Anti-arthritic and Thrombolytic activity of leaves of Cordia dichotoma

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ABSTRACT:-

The present study was designed to investigate the anti arthritic and thrombolytic activity of *Cordia dichotoma* and phytochemical analysis of ethanolic extract of leaves of *Cordia dichotoma*. Inhibition of protein denaturation was evaluated to assess the anti arthritic effect of the selected plant materials. Denaturation was induced by incubating the extract with bovine albumin under various controlled condition. The protein denaturation was calculated by measuring the absorbance of different concentration. The thrombolytic activity has been assessed by human volunteer blood. Then all the activities are done in vitro on leaves of *Cordia dichotoma.* The ethanolic extract of leaves of *Cordia dichotoma* shows significant anti arthritic and thrombolytic activity. The inhibition of protein denaturation was found to be 79.12 % at concentration of 1000µg/ml in anti arthritic activity and that of % clot lysis was found to be 31.23 % in thrombolytic activity. From this above study we concluded that the leaves of *Cordia dichotom*a show significant anti arthritic and thrombolytic activity.

Keywords:-

Cordia dichotoma, Anti arthritic, Thrombolytic activity.

INTRODUCTION:-

Inflammation is a normal tissue response to injury which involves complexes of enzyme activation, mediator release, cell migration, tissue breakdown and repair [1]. Rheumatoid arthritis is a chronic, inflammatory, systemic autoimmune disorder. It is an inflammation of synovial joint due to immune mediated response [2]. Arthritis involves the breakdown of cartilage cause decreased level of cartilage. As a result

causing bone rub together and starts bone pain, swelling and stifiiness [3]. Inflammation is a complex response to a various vascular tissues such as pathogen, damaged cell or irritants such as physical or chemicals. The main aim is to remove the injurious stimuli and initiate the tissue healing process [4]. To fight against the pain and inflammatory disorder the various drugs such as cyclooxygenase (COX) inhibitor are used. These drugs are used in limited otherwise it having various side effects such as dyspepsia, high blood pressure, liver and kidney problem. Inflammatory reaction occurs the production of soluble pro-inflammatory mediators such as cytokines, prostaglandins, leukotrienes [5-7]. Presently the no proper treatment is available for the joint in inflammation. The various types of medicines like steroids, analgesics, non steroidal anti-inflammatory drugs (NSAIDs) glucocorticoids are used in very limited success rates in the treatment of rheumatoid arthritis [811]. *Cordia dichotoma* is a species flowering tree. It belongs to family *Boraginaceae* that is native to China, India, Pakistan, Taiwan, Srilanka etc. The common name includes Indian cherry, lasoda, tenti, bhokar etc. *Cordia dichotoma* is a small to moderate sized tree with a short bole and spreading crown. The stem bark is grayish brown, smooth or longitudinally wrinkled. Flowers are short stalked, or bisexual, white in colour which opens only at night. The fruit is yellow or pinkish yellow which turns black on ripening and pulps gets viscid. It has various advantages such as immature fruits are pickled and also used as vegetable fodder. The leaves are also yield good fodder. The seed kernel has medicinal properties [12].

Thrombosis is the formation of blood clot in blood vessel [13]. When the blood vessel is injured the body uses the platelet and fibrin to form a blood clot to prevent blood loss. When blood vessel is not injured blood clots may form in the body under certain condition [14]. Herbs showing thrombolytic activity have been studied and some significant observations have been studied [15]. Herbal products are extensively perceived as safe because they are natural [16]. They have less or no side effects [17]. Thrombosis is the most important pathophysiological process that underlies the acute coronary disorder such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks which are the main cause of morbidity and mortality in developed countries [18]. Anticoagulation is the best and the proper choice of the thrombolytic drug to decrease platelet aggregation. Intravenous heparin is the first line which is used in the treatment for cerebral venous sinus

thrombosis (CVST), is used in anticoagulation therapy, because it is effective and feasible. There are many drugs have been developed with the purpose of dissolving the clots such as, alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen etc. The Cordia dichotoma has a various biological activities such as antibacterial, antidiabetic, antioxidant, anticancer and anti-inflammatory properties. From this above study it is concluded that the present aimed to evaluate the in vitro anti arthritic and thrombolytic activity of leaves extract of *Cordia dichotoma* [19].

MATERIALS AND METHODS:-

a) Sample collection and preparation:-

The fresh leaves of *Cordia dichotoma* G.forst. Were collected from Sahyadri College of Pharmacy, Methwade Sangola, Solapur. The taxonomical identification of the plant was done by Dr. Tembhurne R.R. Dept. of Botany Sangola College, Sangola with the flora of Solapur District, Maharashtra, India and the voucher specimen were deposited at the herbarium, Department of Botany Sangola College, Sangola. Leaves was dried under shade, powdered and stored in air tight container for further use.

b) Preparation of extracts:-

The preparation of extract was carried out by using soxhlet apparatus. The preparation of different extract such as chloroform, ethyl acetate and ethanol extract were carried out using these solvents. Extraction is carried out in soxhlet extractor at 72 hours at temperature not exceeding boiling point of the solvents. The extract were filtered using Whatman filter paper (No.1) while hot concentrated in vacuums under reduced pressure using rotary flask evaporator and dried under desiccators.

For aqueous extract preparation 250 gm coarse powder is macerated with 500 ml water for 24 hours. Add few drops of chloroform to avoid bacterial growth. Then concentrate the extract for further use.

Drugs and Chemicals:-

All the organic and other reagents were procured from Sona Chemicals Ltd. Islampur and were of analytical grade. Diclofenac sodium and Aspirin was obtained from Pharmaceutical chemistry Laboratory of Sahyadri College of Pharmacy, Methwade, Sangola.

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ANTI-ARTHRITIC ACTIVITY:-

For the current study of anti- arthritic activity of *Cordia dichotoma* in vitro model are evaluated. Diclofenac sodium is used as a standard.

Preparation of reagents:-

1 % Bovine serum albumin:

Dissolve 1 gm of bovine serum albumin in distilled water.

Preparation of Standard solution:-

Diclofenac sodium is used as a standard. Stock solution of Diclofenac sodium in water 1000µg/ml was prepared. From this stock solution 3 different concentrations of 50, 100, 500 µg/ml were prepared.

Preparation of Test solution:-

Stock solution of various leaves extract of 1000 µg/ml was prepared by using ethanol, chloroform, ethyl acetate and water as a solvent. From this stock solution 3 different concentration of 50, 100, 500 µg/ml were prepared.

In vitro Anti-arthritic activity:

This activity was evaluated by using protein denaturation test. The different extraction of plant extract ranging from 100-500 µg/ml was prepared. Reaction

mixture of each concentration was prepared which consisted of 1 ml of test drug and 1 ml of 1% bovine serum albumin solutions. These prepared solutions were incubated at 27 ± 1℃ for 15 minutes. Then the reaction mixture was kept at 70℃ in water bath to induce denaturation. These solutions were cooled and turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as standard drug in the concentration of 50-1000 µg/ml and treated similarly as test extract. % inhibition of denaturation was calculated using control in which no drug was added. Each experiment was done in triplicate manner and average was taken. The % inhibition of protein denaturation was calculated by following equation [20].

% Inhibition of protein denaturation = 100 × [A 1 – A 2] /A 1]

Where,

A1 = Absorbance of control

A2 = Absorbance of test / standard sample with albumin solution.

THROMBOLYTIC ACTIVITY:-

For the study of thrombolytic activity Aspirin is used as a standard.

Preparation of reagents:

Preparation of standard solution:

Stock solution of aspirin in water was prepared of 1000 µg/ml. From this stock solution 4 different concentration of 200, 400, 600, 800 µg/ml were prepared.

Preparation of test solution:

Stock solution of various leaves extract of 1000 µg/ml was prepared by using ethanol, ethyl acetate, chloroform and water is used as solvent. From this stock solution 4 different concentration of 200, 400, 600, 800 µg/ml were prepared.

Thrombolytic Activity:

Venous blood samples (3ml each) drawn from three healthy human volunteers. 500 µl of the blood was transferred to each invitro of five previously weighed Eppendorff tubes for each subject. In the first series the transferred 500 µl allowed to form clot at 37℃ for 45 minutes. After clot formation the serum was completely removed and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each eppendroff tube containing pre-weighed clot 200 -1000 µg/ml of different concentration of plant extract or 100 µl distilled water as a negative control were added. All the tubes were incubated at 37℃ for 90 minutes and observed for clot lysis. After incubation fluid released was removed and tubes were again weighed to observed difference in weight after clot stabilization. The obtained difference in weight was expressed as a percentage of stabled or lysed clots. In second series of experiments simultaneous addition of 500 µl of blood and 100 µl of aspirin incubated at 37℃ for 45 minutes. The obtained clot weight was determined as above [21].

The % clot lysis was measured as,

$$\% \text{ clot lysis} = (W2 - W3 / W2 - W1) \times 100$$

Where,

W1 = Empty weight of eppendorff tube

W2 = Weight of eppendorff tube and clot

W3 = Weight of clot release after addition of plant extract.

Statistical analysis:-

Data was analyzed by ANOVA followed by students‘t’ test with Graph Pad Prism Data Editor for Windows. The values were represented as mean ± S.E.M (standard error of mean).

RESULT AND DISCUSSION:-

In vitro anti arthritic activity:

The anti arthritic activity was also shown in a concentration dependant manner and the activity was increased on increasing the concentration of extracts. So higher activity shown at higher concentration. Ethanol extract shows higher activity as compared to other extract and shown 79.12 ± 1.50 % inhibition of protein while 75.36 ± 1.61 %, 72.17 ± 1.00 % and 70.49 ± 2.52 % inhibition was shown by ethyl acetate, chloroform and aqueous extract respectively at the concentration of 1000 µg/ml which is the highest concentration evaluated. The percentage inhibition by the extracts at different concentrations and their comparison with the standard drug are shown in (table 2). IC50 values for ethyl acetate, ethanol, chloroform and aqueous extracts were 294.17, 73.68, 426.46 and 388.25µg/ml respectively. From this it is concluded that the ethanol extract having significant activity as compare to other extracts.

In vitro thrombolytic activity:-

Addition of 1ml of aspirin as a positive control to the clots with 90 minutes of incubation at 37℃ it showed (64.21± 1.71 %). Clots when treated with 100µl distilled water as a negative control it showed negligible clot lysis (4.00 ± 0.03%). The ethanol extract shows higher degree of thrombolytic activity and clot lysis is (31.23 ± 1.09) at 1000µg/ml. Positive thrombolytic control (aspirin) and negative control (distilled water) is showed in (table 3 and figure 2).

Table 1. % Inhibition of protein denaturation of different leaves extracts of *Cordia dichotoma*

Sr. No.	Plant Extract	Concentration (µg/ml)	% Inhibition ± SEM
1.	Standard (Aspirin)	50	52.17±1.15
		100	64.63±2.23
		500	75.07±1.39
		1000	87.24±1.32
2.	Ethanol	50	40.28±1.89
		100	66.08±2.18
		500	73.62±1.26
		1000	79.12±1.50
3.	Ethyl acetate	50	27.82±1.32
		100	53.91±1.50
		500	61.44±1.89
		1000	75.36±1.61
4.	Chloroform	50	21.73±1.00
		100	43.47±1.00
		500	60.17±1.15
		1000	72.17±1.00
5.	Aqueous	50	23.47±1.00
		100	44.63±1.04
		500	61.91±2.01
		1000	70.49±2.52

(Values are expressed as Mean ± SEM of 3 readings

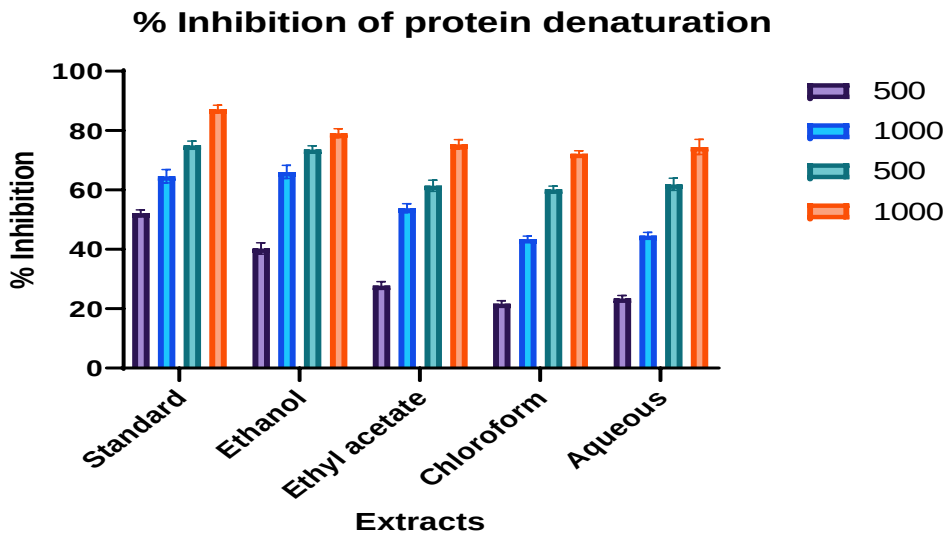

Fig 1. % Inhibition of protein denaturation vs. different extracts.

Table 2. % clot lysis of different leaves extract of *C. dichotoma* in-vitro method.

Sr. No.	Concentration of plant extract (µg/ml)	% clot thrombolysis				Blank negative control	Aspirin positive control
		Ethanol	E.acetate	Chloroform	Aqueous		
1.	200	22.08±1.72	7.55±1.25	5.78±2.16	3.70±1.40	4 ± 0.037	64.24 ± 1.71
2.	400	25.90±2.36	8.39±1.32	7.62±1.48	6.42±1.89		
3.	600	27.39±2.06	10.92±1.22	8.32±1.99	13.02±2.11		
4.	800	30.75±1.95	14.44±2.22	8.83±1.15	14.17±1.15		
5.	1000	31.23±1.09	15.97±1.18	10.89±2.37	22.66±1.88		

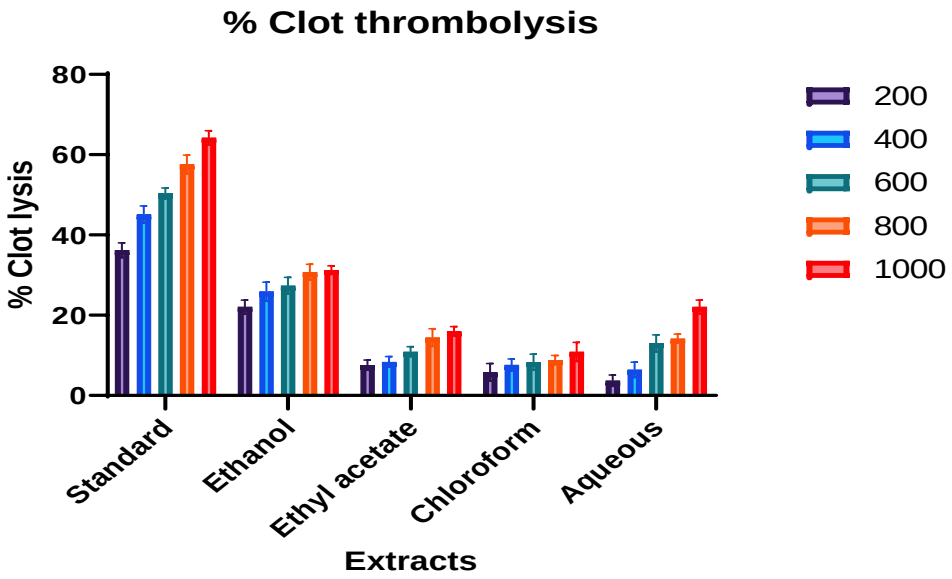

Fig 2. Thrombolytic activity of different extract of *Cordia dichotoma leaves.*

CONCLUSIONS:-

In the field of synthetic drugs during recent era, they are having various side effects. Whereas the plants having no side effects as compare to synthetic drugs. Therefore various types of the plants are used against the arthritis and inflammation so, as to exploit them herbal anti arthritic agents. In % inhibition of protein denaturation of method the *cordia dichotoma* leaves extracts shows concentration dependant inhibition protein denaturation throughout from low to high concentration. In vitro thrombolytic activity of *cordia dichotoma* leaves extract against blood clot. The result of present study is that ethanolic extract *of cordia dichotoma* leaves shows higher thrombolytic activity as compare to other extract.

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