International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 01(A); January 2019; Page No. 16758-16761
DOI: http://dx.doi.org/10.24327/ijcar.2019.16761.3110

Research Article

IN- VITRO ANTI-ARTHRITIC ACTIVITY OFMETHANOLIC EXTRACT OF STEM OF *CALOTROPIS GIGANTEA*

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A R T I C L E I N F O

Article History:
Received 06th October, 2018
Received in revised form 14th November, 2018
Accepted 23rd December, 2018
Published online 28th January, 2019

Key words:
Anti-arthritic, human red blood cell membrane stabilization, inhibition of protein denaturation, phytoconstituents, stem of *Calotropis gigantea*

A B S T R A C T

Objective: To evaluate Anti-arthritic Activity of stem extract of *Calotropis gigantea* by *in vitro* model. **Materials and methods:** *Calotropis gigantea* is a medicinal plant which is indicated for the treatment of arthritis in folklore medicine. The present study was aimed at the investigation of anti-arthritic activity in methanolic extract of stem of *Calotropis gigantea*. The anti-arthritic activity of stem of *Calotropis gigantea* extract was done by Inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) *in vitro* methods. The methanolic extract of stem of *Calotropis gigantea* was subjected to *in vitro* Inhibition of protein denaturation in various concentrations i.e. 20, 40, 60, 80 and 100 µg/ml. HRBC method was also used for the estimation of anti-arthritic activity from in various concentrations 20, 40, 60, 80 and 100 µg/ml. **Results:** The stem of *Calotropis gigantea* stem extract exhibited a concentration dependent inhibition of protein (albumin) denaturation. The stabilization of HRBC membrane showed a concentration dependent anti-arthritic activity, and the protection percent increased with increase in the concentration of methanolic etract of stem of *Calotropis gigantea*. **Conclusion:** The present study is concluded that the stem of *Calotropis gigantea* has more potent anti-arthritic activity.

INTRODUCTION

India has a rich assortment of medicinal plants distributed in different geographical and ecological conditions widespread in the country. Plants have been used since prehistoric times for treatment of various ailments. Today, according to World Health Organization (WHO) as many as 80% of the world's population depend on the traditional medicine for their primary healthcare needs [1, 2]. Rheumatoid arthritis is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads to destruction of cartilages and bone. Rheumatoid arthritis is characterized by inflammation of synovial joints infiltered by CD4 + T cells, macrophages, and plasma cells that play major role in pathogenesis of disease. T cells have direct impact on TNF - alpha, IL-6, IFN- gamma, induction in joints. TNF–alpha is known to play an important role in the pathogenic mechanism of a number of chronic inflammatory diseases, including Rheumatoid arthritis. B cells may play role in pathogenesis of Rheumatoid arthritis through cell-cell interaction with T cells, dendritic cells, synovial nurse like cells and fibroblasts.

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CD4+ CD25+ regulatory T cells are potent suppressors of T cell responses both *in- vitro* and *in-vivo* [3].

Calotropis gigantea commonly known as Mudar or Yercum belongs to the family Asclepiadaceae and is a shrub about 6 m high which is widely distributed in eastern and southern parts of India [4]. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development [5].Plants are reported to have antimicrobial, haemolytic, anticancer, anti-inflammatory, antidiabetic, antioxidant properties etc [6]. The chemical constituent shows that the presence of Root: cardiacglycosides, seven oxypregnane-oligo glycosides, calotroposides A-G. Rootbark: β-amyrin, two isomeric crystalline alcohols, giganteol, isogiganteol and cardenolides. Latex: akundarin, latex contins 0.45% uscharin, 0.15% calotoxin, 0.15% calactin, latex also contains α-calatropeol, β- calotropeol, β- amyrin and calcium oxalate, it also yields a nitrogen and sulphur containing fish and cardiac poison, gigantin [7,8]. Latex also contains traces of glutathione and a proteoclstic enzyme similar to papain. Leaves: alkaloids, glycosides, mudarine. Stembark: β-calotropeol, -amyrin, giganteol. Flower: nα-calatropeol, β-calotropeol, amyrin, cardioactive glycosides, mudarine, asclepin, bitter resins akundarin, calotropin [9-11]. The anti-arthritic activity of these plants has not been reported yet in any of *in-vitro* models. With this background, the present

study was carried out to investigate the anti-arthritic activity of methanolic etract of stem of *Couroupita guianensis* in *in-vitro* models.

MATERIALS AND METHODS

Collection of plant material

The stems of *Calotropis gigantea* were collected in the month of June from the bishop heber college, Trichy, Tamil Nadu, India. The plant was identified and confirmed by Dr. S. John Britto, Director, Rapinat herbarium, St. Joseph College, Tiruchirapalli, Tamil Nadu. The voucher specimen number PP001 dated 14.07.2017.

Preparation of methanol extracts

The stems of *Calotropis gigantea* were washed in running water, cut into small pieces and then shade dried for a week at 35-40ºC, after that it was grinded to a uniform powder of 40 mesh size [12]. The methanol extracts were prepared by soaking 100 g of the dried powder plant materials in 1 L of methanol by using a soxhlet extractor continuously for 10 hr. The extracts were filtered through whatmann filter paper No. 42 (125mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure. The obtained residue 1.2 g (stems) was used to prepare the series (20, 40, 60, 80 and 100 µg/ml) of methanol supernatant concentrations for *in vitro* studies.

Evaluation of anti-arthritic activity

Inhibition of protein denaturation model

2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (PBS, pH 6.4) and 2 ml distilled water were used as control solution (5 ml). 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) (20, 40, 60, 80 and 100 µg/ml) were served as standard drug solution (5 ml). 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and various concentrations of stem of *Calotropis gigantea* (20, 40, 60, 80 and 100 µg/ml) were taken as test solution (5 ml) [13,14].

All of the above solutions were adjusted to pH, 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated using the following formula- Percentage inhibition = (Vt/Vc-1) x 100

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

Human red blood cell (HRBC) membrane stabilization model
Preparation of reagents

2 gm dextrose, 0.8 gm sodium citrate, 0.05 gm citric acid and 0.42 gm sodium chloride were dissolved in distilled water. The final volume was made up to 100 ml with distilled water. This mixture was used as Alsevers solution. Hypotonic saline was prepared by dissolving 0.36 gm of sodium chloride in 100 ml of distilled water. Isotonic saline was prepared by dissolving 0.85 gm of sodium chloride in 100 ml of distilled water. 2.38 gm disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride were dissolved in 100 ml of distilled water. This was served as phosphate buffer (pH 7.4, 0.15 M) [15].

Preparation of suspension (10% v/v) of human red blood cell (HRBC)

The blood was collected from healthy human volunteer who had not taken any NSAID'S for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution [16]. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the study [17].

Assay of membrane stabilizing activity

The assay mixtures contains 1ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension & 0.5 ml different concentrations of extract, reference sample and control were separately mixed.

1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of stem of *Calotropis gigantea* of various concentrations (20, 40, 60, 80 and 100 µg/ml) and 0.5ml of 10% w/v human red blood cells were used as test solution. 1ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline were served as test control.1ml of phosphate buffer, 2ml of hypotonic saline, 0.5ml of standard drug (Diclofenac sodium) of various concentration (20, 40, 60, 80 and 100 µg/ml) and 0.5ml of 10% w/v human red blood cells were taken as standard solution.

All the assay mixtures were incubated at 37oc for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in control as 100% [18, 19]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula-

Percentage protection

100- [(optical density sample/optical density control) × 100]

Statistical Analysis

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at $P < 0.05$ were considered to be significant.

RESULT ND DISCUSSION

Inhibition of protein denaturation model

Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) showed inhibition of protein denaturation. The methanolic extract of Stem of *Calotropis gigantea* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also showed inhibition of protein (egg albumin) denaturation. The effect of stem extracts was found to be more as well as diclofenac sodium. The results are summarized in Table 1 and Fig 1.

Previous study suggested that the Inhibition of protein denaturation model has been used by Chandra *et al*., 2012 for the study of Ashwagandha, *Mikania scandens* and Coffee [20-22].

Table 1 *In vitro* anti-arthritic activity of the stem of *Calotropis gigantea* using protein denaturation method and comparison with standard drug diclofenac sodium.

S.No	Concentrations	Protein denaturation (%)	
		Stem of *Calotropis gigantea*	Diclofenac sodium
1	20 (µg/ml)	79.08±0.35	83.65±0.54
2	40 (µg/ml)	80.60±1.64	89.35±1.77
3	60 (µg/ml)	82.50±1.75	90.49±1.62
4	80 (µg/ml)	88.65±1.52	93.53±1.57
5	100 (µg/ml)	85.55±0.37	95.43±0.27

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM

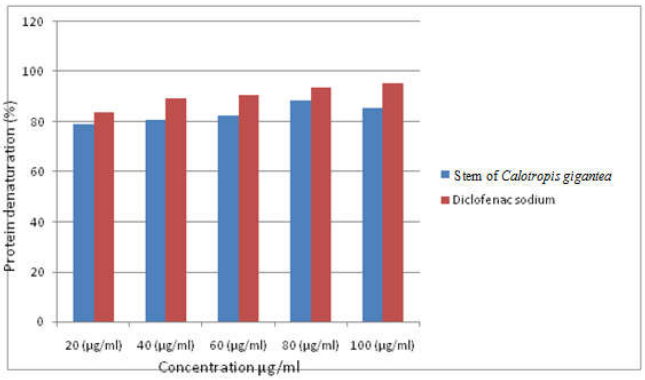

Fig 1 *In vitro* anti-arthritic activity of the stem of *Calotropis gigantea* using protein denaturation method and comparison with standard drug diclofenac sodium.

Human red blood cell (HRBC) membrane stabilization model

Diclofenac sodium was used as standard drug which at different concentrations (20, 40, 60, 80 and 100 µg/ml) exhibited stabilization towards HRBC membrane. The methanolic extract of stem of *Calotropis gigantea* at different concentrations (20, 40, 60, 80 and 100 µg/ml) also exhibited stabilization towards HRBC membrane. The effect of stem extracts was found to be more as well as diclofenac sodium. The results are summarized in Table 2 and Fig 2.

HRBC method was selected for the *in vitro* evaluation because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [18]. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [16]. HRBC membrane stabilization has been used for the study of *Skimmia anquetilia*, *Gendarussa vulgaris*, *Thunnus alalunga* by Kumar *et al*., 2012; Saleem *et al*., 2011; Azeem *et al*., 2010, respectively [16-18].

Table 2 *In vitro* anti-arthritic activity of the stem of *Calotropis gigantea* using HRBC membrane stabilization method and comparison with standard drug diclofenac sodium.

S.No	Concentrations	HRBC membrane stabilization assay (%)	
		Stem of *Calotropis gigantea*	Diclofenac sodium
1	20 (µg/ml)	6.34±0.87	66.66±0.36
2	40 (µg/ml)	17.46±1.68	71.42±1.23
3	60 (µg/ml)	23.80±1.79	76.19±1.22
4	80 (µg/ml)	42.85±1.82	80.95±1.45
5	100 (µg/ml)	50.79±0.47	84.12±0.27

Each value was obtained by calculating the average of three experiments and data are presented as mean± SEM

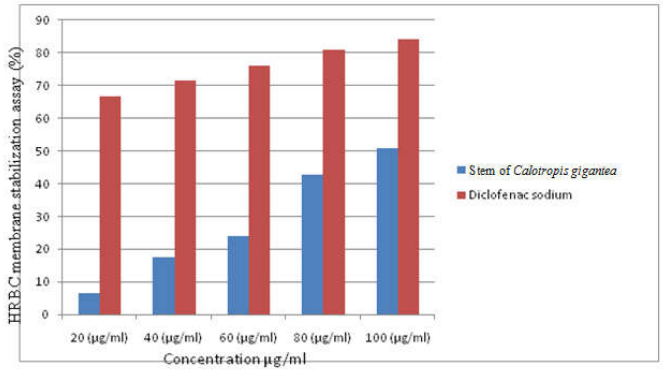

Fig 2 *In vitro* anti-arthritic activity of the stem of *Calotropis gigantea* using HRBC membrane stabilization method and comparison with standard drug diclofenac sodium.

CONCLUSION

It can be concluded that the stem of *Calotropis gigantea* showed anti-arthritic activity by comparing the results of the protein denaturation and HRBC membrane stabilization method under *in-vitro* models, it can be stated that extracts has more potent anti-arthritic activity as compared with the standard drugs.

Acknowledgement

S.P acknowledges K.Manjula, Managing Director, Bio Techno Solutions Training and Research Institute, Tiruchirapalli for providing me the infrastructure to carry out the proposed research work. S.P acknowledges Dr. S. John Britto, Director, rapinat herbarium, St. Joseph College, Tiruchirapalli, Tamil Nadu for identifying the plants. S.P acknowledges Assistant Professor, Venkatesan. C, Dr. N.G.P Arts and Science College, Kalapatti Road, Coimbatore for constant support for this research.

Author Contribution

All authors contribute equally to this manuscript.

Conflicts of Interests

The authors declare that they have no conflict of interest. It has not been published elsewhere. That it has not been simultaneously submitted for publication elsewhere. All authors agree to the submission to the journal.

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How to cite this article:

Santhosh P *et al* (2019) 'In- Vitro Anti-Arthritic Activity of methanolic Extract of stem of Calotropis Gigantea', *International Journal of Current Advanced Research,* 08(01), pp. 16758-16761.
DOI: http://dx.doi.org/10.24327/ijcar.2019.16758.3110

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