ORIGINAL ARTICLE

Anti-inflammatory and anti-helminthic activity of ethanolic extract of *Azadirachta Indica* leaves

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Abstract

Aim: To evaluate membrane stability and anti-helminthic activity of bioactive compounds isolated from the leaves of *A. indica* through soxhlet extraction. **Materials and Methods**: Fresh leaves were collected, cleaned, dried at 37°C and boiled with ethanol for extracting secondary metabolites. The crude extracts were examined for membrane stability by HRBC membrane stabilization and heat induced haemolysis methods using Spectrophotometer. Similarly anti-helminthic activity was evaluated using earthworms of 8-10 cm in length and 0.3-0.4 cm in width and compared the results with standard drug Albendazole. **Results and Discussion**: Ethanolic extract of *A. indica* showed 98.63% for 1:1 dilution and 18.33 % for 1:2 dilution of preservation in hypotonic solution of HRBC and 16.06% protection against heat induced haemolysis. Similarly extract showed anthelmintic activity at lower concentration of 25 mg/ml against *P. posthuma*. The lower concentrations of leaf extract showed good anti-inflammatory activity and anti-helminthic activity, but higher concentrations gave relatively more projecting activity as compared with standard drugs. **Conclusion**: Study indicates that, these extracts could be an alternative for the synthetic drugs available in the market and further studies are needed before the pharmacological properties of *A. indica* can be utilized in therapy.

Key words: *Azadirachta indica*, anti-inflammatory, anti-helminthic, leaves, bioactivity

INTRODUCTION

Infections caused by helminthic parasites are one among the most widespread infections in humans, distressing a huge population of tropical regions and cause an enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia, and pneumonia.[1,2] Other manifestations of helminthic infections include respiratory symptoms, dermatological consequences, and epilepsy as a result of neurocysticercosis. These infections may also subvert immune responses to pathogens of other diseases such as tuberculosis, HIV, and malaria.[3] Gastro-intestinal helminths became resistant to currently available drugs; therefore, there is an increasing demand toward natural anthelmintics.[4-6] Earthworms which are one of the organisms under the same phylum as helminthic parasites share a common origin in the characterization of internal organs and various functionalities. Earthworms are used to study anatomical and physiological characters which resemble with the helminthic parasites and to develop influential anti-helminthic drug.[7,8]

On the other hand, inflammation is the result of activation of the immune system which manages defense mechanism in response to microbial infection or irritation or damage of tissues/organs. Keeping the damage under control is a beneficial consequence of inflammation, but inflammation becomes an annoyance when the body suffers from severe or long-term inflammation.[9] However, chronic inflammation is believed to play crucial roles in the pathogenesis of various diseases, such as cardiovascular diseases,[10] inflammatory bowel disease,[11] cancer,[12] diabetes,[13] asthma,[14] and Alzheimer's disease.[15] To encounter this process, several anti-inflammatory drugs of non-steroidal origin were developed. The word "anti-inflammatory" is defined as the ability of a substance or treatment to minimize inflammation. Many non-steroidal anti-inflammatory drugs are in clinical

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practice, but these drugs cause adverse effects.[16] Hence, the search for safer and better anti-inflammatory agents continues to be an area of interest which led to increase in demand for natural products with anti-inflammatory activity having fewer side effects.

Neem (*Azadirachta indica*) belongs to the Meliaceae family and grows in the Indian subcontinent and its neighboring countries. The species has a long history of medicinal importance having a wide spectrum of biological activity. Its insecticidal properties and low toxicity to mammals have particularly attracted researchers in extracting various bioactive compounds such as nimbin (anti-inflammatory), nimbidin (antibacterial, antiulcer), nimbidol (anti-tubercular, anti-protozoan), gedunin (anti-malaria, anti-fungal), sodium nimbinate (diuretic, anti-arthritic), and salannin (repellent).[17,18] Considering these amazing properties exhibited by the plant, the present study was intended to investigate anti-helminthic and anti-inflammatory activity of ethanolic extract of *A. indica* leaves.

MATERIALS AND METHODS

Ethanol, Whatmann filter paper, dextrose, sodium citrate, citric acid, NaCl, dimethyl sulfoxide (DMSO), KCl, Na_2HPO_4, KH_2PO_4, HCl were of analytical grade and procured from HiMedia India Pvt. Ltd.

Fresh and young leaves were collected from the herbal garden of VFSTR University, Vadlamudi, Andhra Pradesh, India and cleaned to remove all the dirt by tap water followed by distilled water. Leaves were chopped into small pieces and were left to dry at 37°C, and then the leaves were powdered into fine powder; using soxhlet apparatus 10 g of powdered plant material was extracted with ethanol for about 8 h.

Determination of Anti-inflammatory Activity of *A. indica*

Human red blood cell (HRBC) membrane stabilization method

This method mainly determines the percentage of protection. The blood samples were collected from different individuals voluntarily which was stored for about 3 weeks. The blood samples was mixed with equal volume of Alsever solution which contains 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl and it is centrifuged at 3000 rpm for 20 min. The packed cells were washed with isosaline (0.9%) and 10% suspension were made. Various concentrations of plant extract (1:1, 1:2) were prepared using 10% DMSO. To this, add 1 ml of phosphate buffer, 2 ml of hyposaline (0.25%) and 0.5 ml of HRBC and incubated at 37° for 30 min. These mixtures were centrifuged at 3000 rpm for 20 min with that supernatant solution hemoglobin content was estimated spectrophotometrically at 560 nm.[19] For determining the HRBC membrane stabilization (or) the percentage (%) of protection using the formula:

% protection = (1-[O.D of tested samples/O.D of control]) × 100.

Heat induced hemolysis

The reaction mixture consisting of plant extract (1 ml) and 1 ml of 10% RBC suspension was incubated in water bath at 56°C for 30 min, at the end of incubation tubes were cooled under running tap water and centrifuged at 2500 rpm for 5 min and absorbance of supernatant solution were taken spectrophotometrically at 560 nm[19] and percentage (%) of hemolysis was calculated by the formula:

Percentage hemolysis = (O.D of control-[O.D of sample/O.D of control]) × 100.

Antihelminthic Activity

Earthworms

Indian adult earthworms were collected from vermin compost farm near a village in Guntur district. The collected

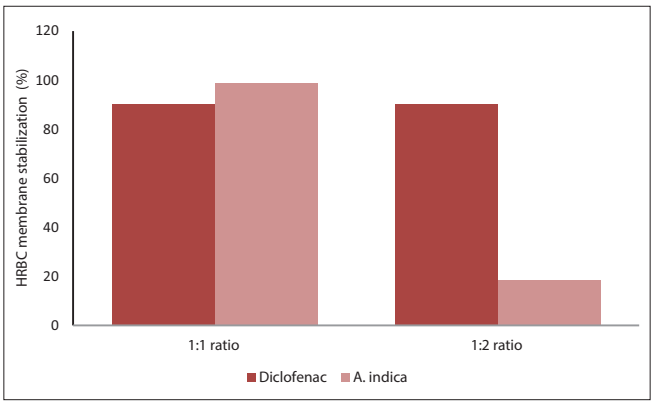

Figure 1: Human red blood cell membrane stabilization method for ethanolic extract of *Azadirachta indica* in comparison with standard diclofenac

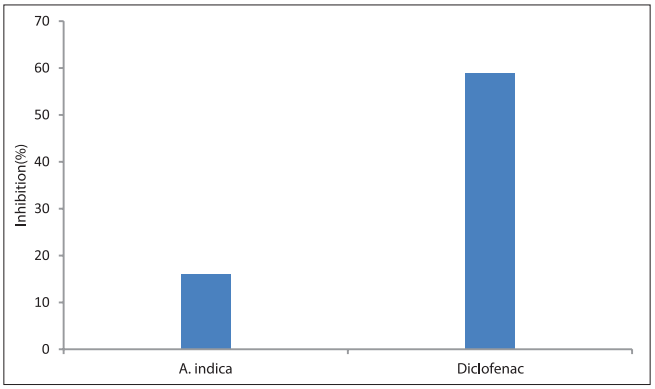

Figure 2: Inhibition of heat-induced hemolysis for ethanolic extract of *Azadirachta indica* with respect to standard diclofenac

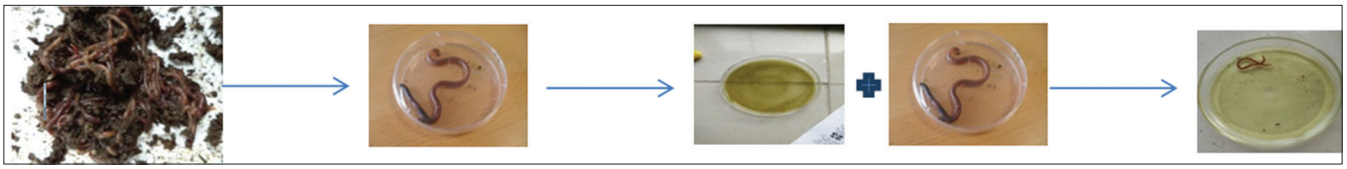

Figure 3: Various steps involved in antihelminthic activity

earthworms were washed thoroughly in saline water to remove the external debris to be used for antihelminthic activity. The earthworms of 8-10 cm in length and 0.3-0.4 cm in width were used for all the experimental protocol. Earthworms serve as preferred replacement as it shares similar anatomical and physiological resemblance with the intestinal parasites of humans which serves the objective of production of influential anti-helminthic drug.

Anti-helminthic Activity

Anthelmintic activity of *A. indica* leaf extract was carried out as described by Ajaiyeoba *et al.*,[20] with necessary modifications. The Indian earthworm (*Pheretima posthuma*) of nearly equal size of three in each group was taken for the experiment. Each type of dried extract was suspended in normal saline water in three different concentrations (25, 50, 100 mg/ml). Albendazole suspension of same concentration was taken as standard, and normal saline water was taken as a control. Worms were placed in petridish containing 15 ml of sample/drug solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50°C). Death was included when the worms lost their motility followed by white secretions and fading away of their body color.

RESULTS AND DISCUSSION

This study is based on the principle of stabilization of HRBC membrane by using an ethanolic extract of *A. indica,* and the same was compared with that of standard diclofenac. Ethanolic extract of *A. indica* showed 98.63% for 1:1 dilution and 18.33% for 1:2 dilution of preservation in hypotonic solution of HRBC [Figure 1]. Similar inhibition of denaturation of hypotonic solution were observed for solvent extracts of *Anthracephalus cadamba* (73.25% for 200 µg/ml),[19] *Pluchea lanceolata* (86.8% for 1000 µg/ml),[21] *Punciagranatum* (91.25% for 100 µg/ml),[22] Garden Coronaria leaves (24.38% for 100 µg/ml),[23] *Mimusops elengi* (86.23% for 250 µg/ml).[24] Heat-induced hemolysis was demonstrated between the ethanolic extract and the standard diclofenac, maximum constrainment was found at 16.06% for *A. indica* extract which was compared with standard diclofenac sodium which demonstrated maximum suppression of 58.92% at 100 mg/ml [Figure 2]. The inhibition of denaturation when compared to the *in vitro* studies of solvent extracts of *A. cadamba* (79.25% for

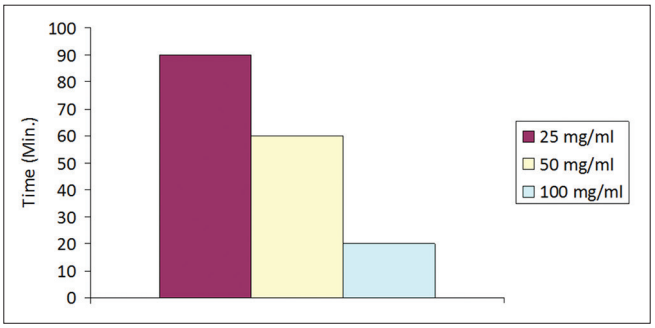

Figure 4: Action of plant extract for different concentrations using saline solution as control

100 µg/ml)[19] was low and slightly better than the solvent extract of *P. lanceolata* with 57.33% for 100 µg/ml.[21]

Similarly, in current study earthworms collected were subjected to antihelminthic activity for *A. indica* plant extract and normal saline was used as control as shown in Figure 3. Earthworms serve as preferred replacement as it shares similar anatomical and physiological resemblance with the intestinal parasites of humans which serves the objective of production of the influential anti-helminthic drug. Based on the current observations, a higher concentration of *A. indica* leaf extract produced paralytic effect much earlier and the time to death was shorter for all worms. Ethanolic extract showed anthelmintic activity in a dose-dependent manner giving the shortest time of paralysis and death with 100 mg/ml concentration, for all worms. The extract exhibited more potent activity at lower concentration (25 mg/ml) against *P. posthuma* [Figure 4], similar results were observed from the leaf extracts of *Luffa cylindrical,*[25] *Zanthoxylum rhoifolium*[26] and *Spigelia anthelmia.*[27] Evaluation of anthelmintic activity was compared with reference standard albendazole, which showed comparatively high efficiency.

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