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Research Article

ANTIARTHRITIC ACTIVITY OF MILK EXTRACT OF *SEMECARPUS ANACARDIUM* NUT

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ABSTRACT
The present study is aimed to evaluate the anti-arthritic activity of milk extract of *Semecarpus anacardium* nut using inhibition of protein denaturation model and human red blood cell Membrane stabilization model. Diclofenac sodium was used as a standard drug. Results revealed that the milk extract of *Semecarpus anacardium* nut at different concentrations possessed significant anti-arthritic activity as compared to standard drug used as Diclofenac sodium. The results obtained in the present investigation Indicate that milk extract of *Semecarpus anacardium* nut showed anti-arthritic activity.
Keywords: *Semecarpus anacardium* nut, anti-inflammatory, Anti-arthritic, protein denaturation, Membrane stabilization.

INTRODUCTION

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage[1]. Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells[2]. It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 times higher preponderance in female.[3] Its prevalence depends upon age.[4] The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.[5,6] Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity.[7] The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas.[8] Number of synthetic medicines has been derived from medicinal herbs.[9] The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost. In the present study we selected a plant namely *Semecarpus anacardium* (Linn.) belonging to the family of Anacardiaceae. It is distributed in the sub-Himalayan tract from the Bias eastwards, ascending in the outer hills up to 3,500 ft., Assam, Khasia hills, Chittagong, Central India and the Western Peninsula. The fruit and seed are acrid in taste, hot, sweetish. In traditional system of medicine it is used as a digestible, aphrodisiac, anthelmintic laxative. It also used treat skin diseases, piles, dysentery, tumors, fevers, loss of appetite, urinary discharges, heals ulcers, and strengthens the teeth, useful in insanity, asthma. The oil is tonic, makes hair black, good for leucoderma, coryza, epilepsy and other nervous diseases. It lessens inflammation, useful in paralysis and superficial pain[10]. Earlier the plant has been studied for its analgesic and anti-inflammatory[11], antiarthritic[12], antimicrobial[13], antibacterial[14], anthelmintic[15], antimutagenic[16], antidiabetic[17], antitumour[18], antioxident[19], fungistatic[20], hepatocellular crcinoma[21-23], diuretic[1,] hypocholesterolemic[24], hypolipidemic[25], immunomodulatory[26] and mammary carcinoma[27] activities.

MATERIALS AND METHODS

Plant Material Collection

Seeds of plant were collected from local regions of Uttar Pradesh, India and the plant was authenticated as *Semecarpus anacardium* by the Dr. A. K. S. Rawat, National Botanical Research institute(NBRI), Lucknow Campus, India. A voucher specimen (Specimen No: NBRI/CIF/328/2012) is preserved in NBRI, Lucknow, India.

Preparation of Milk Extract

Semecarpus anacardium nut extract contains purified nuts of *Semecarpus anacardium* and cow's milk in the ratio as indicated in the Formulary of Siddha Medicine. 200 g of the nut was boiled with 500 mL of milk, which was repeated thrice. The decoction was stored at room temperature and this was used for the study.[1]

Drugs and Chemicals

Diclofenac sodium was obtained from Medley Pharmaceutical Pvt. Ltd., Jammu, J and K, India. Double distilled water from all-glass still was used throughout the study.

Assessment of *in-vitro* Anti-arthritic activity

Inhibition of albumin denaturation[1,28,29]

The reaction mixture (5 ml) 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 of extract so that final concentrations become 50, 100, 200, 400, 800 µg / ml. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37 ± 2ºC in a BOD incubator for 15 minutes and then heated at 70ºC for 5 minutes. After cooling, their absorbance was measured at 660 nm (Shimadzu, UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of (50, 100, 200,

400, 800 µg / ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ of Inhibition} = 100 \text{ X } [Vt / Vc - 1]$$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Membrane Stabilization Test

Preparation of Red Blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10 % v/v suspension with normal saline.[1,30,31]

Heat Induced Hemolysis

The reaction mixture (2 ml) consisted of 1 ml of test drug solution and 1 ml of 10 % RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56^{o}C for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by the formula mentioned above.[31,32]

RESULT

Anti-arthritic effect of *Semecarpus anacardium* was studied significantly by testing various *in-vitro* parameters. The effect of *Semecarpus anacardium* on inhibition of protein denaturation and membrane stabilization is shown in table. *Semecarpus anacardium* at different dose levels (50, 100, 200, 400 and 800 µg / ml) provided significant protection against denaturation of proteins and hypotonic saline induced RBC membrane damage.

Table 1: *in vitro* Anti-arthritic Activity by Inhibition of Protein Denaturation Method

Test Sample	Conc. (µg / ml)	% Protection
Milk extract of *Semecarpus anacardium* nut	50	72.7
	100	88.4
	200	102.2
	400	126.4
	800	158.8
Effect of Diclofenac Sodium (Std. drugs)	50	113.54
	100	137.43
	200	168.45
	400	198.35
	800	245.4

Table 2: *in vitro* Anti-arthritic Activity by Membrane Stabilization Method

Test Sample	Conc. (µg / ml)	% Protection
Milk extract of *Semecarpus anacardium* nut	50	22.43
	100	35.6
	200	53.32
	400	71.6
	800	88.4
Effect of Diclofenac Sodium (Std. drugs)	50	68.4
	100	76.53
	200	89.74
	400	92.52
	800	98.65

DISCUSSION

There are certain problems associated with animal use in experimental pharmacological research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-arthritic property of Milk extract of *Semecarpus anacardium* nut. Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*.[33,34]. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding.[35]. From the results of the present study it can be stated that Milk extract of *Semecarpus anacardium* nut is capable of controlling the production of auto-antigens due to *in vivo* denaturation of proteins in rheumatic diseases. Protective effect on heat and hypotonic saline-induced erythrocyte lysis is known to be a very good index of anti-arthritic activity of any agent.[36]. Since the membrane of RBC is structurally similar to the lysosomal membrane, the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane.[36] Further studies are needed to elucidate other mechanisms of the in-vitro Anti-arthritic activity of the *Semecarpus anacardium* extract and to identify the active constituents responsible for the anti-arthritic effect.

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