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IN VITRO ANTI - INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA LINN*

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Abstract:

Nature has been a source of medicinal agents for thousands of years and impressive numbers of modern drugs have been isolated from natural sources. Different parts (root, stem, leaves, flower, seeds etc.) of Calotropis gigantea are traditionally used to cure a number of diseases such as rheumatism, diabetes, cancer. Research has developed into investigating the potential properties and use of extracts for preparation of drug for treating arthritis. Hence the present study envisages the anti-inflammatory activity of ethanolic leaf extract of Calotropis gigantea. The results of the present study indicate that the herbal plant Calotropis gigantea has significant anti-inflammatory activity.

Key words: *Calotropis gigantea, rheumatism, Arthritis.*

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INTRODUCTION:

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as-the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion. [1]

Calotropis gigantea R.Br. (Asclepiadaceae) known as Arka and Jayanti in ayurveda has been widely documented in the ayurvedic and traditional medical literature for various therapeutic applications. *C. gigantea* has been used as a violent purgative, gastrointestinal irritant, inducing abortion and treatment of earache, toothache, headache, anxiety, sprain and stiff joints to cure pain.[2] It has been also reported as analgesic and anti-inflammatory agent.

A total of 40 compounds have been identified in the ethanolic extract of leaves. The determination of biologically and pharmacologically active compounds from plants and their pharmaceutical potential for human use is necessary to challenge the life threatening diseases like human arthritis. [3]

Recent studies with a number of herbal extracts have shown promising results. It has been shown that these compounds isolated from various medicinal plants express their anti-inflammatory activities by down regulating expression of several crucial proinflammatory mediators like inducible NOS synthase (INOS), PGs, interleukin-1 β (IL-1β), TNF-α and IL-10. Due to the adverse effects of nonsteroidal anti-inflammatory drugs and opioids, the search is on for new drugs with lesser side effects. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine, aspirin, vincristine, morphine, and quinidine) came into use through the study of herbal and indigenous remedies. [4, 5]

MATERIALS AND METHODS:

Collection of medicinal plant

The Indian medicinal plant *Calotropis gigantea was* collected from the medicinal garden of DPS, CPAS, puthuppally Kottayam, Kerala India. The plant was authenticated by Rogimon P.Thomas, Assistant professor Department of botany, CMS College Kottayam, Kerala.

Preparation of Plant extract

The ethanolic extract of leaves of *Calotropis gigantea* was used in the study. The leaves were separated, freed from adhering moisture, dried in sunshade and powdered. The powdered material (32gm) [6] was packed in Soxhlet apparatus and extraction was done using 450 ml of ethanol (60-70○C) for 56 hours. The extract was filtered using filter paper while hot, then concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in vaccum. The ethanolic extract yielded a dark greenish solid residue. The extract was then kept in sterile bottle, under refrigerated conditions, until further use. The dry weight of the plant extract was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2 to 4°C.

DETERMINATION OF ANTI - INFLAMMATORY ACTIVITY

Cyclooxygenase (COX) Inhibitory activity

The COX inhibitory activity was assayed by the method of Walker and Gierse [7]. 100µl cell lysate was incubated in Tris-HCl buffer (pH 8), glutathione 5 mM/L, and hemoglobin 5 mM/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200 mM/L and terminated after 20 minutes incubation at 37°C, by the addition 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 200µL of 1% thiobarbiturate, the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for three minutes. COX inhibitory activity was determined by reading absorbance at 632nm. [8]

The results are shown in table number 1 (Figure number 1)

Inhibition of Lipoxygenase (LOX) activity

The determination of LOX activity was as per Axelrod *et al*. Briefly, the reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of cell lysate, and sodium linoleate (200 µL). The LOX inhibitory activity was monitored as an increase of absorbance at 234 nm (Shimadzu), which reflects the formation of 5-hydroxyeicosatetraenoic acid. [9]

Percentage inhibition of the enzyme was calculated using the formula:

% inhibition = Absorbance of control – Absorbance of test/Absorbance of control × 100

The results are shown in table number 2 (figure number 2)

Inhibition of Myeloperoxidase (MPO) activity

Cell lysate was homogenized in a solution containing 50 mM potassium phosphate buffer and 0.57% hexadecyltrimethyl ammonium bromide (HTAB) the samples were centrifuged at 2000 g for 30 minutes at 4°C, and supernatant was assayed for MPO activity. MPO in the sample was activated by the addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% H2O. The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. One unit of MPO activity was defined as that degrading 1 µM of peroxide per minute at 25°C. [10]

The results are shown in table number 3 (figure number 3)

Inducible nitric oxide synthase inhibitory activity

Nitric oxide synthase was determined by the method described by Salter et.al 1997.Cell lysate was homogenized in 2ml of HEPES buffer. The assay system contained substrate 0.1ml L-Arginine, 0.1ml manganese chloride, 0.1ml 30µg dithiothreitol (DTT), 0.1ml NADPH, 0.1ml tetrahydropterin, 0.1 ml oxygenated hemoglobin and 0.1ml enzyme (sample). Increase in absorbance was recorded at 401nm. [11]

The results are shown in table number 4 (figure number 4)

Estimation of Cellular Nitrite Levels

The level of nitrite level was estimated by the method of Lepoivre et al. (Lepoivre et. al. 1990) To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves. [12]

The results are shown in table number 5 (figure number 5)

RESULTS:

Table number: 1

Sample Concentration (µg/ml)	Percentage inhibition (%)	
	Extract	Diclofenac
25	22.95	72.02
50	30.29	80.19
100	49.86	84.91

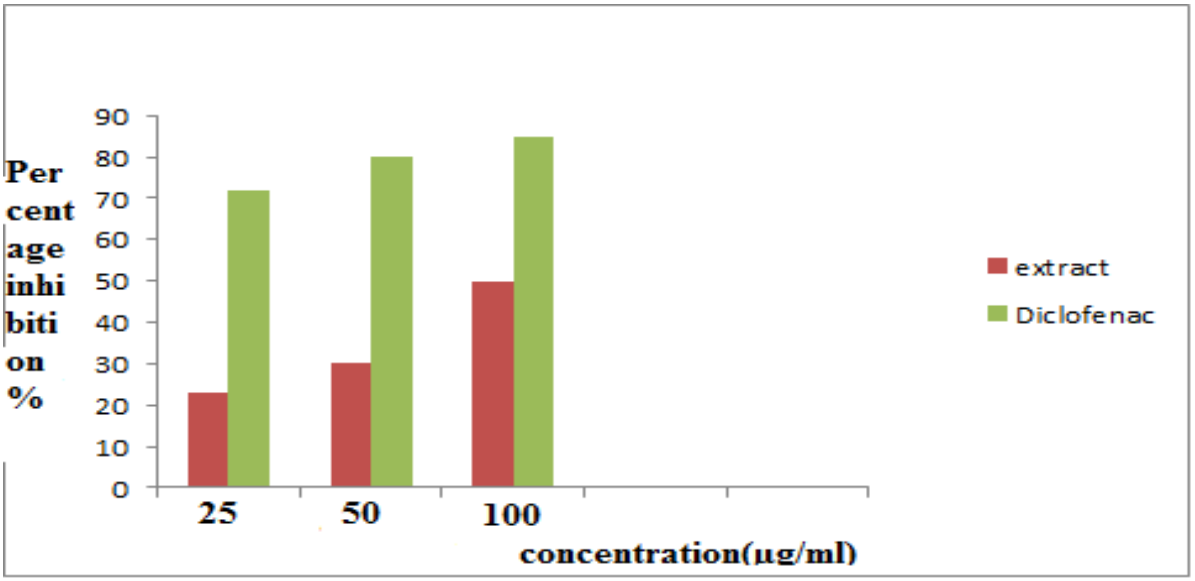

Figure 1: shows inhibition of cyclooxygenase activity

Table number: 2

Sample Concentration (µg/ml)	Percentage inhibition (%)	
	Extract	Diclofenac
25	12.62	71.93
50	35.90	86.56
100	69.85	98.02

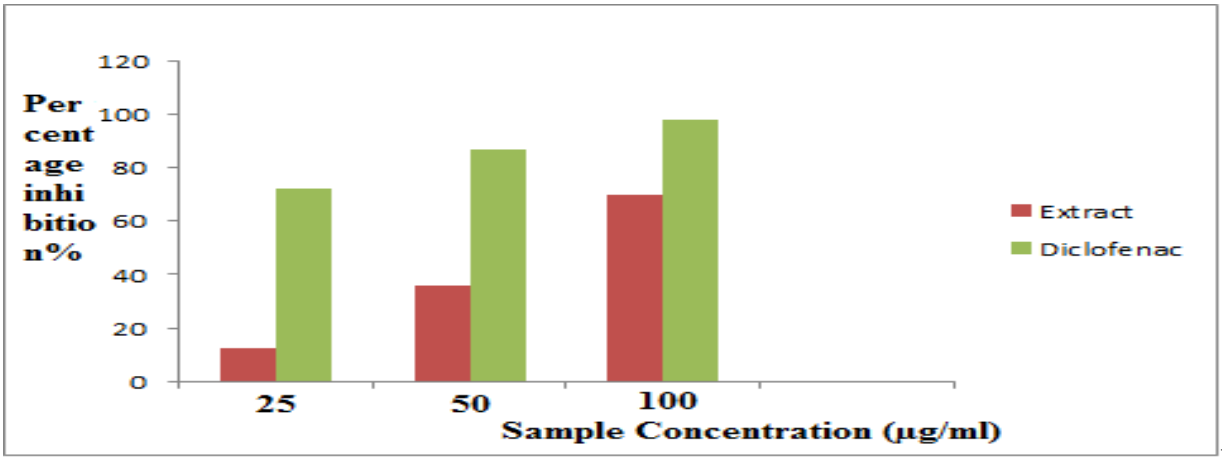

Figure 2 shows lipoxygenase inhibitory activity

Table number: 3

Sample Concentration (µg/ml)	Enzymatic activity (U/ml)	
	Extract	Diclofenac
25	0.006851	0.001419
50	0.003366	0.00099
100	0.0030	0.000759

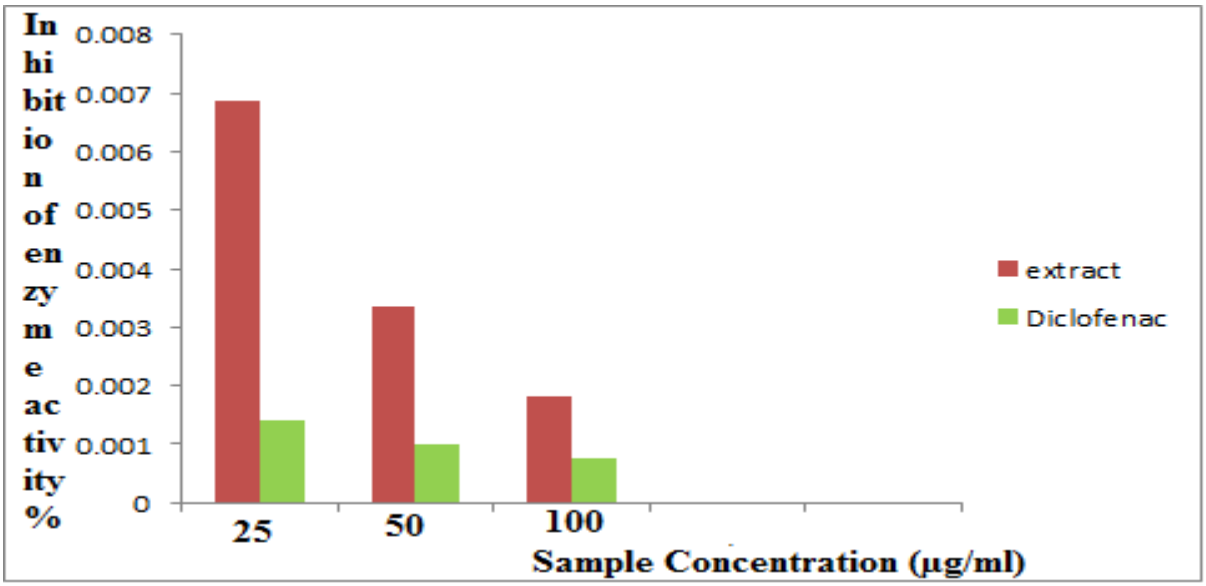

Figure 3 shows inhibition of myeloperoxidase activity

Table number 4

Sample Concentration (µg/ml)	Δ OD	
	Extract	Diclofena
25	0.0208	0.029
50	0.0089	0.010
100	0.0034	0.006

Control Δ OD = 0.072

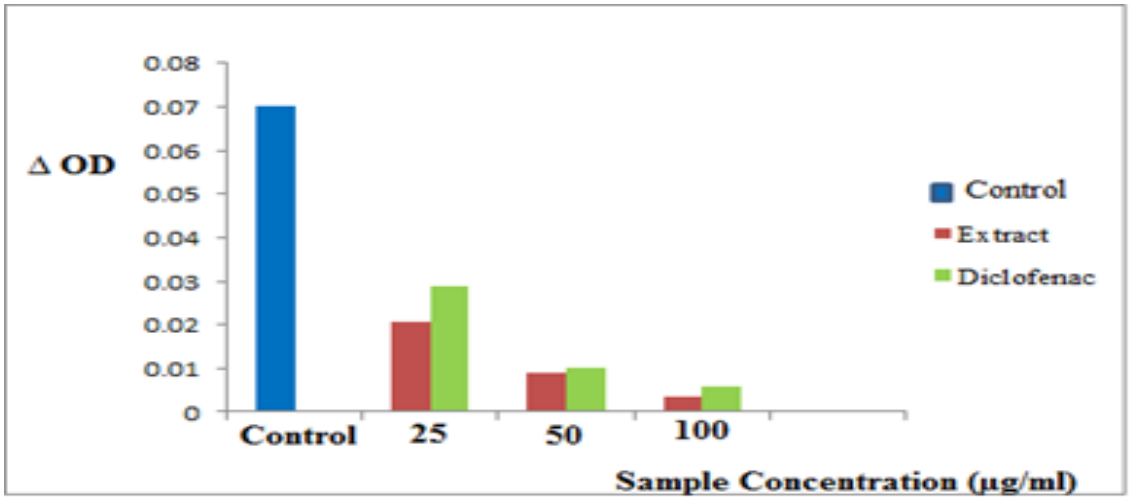

Figure 4 shows inducible nitric oxide synthase inhibitory activity

Table number: 5

Sodium Nitrite (Standard graph)

Concentration(µg)	OD Values
100	0.021
200	0.042
300	0.063
400	0.080
500	0.170

Table no: 6

Sample Concentration (µg/ml)	Concentration of nitrate (µg/ml)	
	Extract	Diclofenac
25	442.035	729.596
50	394.515	588.448
100	313.83	339.948

Control =520.74

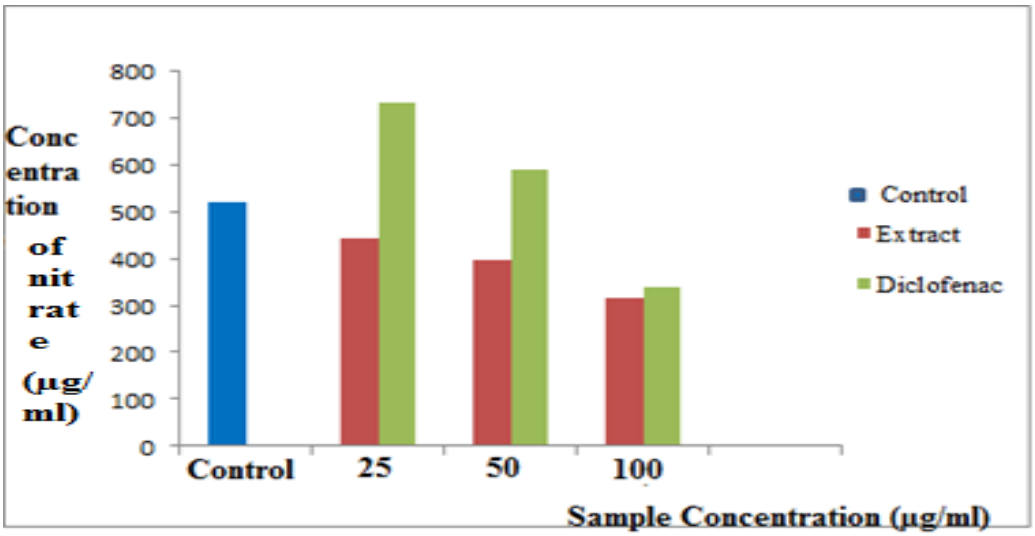

Figure 5 shows estimation of cellular nitrate level

DISCUSSION:

Acute inflammation is the initial response of the body to harmful stimuli and is brought about by the increased movement of leukocytes and plasma into the injured tissues. Chronic inflammation is the prolonged inflammation, where there is a progressive shift in the type of cells present at the site of inflammation, like mono nuclear cells which is characterized by simultaneous destruction and healing of the tissue. The features of inflammatory response are vasodilatation, increased vascular permeability, cellular infiltration, changes in the biosynthetic, metabolic and catabolic profiles of many organs and activation of cells of immune system and enzyme systems of blood plasma. The metabolism of arachidonic acid has a significant role in the inflammation mechanism. Arachidonic acid can be metabolized to prostaglandins and thromboxane A2 by cyclooxygenase (COX) pathway and to hydroperoxy-eicosatetraenoic acids (HPETE's) and leukotriene's (LT's) by 5lipoxygenase (5-LOX) pathway. Inhibition of 5-LOX and COX decreases the production of these biologically active mediators of inflammation. So agents that inhibit these enzymes can act as anti-inflammatory agents. Inflammation is implicated in the pathogeneses of arthritis, cancer, stroke, neurodegenerative and cardiovascular disease. So it is necessary to explore plant to obtain traditional herbal medicines. The total ethanolic extract of *Calotropis gigantea* used in the present study exhibited good inhibitory activity against metabolism of arachidonic acid which is mediated by the enzymes COX and 5-LOX, which is indicated by the reduction of cellular nitrite level, inducible nitric oxide synthase and myeloperoxidase significantly. In all the five methods studied the ethanolic extract was showing dose dependent anti -inflammatory activity which is comparable with that of standard drug diclofenac.

CONCLUSION:

A variety of mechanisms are involved in inflammation. In the present study the ethanolic extract of leaves of *Calotropis gigantea* exhibited COX, 5-LOX, i NOS, cellular nitrite and MPO inhibition properties. This indicates the ability of the plant under study to act as anti-inflammatory agents by various mechanisms. Further studies are also recommended to find the exact mechanism behind the anti- inflammatory activity and also to identify the active component responsible for the particular activity. Based on the future investigations the plants can be utilised as components of a polyherbal formulation for treating inflammatory conditions.

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