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Invitro Anti-Inflammatory Activity of Anacardium Occidentale Seed Extract.

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Abstract: *Anacardiumoccidentale (Cashew) is a plant native to Brazil, but is cultivated in tropical regions worldwide. It was imported to India by Portuguese. According to the FAO, India is one of the top five producers of cashew in the world. Antibacterial, antidiarrheal, anti-ulcerogenic, wound healing, anticancer and antioxidant activities of extracts and phytochemical derivatives from Anacardiumoccidentale have been reported by different authors. In this study we evaluated that anti-inflammatory activity of Anacardium occidentale seed extract "In vitro" using HRBC membrane stabilization method and Protein denaturation using egg albumin method. The EEAOS (EthanolicExtract of Anacardiumoccidentale**S**eed)extractshowed dose dependent percentage inhibition of HRBC (Human Red Blood Cell) membrane lysis and inhibition of protein denaturation. EEAOS extract exhibited membrane stabilization effect at 150 and 200 µg/ml have anti-inflammatory activity and it is almost equal to Diclofenac at 200 µg/ml. At 200 µg/ml dose EEAOS extract has almost equal inhibition of protein denaturation action like that of diclofenac.*

Keywords:*Anacardiumoccidentale seed extract, HRBC membrane stabilization method, Protein denaturation using egg albumin method.*

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I. Introduction

Inflammation is a protective response to tissue injury by physical, chemical or biological injury. Inflammatory changes are produced by the inflammatory mediators. These mediators are released from neutrophils, monocytes, macrophages, mast cells and platelets. Histamine, serotonin, prostaglandin, leukotrienes and interleukins and TNF (Tumor necrosis factor) alpha are important inflammatory mediators. Apart from these inflammatory mediators, lysosomal enzymes are also released during inflammation[1].Anti-inflammatory drugs are group of drugs which inhibit the inflammation either by preventing the production of inflammatory mediators or stabilizing the lysosomal membrane. Currently Glucocorticoids and NSAIDs are two important groups of drugs available as anti-inflammatory drugs. Glucocorticoids are considered as powerful anti-inflammatory and immunosuppressive agents. But these drugs produce many adverse effects such as cushing syndrome, HPA (Hypothalamic–Pituitary–Adrenal) axis suppression, precipitation of diabetes, hypertension and delayed wound healing[2]. Nonsteroidal anti-inflammatory drugs are commonly used anti-inflammatory and analgesic drugs but these are associated with development of gastritis, peptic ulcer andnephrotoxicity[3]. In this situation treatment with drugs or agents which are devoid of side effects and has efficacy are important. Many plants and plant products were screened for anti-inflammatory activity. Cashew tree is one among them.Anacardiumoccidentale.Lis belonging to family anacardiaceae. Cashew nut is the seed part of this plant. This is one of the commonly consumed dry nut due to rich in nutrients. The stem bark is having astringent action and rich in tannins. It has been reported that cashew nut shell liquid has larvicidal, antifungal, antimicrobial and anticancer activity.Tannins isolated from stem bark has been reported to have analgesic and anti-inflammatory activity[4].Pawar reported the anti-inflammatory and analgesic activity of dried leaf extracts of Anacardium Occidentale[5].He also reported that dried root also possesses the anti-inflammatory and analgesic activity.

II. Phytochemical composition

Stem bark: Polyphenolic acids, protocatechuic acid, gentacic acid, gallic acid,tannins[6]
Leaves: Flavonoids, rhamnosides, arabinosides, glycosides quercetol[7]
Nutshell of cashew: Polyphenols
Flowers: Polyphenols & flavonoids

1.2 Traditional uses: All parts are used for treatment of hypertension[8], inflammatory diseases, asthma, peptic ulcer and renal diseases and for wound healing. Cashewnut shell liquid used for tooth abscess also have antifungal action. Bark for anti-diarrhoeal action and seeds for snake bites have been used.

1.3 Pharmacological actions reported: Antibacterial, antidiarrheal, anti-ulcerogenic, wound healing, anticancer and antioxidant activities[9].

1.4 Aim: To evaluate the invitro anti-inflammatory activity of Anacardiumoccidentale seed extract.

1.5 Objectives:

1. To prepare EEAOSby soxhlet apparatus
2. To test anti-inflammatory activityof EEAOS by HRBC membrane stabilization methods
3. To test anti-inflammatory activityof EEAOS by Protein denaturation using egg albumin method

III. Methodology

3.1 Preparation of ethanolic extract of Anacardium occidentale seed:

One kg of cashew nut was collected, washed and dried in shade at room temperature for 5 days. Nuts were milled into coarse powder. The dried powder obtained was subjected to continuous extraction with 95%v/v ethanol in Soxhlet apparatus for 15 cycles. The extract was dried in a flash evaporator. The reside was collected and stored in a separate container.

I. In vitro studies for determination of anti-inflammatory potential

3.3 HRBC membrane stabilization method

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared (50,100, 150 and 200 µg/ml) using distilled water and to each concentrations, 1 ml of phosphate buffer, 2 ml ofhyposaline and 0.5 ml of HRBC suspension were added. It was incubated at 370^0C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (10 mg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization[10,11] or protection was calculated using the following formula:

Percent Protection (%) = (100- OD of drug treated sample/OD of Control) X 100(OD-absorbance)

3.4 Protein denaturation using egg albumin method:

The reaction mixture (5ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations (50,100, 150 and 200 µg/ml) of extract. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37 ± 2°C in a BOD(Bio-Oxygen Demand)incubator for 15minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium in the concentrations of 50, 100, 150, 200 µg/ml was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula: % inhibition = 100 × [V t / V C - 1] Where, V t = absorbance of the test sample, V c = absorbance of control[12].

IV. Statistical Analysis

Results are shown as Mean ± SD. Significance of difference between groups was evaluated by using ANOVA with Dunnett's test. $P<0.05$ was considered as statistically significant.

V. Results

The EEAOS was tested for its anti-inflammatory activity by its ability to inhibit denaturation of egg albumin protein and the ability to stabilize HRBC membrane**.**

5.1 HRBC membrane stabilization method:

The EEAOS showed dose dependent percentage inhibition of HRBC membrane lysis. These results were compared with standard drug Diclofenac. Lowest percentage of inhibition was observed at a concentration of 50 µg/mlandhighest with 200 µg/ml.There was significant difference betweendiclofenac and test drug with $p<0.05$ was seen at 50 and 100 µg/mland not significant with 150 and 200 µg/ml. There was almost equal

percentage of inhibition was noticed at 200µg/ml.EEAOS extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane.
From the results it can inferred that EEAOS extract at 150 and 200 µg/mlhave anti-inflammatory activity and it is almost equal to Diclofenac at 200µg/ml(Table 1& fig.1).

Table 1: Percent activity of HRBC membrane stabilization

	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
STD	63.55±2.1	80.93±2.6	83.71±5.0	85.68±4.4
Test	54.54±2.0	70.78±0.4	78.66±2.2	82.72±6.2
P Value	<0.05	<0.05	>0.05	>0.05

N=3, values expresses ad Mean ± SD, p<0.05 significant difference, >0.05 not significant

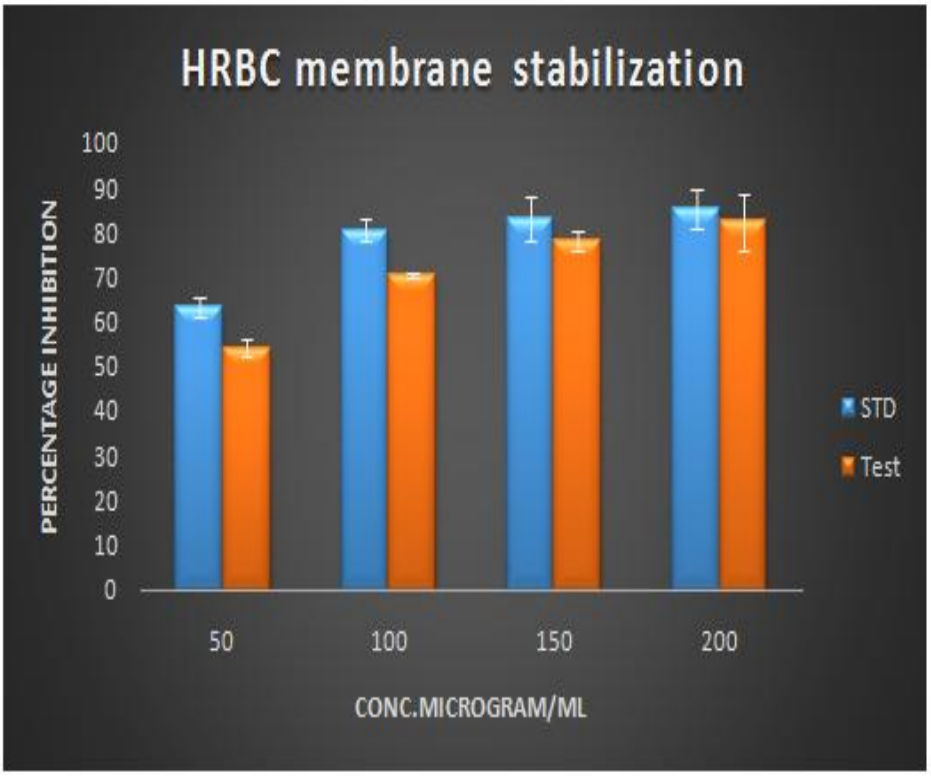

Fig 1. HRBC membrane stabilization assay

5.2 Inhibition of Albumin denaturation:

In this study, the extract showed the inhibition of denaturation proteins at doses 50, 100, 150, and 200 µg/ml. Maximum percentage of inhibition(45 %)was observed at 200 µg/ml. In this method also EEAOS extract showed dose dependent inhibition of protein denaturation. 20.92%, 27.75 %, 41.11 & 45.09% of protein denaturation inhibition was seen with 50, 100, 150 and 200 µg/ml respectively. The results were compared with Standard Diclofenac sodium which showed 32.22%, 40.55%, 46.22% and 47.73% inhibition in albumin denaturation at 50, 100, 150 and 200 µg/ml respectively. There was significant difference seen with 50, 100 µg/ml and 150 µg/ml but not with 200 µg/ml. At high dose EEAOS extract has almost equal action like that of diclofenac. From this EEAOS has anti-inflammatory activity and almost equal to that of diclofenac at 200 µg/ml (table 2 and fig 2).

Table 2: Percent inhibition of albumin denaturation

	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
STD	32.22 ±1.43	40.55 ±5.27	46.22 ±1.59	47.73 ±2.77
Test	20.92 ±0.29	27.75 ±1.27	41.11 ±1.12	45.09 ±2.55
p value	<0.05	<0.05	<0.05	>0.05

N=3, values expresses ad Mean ± SD, p<0.05 significant difference, >0.05 not significant

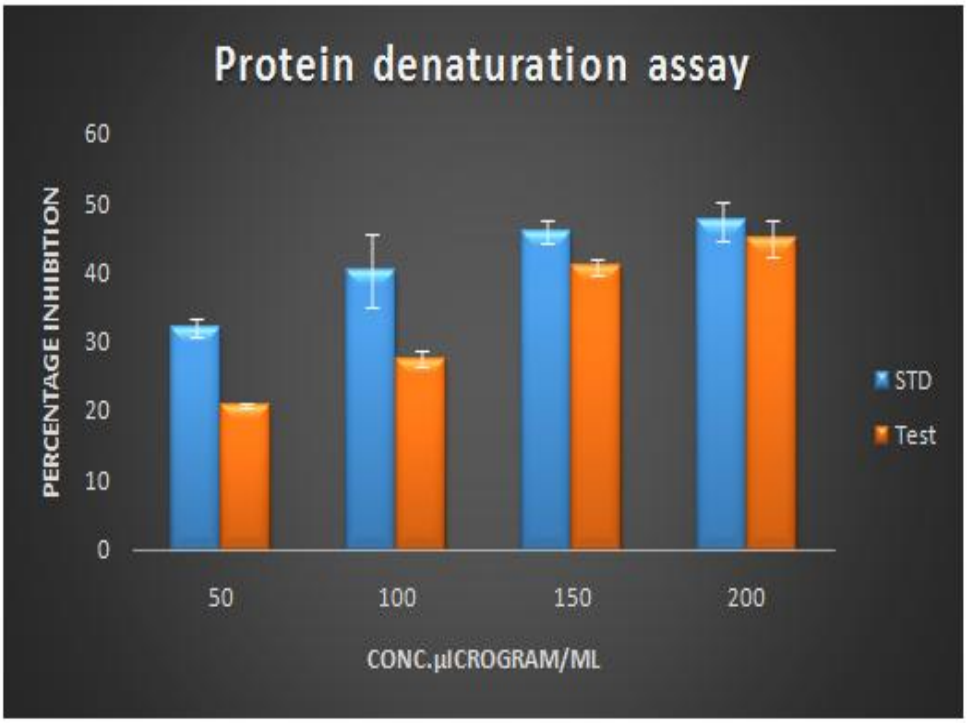

Fig 2: Percent inhibition of albumin denaturation

VI. Discussion

The present study was evaluated the anti-inflammatory activity of Anacardium occidentale seed extract by in vitro methods.HRBC membrane stabilization method and protein denaturation methods are two commonly used in vitro methods for testing anti-inflammatory activity.Human RBC membrane closely resembles lysosomal membrane. Lysosomal membrane damage or its increased permeability will lead to release of inflammatory mediators and free radicals which will induce oxidative damage. Hence stabilization of this membrane will inhibit the process of inflammation. Drugs and agents which stabilizes the HRBC membrane will have the same effect on lysosomal membrane[13]. In our study HRBC membrane stabilization was checked by exposing RBC to hypotonic solution. Hypotonic solution will cause swelling of cells and finally lysis of the cells.The viability of the cells depends on the integrity of their membrane.Drugs or chemicals having membrane stabilizing action can offer significant protection to the cell membrane against injurious substances[14].EEAOS was tested for its anti-inflammatory activity against standard drug Diclofenac. Diclofenac belongs to NSAID (Nonsteroidal anti-inflammatory drug) group have potent anti-inflammatory activity. EEAOS showed highest percentage inhibition of RBC membrane lysis & protein denaturation at 200^0C and it was almost equal to standard drug Diclofenac at this concentration.It has been reported that Anacardium occidentlae leaf extract has cytoprotective effect on erythrocyte membrane[15] and this could be due to inhibition of calcium influx. Cashewnuts are rich in flavonoids. Flavonoids are well reported to have anti-inflammatory activity. Cashew nuts are also rich in vitamins such as A, E, C and D which can contribute to its antioxidant activity.Protein denaturation is a well-documented process of inflammation. It has been reported that NSAIDS act not only by inhibition of COX (cyclooxygenase) enzyme but also by prevention of denaturation of proteins. These drugs showed dose dependent inhibition of heat induced protein denaturation. Grant et al 1970EEAOS exhibited anti-inflammatory activity by inhibiting denaturation of proteins. But highest percentage and almost equal to standard drug was seen at 200 µg/ml.The percentage of inhibition of HRBC membrane lysis was highest when compared with almond (51.35%), pea nut (9.27%) and corn (56.52%). Protein denaturation inhibition was less than almond (54.72%) and corn (114.15%).The anti-inflammatory activity of EEAOS could be due to the presnce of phytochemical compounds. It contains antioxidants such as vitamins C, E and anti-inflammatory compounds such as flavonoids and sterols which would have contributed to anti-inflammatory action.Further in vivo studies are required to confirm the action and to find exact phytochemical compound responsible for the action.

V. Conclusion

The present study was evaluated the anti-inflammatory activity of ethanolic extract of Anacardiumoccidentale seed in HRBC membrane stabilization and protein denaturation methods. In both the test the extract showed dose dependent anti-inflammatory activity. At 200 µg/ml the action is almost equal to standard

drug Diclofenac. Anti-inflammatory action could bedue to the presence of phytochemical compounds. Further in vivo studies are required to find its action and studies to find exact compound responsible for the action.

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