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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

IN-VITRO ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF *CARDIOSPERMUM HALICACABUM* AND *MORINGA OLEIFERA* LEAVES EXTRACT

V. Balamurugan*, L . Muruganandam

Department of Chemistry, Saranathan College of Engineering, Tiruchirapalli, Tamil Nadu, India

**Corresponding author: manibala_mb@yahoo.co.in*

ABSTRACT

The objective of present work is to study the *in-vitro* anti-inflammatory and anti-arthritic activity of mixture of *Cardiospermum halicacabum* and *Moringa oleifera* leaves extract. The ethanolic extract of leaves mixture was studied for *in-vitro* anti-inflammatory activity by human red blood cell (HRBC) membrane stabilization method and *in-vitro* anti-arthritic activity by bovine serum protein denaturation method and egg albumin denaturation method. The activity of ethanolic extract was compared with standard anti-inflammatory drug diclofenec sodium. It was found that ethanolic extract of mixture of *Cardiospermum halicacabum* and *Moringa oleifera* was more potent in inhibition of egg albumin denaturation than diclofenac sodium. It can be concluded that mixture of *Cardiospermum halicacabum* and *Moringa oleifera* possess good *in-vitro* anti-inflammatory and anti-arthritic activities.

Keywords: *In-vitro,* anti-inflammatory, Anti-arthritic activity, *Cardiospermum halicacabum*, *Moringa oleifera*.

1. INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are a major cause of morbidity throughout world. Inflammation is a bodily response to injury, infection or destruction characterised 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 [1]. Prolonged inflammation leads to the auto-immune diseases like rheumatoid arthritis (RA), atherosclerosis [2, 3]. The inflammation of RA can also occur in tissues around the joints, ligaments and muscles [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]. For these reason, there is a need for ant-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is a lack of proper scientific evidence. In South India, many Siddha practioners are using various plants for the treatment of different types of arthritic conditions. *Cardiospermum halicacabum* Linn. (Sapindaceae), English name: Balloon Vine, is an annual or sometimes perennial climber, commonly found as a weed throughout India [7]. Previous studies which have reported phytochemical investigations of the plant revealed the presence of flavones, aglycones, triterpenoids, glycosides, carbohydrates, fatty acids and volatile esters in the different extracts of the plant [8-11]. Apart from this, pharmacological evaluation of *C. halicacabum* extracts showed that the plant possesses antimalarial [12], antifilarial [13] antiparasitic [14], antipyretic [15] anti-inflammatory [16] and anti-arthritic [17] activity. *Moringa oleifera* Lam is one of the broadly used plants in traditional medicine. *Moringa oleifera* is the most cultivated species of a mono-generic family, the *Moringaceae* [18]. Chemical analysis showed that methanolic and aqueous extracts o *Moringa oleifera* contained the highest total phenolic and flavonoid contents. Kaempferol, gallic acid, vanillic acid, coumaric acid and quercetin were detected and quantified in the *Moringa oleifera* extracts. *Moringa oleifera* leaf extracts exhibited *in vitro* free radical scavenging activity, anti-inflammatory and anti-arthritic activity [19, 20]. The anti-inflammatory activity of mixture of *Cardiospermum halicacabum* and *Moringa oleifera* have not been evaluated till now.

The objective of present work is to study the *in-vitro* anti-inflammatory and anti-arthritic activity of mixture of *Cardiospermum halicacabum* and *Moringa oleifera* leaves extract.

2. MATERIAL AND METHODS

2.1. Chemicals and Instruments

Diclofenac sodium, all other reagents used were of analytical grade. Instrument: UV Spectrophotometers (SHIMADZU 1800).

2.2. Plant Material and Extraction Procedure

Cardiospermum halicacabum and *Moringa oleifera* leaves were collected from Tiruchirapalli, Tamil Nadu, India. The collected leaves material shade dried and powdered. Each plant powder was weighed accurately and mixed together in equal proportions. One kg of the obtained powder (500 g each) was extracted for 48 h in one litre of absolute ethanol, and filtered. The resulting extract was evaporated using a rotary evaporator at 50°C. The obtained residue was reconstituted in distilled water at the beginning of the experiment and was kept in a brown bottle at 4°C all over the experimental period.

2.3. *In-vitro* Anti-inflammatory Activity-Human red blood cell (HRBC) membrane stabilization test

Fresh human blood (10 ml) was collected in heparinised centrifuge tubes and centrifuged at 3000 rpm for 10 min and washed 3 times with an equal volume of normal saline solution. The volume of the blood was measured and reconstituted as a 10%v/v suspension with normal saline [21]. The reaction mixture (2 ml) consisted of 1 ml ethanolic plant extract and 1 ml of 10% red blood cell suspension. For the control, saline was added instead of plant extract. Diclofenac sodium was used as a standard drug. The samples were incubated at 56°C for 30 min, centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant measured at 560 nm. The experiment was performed in triplicate. Percent membrane stabilization activity was calculated by the formula given in Inhibition of protein denaturation method section [22], while the percentage of protection was calculated using the following formula:

$$\% \text{ inhibition} = 100 - A_S/A_C \times 100$$

Where, A_C and A_S are the absorbance (at 560 nm) of the control and sample, respectively.

2.4. *In-vitro* Anti-arthritic Activity

The *in-vitro* anti-arthritic activity was studied using Egg Albumin denaturation method [23, 24]. A mixture of 0.2 ml of egg albumin, 2.8 ml of PBS (pH 6.4) and 2 ml of varying concentrations (10, 20, 30, 40 and 50 µg/ml) of leaves extract was used as the test sample. The same mixture replacing the plant extract with Diclofenac sodium (20, 40, 60, 80 and 100 µg/ml) was used as the reference drug. A similar volume of double distilled water served the control (represents 100% denaturation). The above mixtures were incubated at 37±2°C, for 15 min and then heated at 70°C for 5min. The test procedure was repeated 6 times. After cooling the absorbance was measured at 660 nm using UV Spectrophotometers (SHIMADZU 1800). The percentage inhibition of denaturation, which is an index of anti-inflammatory activity, was calculated using the following formula.

$$\% \text{ Inhibition} = 100 \times (V_t/V_c - 1)$$

Where, V_t = absorbance of the test sample, V_c = absorbance of control. Each experiment was done in triplicate and the average was taken. The extract concentration for 50% inhibition (IC_{50}) was determined by the dose-response curve.

3. RESULTS

3.1. In-vitro Anti-Inflammatory Activity by HRBC Method

In *in-vitro* anti-inflammatory activity, *Cardiospermum halicacabum* and *Moringa oleifera* leaves extract at concentration 10, 20, 30, 40 and 50 µg/ml showed 32.26, 43.18, 55.16, 69.19 and 78.25 % protection of HRBC in hypotonic solution respectively (IC_{50} 25.05) (Fig.1); whereas, standard diclofenac sodium at concentration of 20, 40, 60, 80 and 100 µg/ml showed 56.28, 60.14, 67.49, 72.78 and 78.69% (IC_{50} 0.592) (Fig.2).

3.2. *In-vitro* Anti-arthritic Activity by Egg Albumin Denaturation Method

In *in-vitro* anti-arthritic activity by Egg Albumin denaturation method at concentration of 10, 20, 30, 40 and 50 µg/ml showed 41.12, 49.49, 62.23, 73.31 and 83.28 % inhibition of egg albumin denaturation (IC_{50} 19.02) (Fig.3); whereas, standard diclofenac at concentration of 20, 40, 60, 80 and 100 µg/ml showed 47.76, 57.71, 63.89, 75.87 and 84.81 % inhibition of egg albumin denaturation (IC_{50} 21.31). It was found that ethanolic extract of leaves extract was more potent in inhibition of egg albumin denaturation than diclofenac (Fig.4).

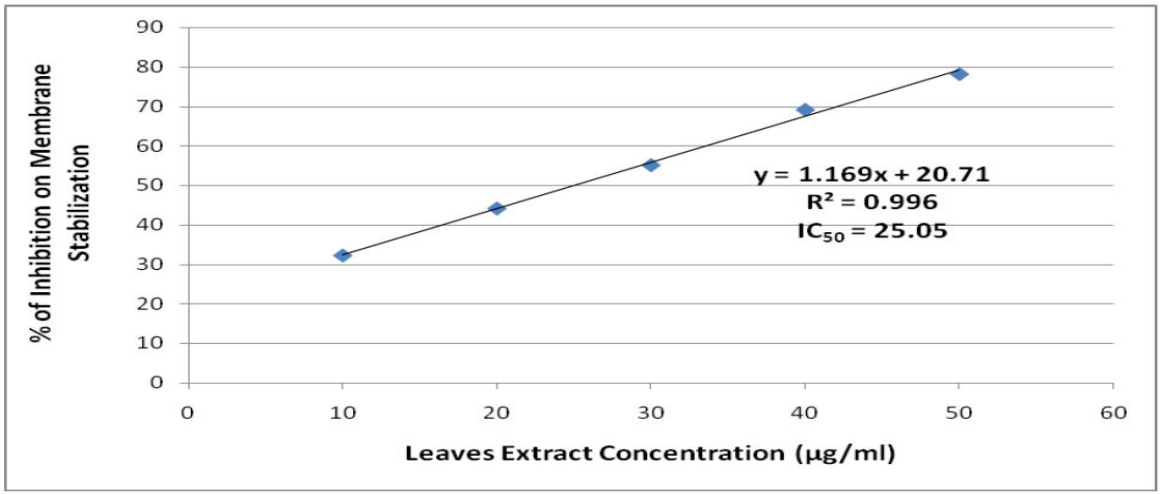

Fig. 1: Effect of Ethanol Extract of *Cardiospermum halicacabum and Moringa oleifera* Leaves on Human RBC membrane stabilization Activity

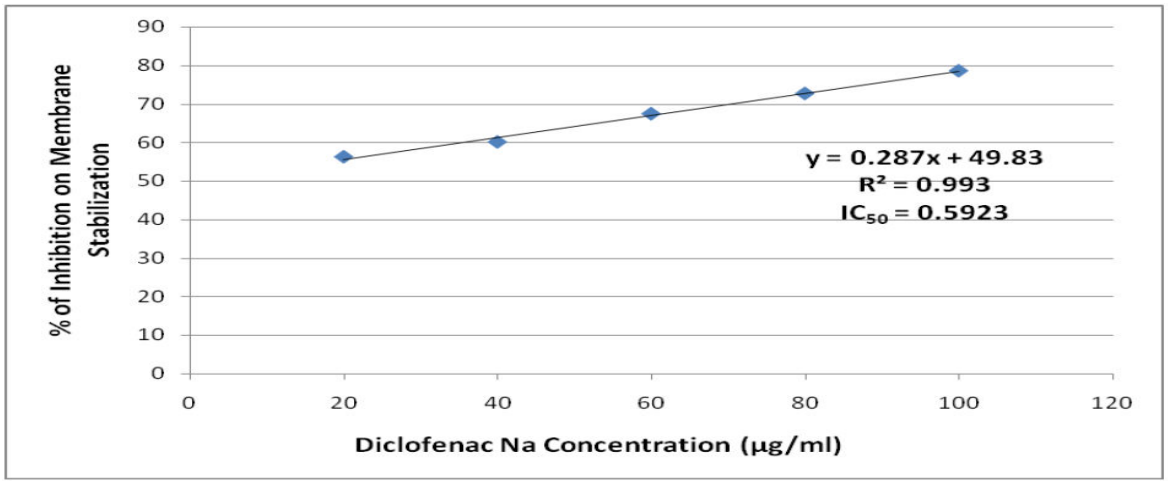

Fig. 2: Effect of Diclofenac sodium (Standard) on Human RBCs Membrane Stabilization Activity

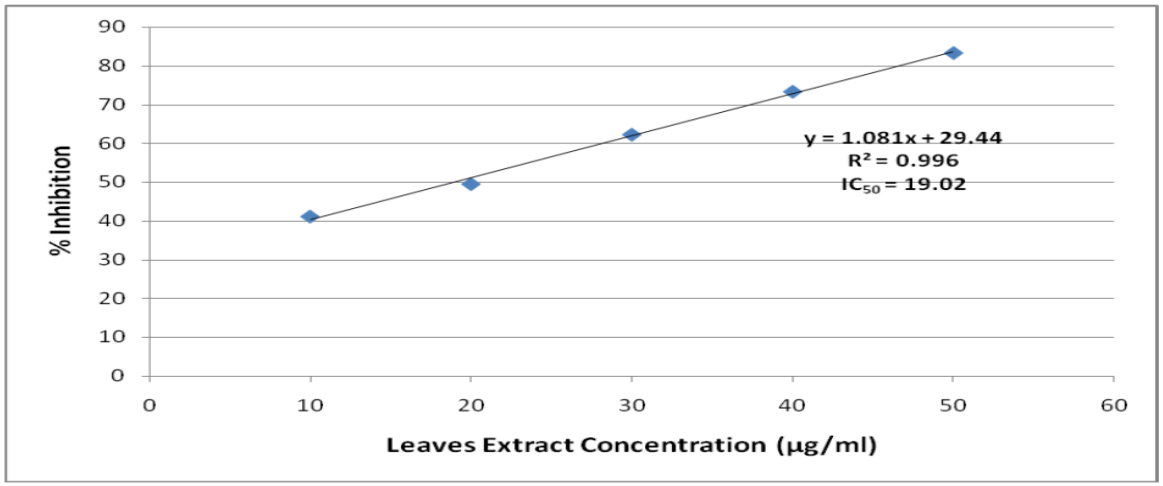

Fig. 3: In-vitro Anti-Arthritic Activity Ethanol Extract of *Cardiospermum halicacabum and Moringa oleifera* Leaves on Egg Albumin Denaturation Method

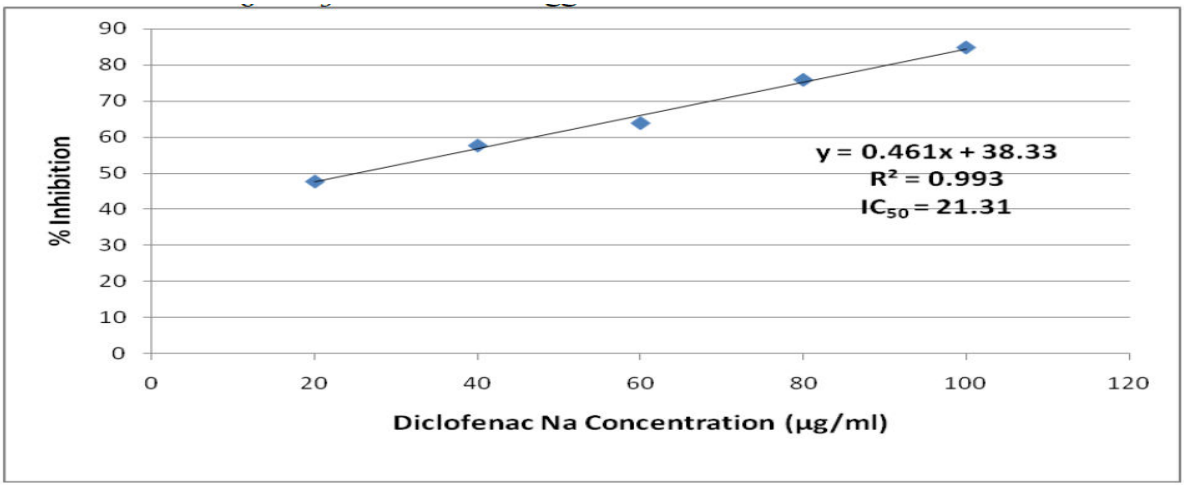

Fig. 4: *In-vitro* Anti-Arthritic Activity of Diclofenc sodium (Standard) on Egg Albumin Denaturation Method

4. DISCUSSION

Disease-modifying anti-rheumatic and anti-inflammatory non-steroidal drugs, either individually or in combination, have been the primary therapy for controlling RA with severe side effects [25]. Compared with synthetic drugs, herbal formulations are considered to be holistic and rather safe, and therefore are considered more suitable for long-term treatments [26]. However, these herbal medicines lack scientific evidence in the form of detailed clinical and preclinical studies to prove their efficacy in healing chronic and acute diseases. Traditionally, *Cardiospermum halicacabum* and *Moringa oleifera* has been prescribed as a medicine for the treatment of RA [17, 19]. Protein denaturation and stabilization of human red blood cell membranes were studied to further establish the mechanism of anti-inflammatory action of the traditionally used medicinal plant *Cardiospermum halicacabum* and *Moringa oleifera*. Inflammation is usually associated with the denaturation of proteins. Results from the present study revealed that *Cardiospermum halicacabum* and *Moringa oleifera extract* significantly inhibited protein/albumin denaturation. *Cardiospermum halicacabum* and *Moringa oleifera* extract was also effective in stabilizing RBC membranes or inhibiting the heat-induced hemolysis at different concentrations. Mohan et al., (2020) [27] study revealed that ethanolic extract of *Barringtonia acutangula* significantly inhibited protein/albumin denaturation , also their study revealed that *B.autangula* extract was effective in stabilizing RBC membranes or inhibiting the heat-induced hemolysis at different concentrations. Chowdhury et al., [28] also reported that methanolic leaf extracts of *Gardenia coronaria* promoted RBC membrane stability. Results from the present study provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory potential.

5. CONCLUSION

It is concluded that *Cardiospermum halicacabum and Moringa oleifera* is a plant mixture with anti-inflammatory and anti-arthritic potential. These properties have been evaluated by *in vitro* studies. *In vitro* studies have shown that mixture of *Cardiospermum halicacabum* and *Moringa oleifera* extract has a very strong anti-inflammatory property by human RBCs membrane stabilization method and *in vitro* studies that have shown anti-arthritis activity of the plant on a egg albumin denaturation method. These results suggest that *Cardiospermum halicacabum* and *Moringa oleifera* mixture may be a potent candidate for anti-inflammatory and anti-arthritic drug.

6. ACKNOWLEDGEMENT

Authors sincerely thank to Acme Progen Biotech India Pvt. Ltd, Salem, Tamil Nadu, India.

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