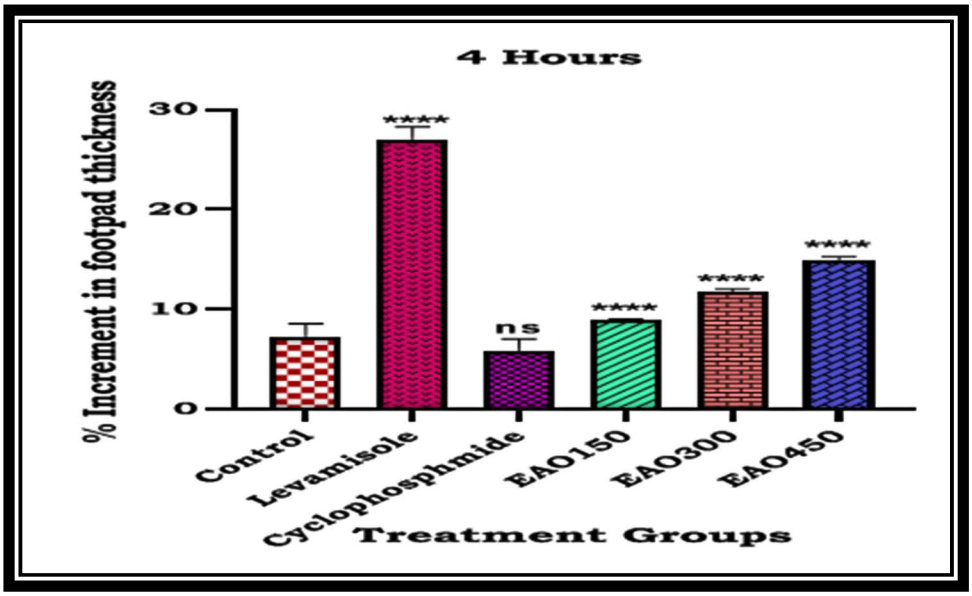

Figure 2: Graphical representation of delayed type hypersensitivity test (4 h)

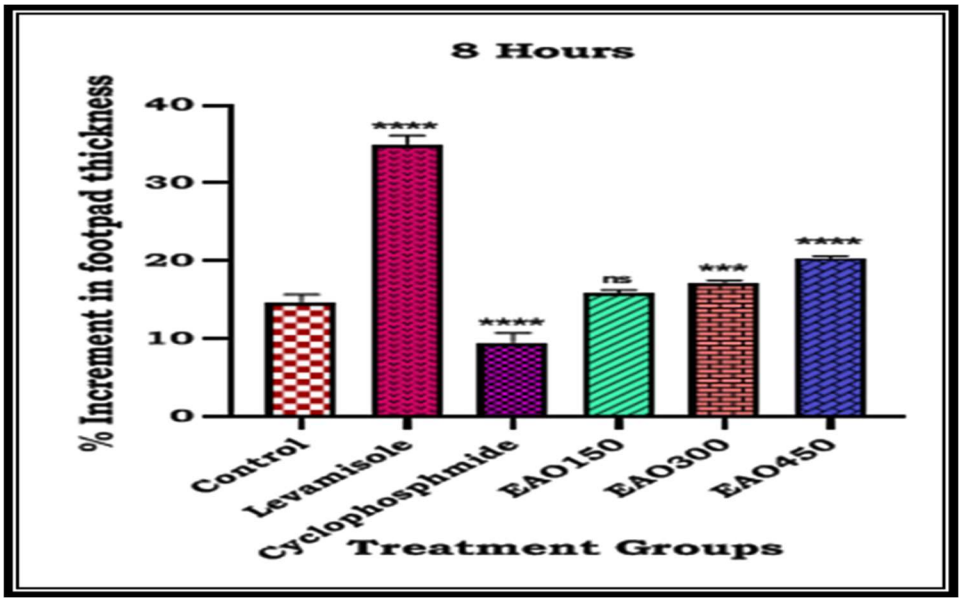

Figure 3: Graphical representation of delayed type hypersensitivity test (8 h)

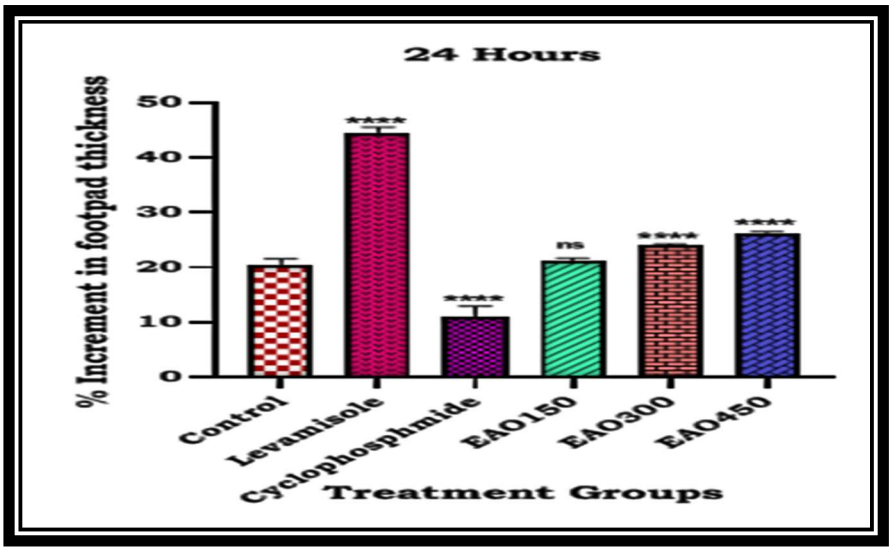

Figure 4: Graphical representation of delayed type hypersensitivity test (24 h)

3) Neutrophil Adhesion Test: (Table 1.5, Figure 5)

Table: 1.5. Result of neutrophil adhesion test

Sr. No	Group	Treatments	Dose and route of administration	Neutrophil Index before Treatment (NIU)	Neutrophil Index after Treatment (NIT)	%Neutrophil Adhesion (Mean±SEM)
1	I	Control	10 ml/kg (P.O)	240.31±3.42	216.96±3.27	10.09±0.45
2	II	Levamisole (Std-1)	50 mg/kg (P.O)	280.4±4.6	128.16±3.02	54.31±0.45****
3	III	Cyclophosphamide (Std-2)	50 mg/kg (P.O)	188.8±7.04	175.98±7.66	6.88±0.79**
4	IV	EAO	150 mg/kg (P.O)	234.9±9.12	200.76±8.7	14.60±0.93***
5	V	EAO	300 mg/kg (P.O)	221.18±8.00	177.8±6.70	19.62±0.68****
6	VI	EAO	450 mg/kg (P.O)	230.9±3.82	177.66±3.14	23.05±0.56****

Values are expressed as (Mean ±SEM) n=6 ****p<0.0001 statistically significant when compared with control group by ANOVA followed by Dunnett test

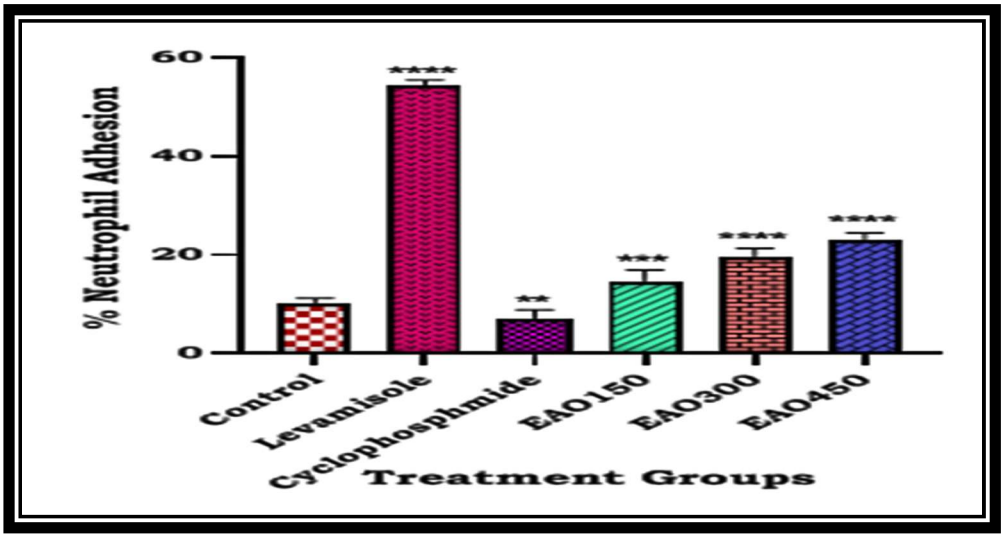

Figure 5: Graphical representation of neutrophil adhesion test

4) *In vivo* antibody (HA) titer response to SRBCs. (Table 1.6, Figure 6)

Table: 1.6. Result of *in vivo* antibody (HA) titer response to SRBCs.

Sr. No	Group	Treatments	Dose and route of administration	HA Titer (Mean±SEM)
1	I	Control	10 ml/kg (P.O.)	9.33±1.3385
2	II	Levamisole (Std-1)	50 mg/kg (P.O.)	469.33±42.8326****
3	III	Cyclophosphamide (Std-2)	50 mg/kg (P.O.)	5.33±0.8465^{ns}
4	IV	EAO	150 mg/kg (P.O.)	14.66±1.3385^{ns}
5	V	EAO	300 mg/kg (P.O.)	53.33±6.7724^{ns}
6	VI	EAO	450 mg/kg (P.O.)	128.00±0.00***

Values are expressed as (Mean ±SEM) n=6 ****p<0.0001 statistically significant when compared with control group by ANOVA followed by Dunnett test

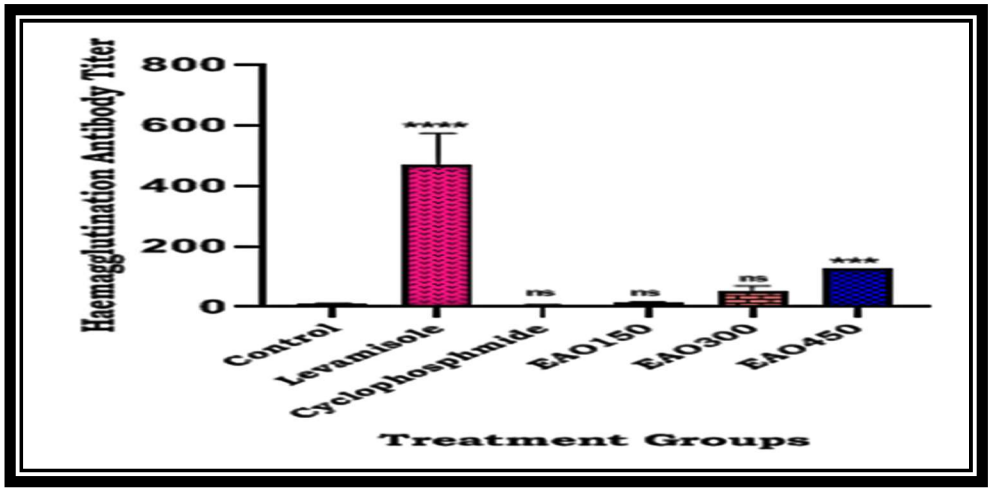

Figure 6: Graphical representation of *in vivo* antibody (HA) titer response to SRBCs

5) T cell Population Assay: (Table 1.7, Figure 7, 8)

Table: 1.7. Result of T cell population assay

Sr. No	Group	Treatments	Dose and route of administration	Total Lymphocyte Count (10^3/mm^3) (Mean±SEM)	Number of Rosette (Mean±SEM)
1	I	Control	10 ml/kg (P.O.)	4.81±0.1254	6.16±0.3346
2	II	Levamisole (Std-1)	50 mg/kg (P.O.)	6.4±0.1904****	23.5±0.9255****
3	III	Cyclophosphamide (Std-2)	50 mg/kg (P.O.)	3.73±0.2035**	5.16±0.4791^{ns}
4	IV	EAO	150 mg/kg (P.O.)	4.98±0.2220^{ns}	6.66±0.7630^{ns}
5	V	EAO	300 mg/kg (P.O.)	5.18±0.2128^{ns}	8.83±0.8365*
6	VI	EAO	450 mg/kg (P.O.)	5.26±0.1260^{ns}	12.5±0.4298****

Values are expressed as (Mean ±SEM) n=6 ****p<0.0001 statistically significant when compared with control group by ANOVA followed by Dunnett test

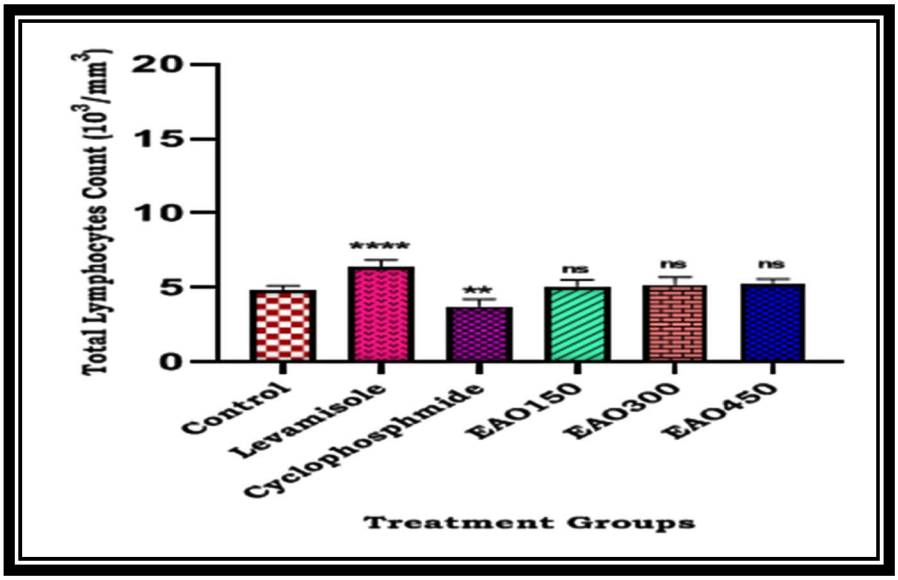

Figure 7: Graphical representation of T cell population assay (Lymphocyte count)

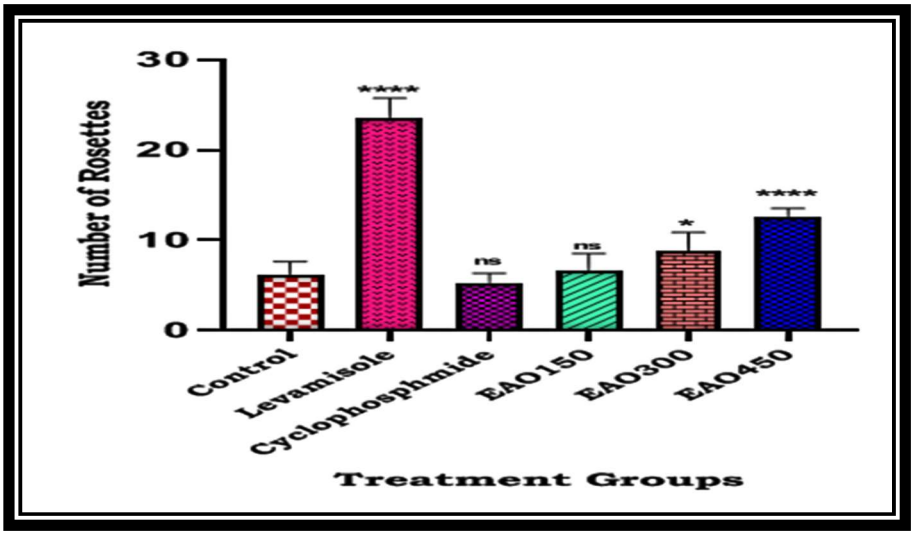

Figure 8: Graphical representation of T-cell population assay (Rosettes count)

DISCUSSION:

The various plants, fruits, vegetables, nutraceuticals used in the Indian medical system of Ayurvedic which exhibit a wide range of pharmacological properties **[16]**. Finding plant phytochemicals with immunomodulatory action that might one day be used as alternative medications is gaining more and more attention in the modern era. The scientific findings from the various studies of nut of *Anacardium Occidentale* Linn plant is widely grown in for therapeutic purposes **[17]**. The major goal of present study was to concentrate on immunomodulatory activity of ethanolic extract of nuts of *Anacardium Occidentale* Linn with particular attention to its potential immunomodulatory and protective effects in animal models. Acute oral toxicity studies of ethanolic extract of *Anacardium Occidentale* Linn was performed by OECD guideline 425 we had selected doses 150, 300 and 450 mg/kg which was safe and does not showed any sign of toxicity and mortality **[17, 18]**. The study was conducted using five different methods, each of which offers details on the impact on various immune system components. The results of the study suggested that selected dose 450 mg/kg of the Ethanolic extract of *Anacardium Occidentale* Linn stimulated overall humoral and cell mediated immune response due to presence of different phytoconstituents like Vitamin (A and C), fat, protein, carbohydrate, calcium, phosphorus, iron tannins, cardol, anacardic acid, triglycerides, fatty acids, alkyl-substituted phenol, water, cholesterol and the main constituents of the free fatty acids are palmitic and oleic acids which also different pharmacological properties like anti-microbial, anti-bacterial ,anti-septic, anti-oxidant, anticancer, anti-diabetic and anti-ulcerative property.

Carbon Clearance Test:

Reticuloendothelial system (RES) consists of mononucleated phagocyte and which are mainly responsible to causes phagocytosis. Exogenously administered antigen *i.e.* colloidal carbon ink preparation, are recognized as foreign invaders and serve by RES through this process of phagocytosis. Increase in removal of carbon particle from blood stream indicated increase in phagocytic activity **[19]**. Effect of EAO on the phagocytic activity by carbon clearance test is shown in **[Table 1.3]**. In carbon clearance test EAO treated all group exhibited significantly high phagocytic index ($P<0.0001$) when compare with control group. EAO treated group showed phagocytic index 0.0230±0.001807****of dose 150 mg/kg, 0.0365±0.0014**** of dose

300 mg/kg and 0.0489±0.0030****of dose 450 mg/kg which indicated stimulation of reticuloendothelial system to when compare with control. Similarly Cyclophosphamide treated group showed phagocytic index 0.0067±0.00005^{ns} which showed not significant effect on reticuloendothelial system when compare with control group **[5]**. Increases phagocytic index of ethanolic extract of nuts of *Anacardium occidentale* Linn of dose 450 mg/kg somewhat close to results obtained by standard levamisole which is the indicator of increased in vivo phagocytic activity and granulopoetic system competency in removing foreign particles, which is a sign of increased immune response to foreign particles or antigens.

Delayed type hypersensitivity test:

Delayed hypersensitivity test (DTH) is a type IV hypersensitivity reaction which was used to evaluate the skin hypersensitivity reaction after inoculation of the foreign antigen **[20]**. This antigen triggers immune response specifically memory T cells that in responsible for margination and inflammation at a site of antigen challenge (SRBCs) that more prominently observed after 24 hrs. Further, DTH response also helps to improve lytic enzymes concentration as well as phagocytic activity for more efficient destruction of microbes. Effects of EAO on cell mediated immune response by DTH induce footpad edema is shown in **[Table: 1.4]**. EAO treated groups significantly showed increase in footpad edema (p<0.0001) potentiating delayed type hypersensitivity response when compare with control group at of interval of 4 h, 8 h and 24 h which indicates the activation of cellular immune response, release of cytokine increases permeability to vessels and leads to vasodilation, increase in accumulation of phagocyte leading to inflammation. EAO treated group showed footpad edema 8.93±0.0435****, 11.73±0.01334**** and 14.29±0.1414**** of dose 150, 300, 450 mg/kg respectively. After 8 h EAO treated group showed positive response over % increment in foot pad thickness 17.15±0.1459*** 20.38±0.1001****at dose 300 mg/kg and 450 mg/kg respectively where EAO150 mg/kg dose showed not significant response over % increment in foot pad thickness *i.e.* 15.95±0.1407^{ns}. DTH response prominently showed after 24 h which showed significant repose on foot pad thickness. EAO treated group showed 24.00±0.1104**** and 26.22±0.1070**** at dose of 300 and 450 mg/kg but EAO 150 mg/kg showed not significant response over % increment in foot pad thickness *i.e.* 23.63±0.1476^{ns} when compare with control.

Increases DTH response of ethanolic extract of *Anacardium occidentale* Linn of dose 450 mg/kg Somewhat close to results obtained by standard levamisole at 24 h which is the indicator of increased in cell mediate immune response to foreign particles or antigens.

Neutrophil Adhesion Test:

Neutrophils are the fighting cells of human body responsible for various cell mediated immune responses such as phagocytosis, exocytosis, chemotaxis, both intracellular and extracellular killing. Significant contribute to the clearance of foreign bodies by recognition and migration towards the foreign body, phagocytosis and destroying foreign agent **[21]**. In the present study the result showed dose depend action over the migration of neutrophil granulocytes into the blood vessels and the accumulation of neutrophils at the site of inflammation are both indicated by neutrophils adhering to the nylon fibers **[22]**. Effect of EAO on neutrophils activation by the neutrophils adhesion test is shown in **[Table: 1.5]**. Cytokines are secreted by activated immune cell for margination and extravasation of the phagocytes mainly Polymorphonuclear neutrophils. The percentage neutrophils adhesion was significantly ($P<0.0001$) increase by EAO treated groups showed % neutrophil adhesion 14.60±0.93***, 19.62±0.68****and 23.05±0.56**** of dose 150, 300 and 450 mg/kg respectively when compare with control group.

***In vivo* antibody (HA) titer response to SRBCs.**

HA titer response is used to assay humoral immune response, it involve interaction of B-cell with the antigen and their subsequent proliferation, differentiation into plasma cell which secrete antibodies **[20, 23]**. The augmentation of the humoral immune response to SRBCs indicated the enhanced responsiveness of T and B lymphocyte, increase in antibody titer. Humoral antibody response to SRBCs challenge was found to be significantly ($P<0.0001$) increase by EAO treated groups when compare with control **[Table: 1.6]**. Similarly EAO 450 mg/kg showed significant response for HA Titer of 128.00±0.00*** where EAO 150 mg/kg and EAO 300 mg/kg showed not significant effect on haemagglutination *i.e.* 14.66 ± 1.3385^{ns} and 53.33 ± 6.7724^{ns} when compare with control.

T cell population Assay:

T cell coordinate multiple aspects of adaptive immunity including response to pathogens, allergens and tumor, it provides essential immune protection at different life

stages according to kumar *et al.* establishment and maintenance of immune response, homeostasis and memory depends on T-cell. Activation of T-cell response occurs when any foreign invaders enters to the body **[24]**. EAO treated groups dose not showed a significant response to increase in lymphocytes when compared with control. **[Table: 1.7]** EAO treated all groups 150,300,450 mg/kg showed not significant effect over increasing lymphocyte count. Lymphocytes are involved in both the cellular and humoral immune response and T cell formation is a very important factor. These cells do not secrete the antibody but attack the tissue cells that have been transplanted from one host to other. Therefore, only T cells come into close contact with foreign or infected cell in order to destroy them and to provide cell mediated immunity. Attachment of lymphocytes to foreign or infected cell is represented as rosette. Formation of rosette when interaction with antigen showed significantly (p<0.0001) by EAO 450 mg/kg, 300 mg/kg, showed response 12.5±0.4298**** 8.83±0.8365* respectively where EAO 150 mg/kg showed not significant response over rosette formation when compare with control.

Overall Immunostimulant effect were observed by EAO treated groups of different doses positive response were observed over reticuloendothelial system, delayed type hypersensitivity reaction , increase in % of neutrophil adhesion , increase in antibody response and increase in T-cell count with formation of rosette when interaction with antigen, when compare with control as well as Levamisole (Std-1) it indicate ethanolic extract of nuts of *Anacardium occidentale* Linn showed some immunomodulatory activity.

CONCLUSION:

Nutraceuticals play very important role in day to day life for maintaining normal healthy body function it also having important role for boosting immune response of our body to defense against pathogenic infection. According to the Academy of Nutrition and Dietetics, good nutrition habits such as eating a healthy and balanced diet are crucial to promoting a strong immune system, and suggested essential nutrients that support immune health include vitamins A, B6, C, and E, as well as folic acid, zinc, selenium, and iron. *Anacardium occidentale* Linn is nutraceuticals which possesses number of beneficial effect on human body it shows anti-microbial, anti-oxidant, anticancer, anti-diabetic and anti-ulcerative property. Immunomodulatory potential of *Anacardium occidentale* Linn could be attributed for the

presence of carbohydrate, flavonoids, vitamins polyphenols , alkaloid, glycoside and terpenoids which may modulate and potentiate humoral as well as cell mediated immunity. This emphasizes the future scope of this study.

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REFERENCE:

[1] C. Yapijakis, "Hippocrates of Kos, the father of clinical medicine, and Asclepiades of Bithynia, the father of molecular medicine. Review," In Vivo, 2009; 23(4): 507–514.

[2] https://www.who.int>docs coronavirus diseases (covid -19) weekly report (dated as 8/8/22)

[3] Silpi chanda, Rajkumar Tiwari, Arunkumar and kuldeep singh. Nutraceuticals Inspiring the Current Therapy for Lifestyle Diseases. Advances in Pharmacological Sciences, 2019; 2019: 1-5.

[4] Bhokare P, Khadke A, Kuchekar G, Kulkarni S. Comparative study of different extraction technique and phytochemical screening of Delonixregia. J Pharmacognosy Phytochem. 2018; 7(4): 133-8.

[5] Kumar AR, Shaik R, Yeshwanth D. Phytochemical evaluation of *Delonixregia, Samaneasaman, Bauhinia variegatga.* International Journal of research in Pharmacy and Chemistry. 2013; 3(4): 768-72.

[6] Dr. Khandelwal KR (2012) Practical Pharmacognosy, 22nd edition, Nirali Prakashan Pune

[7] Shewale VD, Deshmukh TA, Patil LS, Patil VR. Anti-inflammatory activity of *delonixregia* (boj. Ex. Hook). Advances in pharmacological sciences. 2011 Nov 1; 2012.

[8] Ahmed J, Nirmal S, Dhasade V, Patil A, Kadam S, Pal S, Mandal S, Pattan S. Hepatoprotective activity of methanol extract of aerial parts of Delonixregia. Phytopharmacology. 2011; 1(5): 118-22.

[9] Dashputre N.L and Naikwade N.S. Immunomodulatory activity of *Abutilon indicum* Linn. On Albino Mice. *IJPSR.* 2010; 1(3): 178-184.

[10] Tripathi S, Maurya A.K, Kahrana M, Kaul A and Sahu R.K. Immunomodulatory property of ethanolic extract of *Trigonellafoenum- graeceum* leaves on mice. *Scholar Research Library.* 2012; 4(2): 708-713.

[11] Sharma A, Rangari V. Immunomodulatory activity of methanol extract of *Adansonia digitata L. Trop J Pharm Res 2016; 15(9):1923-1927*

[12] Savant C, Joshi N, Reddy S, Mannasaheb BA, Joshi H. Immunomodulatory medicinal plants of India: A review. Int. J. Pharma. Toxicol. 2014 Jan 1; 4: 109-15.

[13] Ganeshpurkar A, Saluja AK. Experimental animal models used for evaluation of potential immuno-modulators: A mini review. Bulletin of Faculty of Pharmacy, Cairo University. 2017 Dec 1; 55(2): 211-6.

[14] Shruti Shukla & Archana Mehta. Comparative phytochemical analysis and *invivo* immuno-modulatory activity of various extracts of *Steviare baudiana* leaves in experimental animal model, Frontiers in Life Science,(2015) 8:1, 55-63, DOI: 10.1080/21553769.2014.961615

[15] Sneha J Anarthe, Sunitha D. Sandhyarani. Immunomodulatory activity of methanolic extract of *Trigonella foenumgraecum* whole plant in Wistar albino rat.

[16] Kumar D, Arya V, Kaur R, Bhat ZA, Gupta VK, Kumar V. A review of immunomodulators in the Indian traditional health care system. Journal of Microbiology,

[17] Ghase Mayuri, Devkate Reshma, Fargade Dipika. The *flamboyant delonixregia (*Gulmohar). International Journal of Creative Research Thoughts. 2022 March: 10(3): 586-93

[18] Ajith Y, Dimri U, Dixit SK, Singh SK, Gopala krishnan A, Madhesh E, Rajesh JB, Sangeetha SG. Immuno-modulatory basis of antioxidant therapy and its future prospects: An appraisal. Inflammo-pharmacology. 2017 Oct;25(5):487-98.

[19] Patel P, Asdaq SM. Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals. Saudi Pharmaceutical Journal. 2010 Jul 1;18(3):161-5

[20] Sudha P, Asdaq SM, Dhamingi SS, Chandrakala GK. Immuno-modulatory activity ofmethanolic leaf extract of *Moringa oleifera* in

animals. Indian J Physiol Pharmacol. 2010Jun; 54(2): 133-40.

[21] Pasala PK, Reddy LS, Silvia N, Reddy YD, Sampath A, Dorababu N, Mulukuri NS, Kumar KS, Chandana MS, Chetty CM, Bendale AR. Molecular docking and in vivo immunomodulatory activity of *Albizia procera* bark on doxorubicin induced immunosuppressive rats. Journal of King Saud University-Science. 2022

[22] Kumar H, Vasudeva N. Immunomodulatory potential of *Nyctanthes abrortristis* stem bark. Journal of Ayurveda and Integrative Medicine. 2022 Apr 1; 13(2): 100556.

[23] Janeway, CA.; Travers, P., Jr; Walport, M.; Shlomchik, MJ. The immune system in health and disease: immunobiology. 5. New York: Garland Publishing; 2001.1-312.

[24] Brahma V.Kumar, Thomas Connors and Donna L. Farber. Humman T-cell development and function through life.1; 34(3): 101828.