International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 4 Issue 4, June 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

In -Vitro Anti-Arthritic Activity of Acacia Catechu Willd

Priyanka Karande, Ashapak Tamboli, Swapnil More, Arti Chandanshive, Shweta Bahire, Mahadevi Bhosale

Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methvade, Maharashtra, India

ABSTRACT

Rheumatoid arthritis is a major ailment among rheumatic disorders. A large number of herbal extracts are in vogue used for treatment of various types of rheumatic disorders. Acacia catechu willd, an Indian herb was reported to have anti-inflammatory as well as analgesic activity, in-vitro as well as in-vivo. The present study deals with anti-arthritic activity in-vitro. Various in-vitro anti-arthritic pharmacological models were studied, such as, inhibition of protein denaturation, effect of membrane stabilization, and proteinase inhibitory action Herbal extract. All the in-vitro models i.e. inhibition of protein denaturation, membrane stabilization and proteinase inhibition were carried out with standard reference drug diclofenac sodium.

KEYWORDS: *Acacia catechu willd, Anti-arthritic, Proteinase inhibitory, protein denaturation, Membrane Stabilization*

How to cite this paper: Priyanka Karande | Ashapak Tamboli | Swapnil More | Arti Chandanshive | Shweta Bahire | Mahadevi Bhosale "In -Vitro Anti-Arthritic Activity of Acacia Catechu Willd" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-4 | Issue-4, June 2020, pp.1310-1312, URL: www.ijtsrd.com/papers/ijtsrd31469.pdf

IJTSRD31469

INTRODUCTION

Rheumatoid arthritis is one of the chronic systemic disease which affects majority of population. It leads to irreversible joint damage and systemic complications. It may involve nociceptive and non-nociceptive components, including neuropathic components due to sensitization and peripheral inflammation. Despite the modern wealth of analgesic options available in our country, treating acute to chronic patients still remains clinically challenging. NSAIDS are effective in treating nociceptive arthritis related pain. But because of the side effects and toxicity caused by NSAIDS even after the discontinuation of the drug, the use of NSAIDS has been relatively reduced. Acacia catechu willd. belongs to the family Fabaceae and subfamily mimosoideae. It is widely used in Ayurveda for many diseases and mainly skin diseases. Many Ayurvedic oil preparation use khadira as one of its active ingredient. Acacia catechu has strong astringent and antioxidant activity. It is most commonly known as katha which is an ingredient of Pan, a beetle leaf preparation chewed in India. It is used to reduce the oozing from chronic ulcers and as an astringent In throat, dental and oral infections. Acacia catechu extracts exhibits various pharmacological effects like antidiarrheal, hypoglycemic, antipyretic, anti inflammatory, hepatoprotective, antioxidant and antimicrobial activities.

Material and Method

Fresh aerial parts of acacia catechu, used for the study was collected from the sangola resion sangola district during August 2019. The plant was identified and Authenticated by Botanist DR. Tembhurnikar sir, Sangola mahavidyalaya, sangola. The leaves of acacia catech. was shade dried and made the fine powder of the leaves and store in the polythene bags

The use of in-vitro studies herbal extract of Acacia catechu willd as show following

1. Inhibition of protein denaturation

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Acacia catechu willd.* extract (100,200 and 500 m/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37o C for 20 min and then heated at 57o C for 3min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm4 for control tests 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin.The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percent stabilization} = 100 - \frac{(\text{O. D. of test} - \text{O. D. of product control}) \times 100}{\text{O.D of control}}$$

2. Proteinase inhibitory action:

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0ml. 25 mM tris-HCl buffer (pH 7.4) and1.0 ml aqueous solution of Acacia catechu willd extract (100, 200 and 500

mcg/ml of final volume). The mixtures were incubated at 37oC for 5 minutes. then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 minutes. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated as follow:

$$\text{Percent stabilization} = 100 - \frac{(\text{O. D. of test} - \text{O. D. of product control}) \times 100}{\text{O.D of control}}$$

Result and discussion

1. Inhibition of protein denaturation:-

Protein denaturationis process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base.Denaturation of proteins is a well documented cause of inflammation. The mechanism of In-Vitro anti-inflammatory activity of various extracts of Vitex nagundo linn. The ability of this various plant extract to inhibit protein denaturation was studied. The maximum inhibition was observed by the ethanolic extract 68.47% at 500µg/ml.as compare to another extract.Diclofenac sodium as standard anti-inflammatory drug showed the maximum Inhibition of protein denaturation of different extract of Acacia catechu Willd in-vitro inhibition65.65. % at concentration 500µg/ml.In the study of protein denaturation assay study that concentration increases the percent of inhibition also decreases.

Table No.1 Inhibition of protein denaturation of different extract of Acacia catechu Willd in-vitro

Sr. No.	Plant Extract	Concentration µg/ml	% inhibition	Mean±SD
1	control			0.460±0.005
2	Pet Ether Extract	100	46.73%	0.246±0.0015
		200	60.01%	0.184±0.0015
		500	67.60%	0.147±0.0015
3	Chloroform Extract	100	61.73%	0.176±0.002
		200	66.08%	0.157±0.0015
		500	71.08%	0.135±0.0026
4	Ethyl Acetate Extract	100	43.04%	0.262±0.0011
		200	51.52%	0.222±0.0015
		500	60.65%	0.182±0.001
5	Ethanol	100	41.52%	0.269±0.0015
		200	56.95%	0.198±0.0057
		500	68.47%	0.144±0.0015

Table No.2 Inhibition of protein denaturation by Diclofenac sodium In-vitro

Sr. No.	Standard	Concentration µg/ml	% inhibition	mean±SD
		100	59.13%	0.124±0.001
		200	61.30%	0.175±0.002
		500	65.65%	0.158±0.002

2. Trypsinase inhibitory assay:-

The trypsinase or protianase inhibitory activity is the second model of anti-inflammatory activity by In-Vitro.Leukocytes proteinase play most important role in development of tissue damage during inflammatory reactions and significant protection was provided by proteinase inhibitors. In table no. 3 showed maximum inhibition of ethanolic extract 79.06% at 500µg/ml. as compare to other extract. The Diclofenac sodium showed 71.75% of inhibition at 500µg/ml concentration. In this study clearly showed that if the concentration increases, the percentage inhibition also increases.

Table No.3The Trypsinase inhibitory activity of Acaciac catechu Willd leaves Extract In-vitro

Sr. No.	Plant Extract	Concentrationµg/ml	% inhibition	Mean±SD
1	Control			0.517±0.0015
2	Pet Ether Extract	100	59.10%	0.210±0.005
		200	61.82%	0.196±0.0015
		500	71.51%	0.262±0.197
3	Chloroform	100	58.04%	0.192±0.0015
		200	64.72%	0.143±0.001
		500	70.10%	0.155±0.001
4	Ethyl Acetate Extract	100	61.82%	0.198±0.002
		200	64.72%	0.125±0.0015
		500	75.96%	0.125±0.0015
5	Ethanol Extract	100	64.14%	0.187±0.002
		200	69.37%	0.159±0.0015
		500	79.06%	0.109±0.0015

Table No.4 Inhibition of protein denaturation by Diclofenac sodium In-vitro

Sr. No.	Standard drug	concentrationµg/ml	%inhibition	Mean±SD
1	Diclofenac sodium	100	68.02%	0.166±0.0015
		200	71.51%	0.149±0.0032
		500	71.75%	0.130±0.0015

Conclusion

In this study clearly showed that if the concentration increases ,the percentage inhibition also increases. In the protein denaturation and trypsinase inhibitory assays shows that the maximum percentage of inhibition at maximum concentration.

The ethano medical use of vitex negundo as a useful remedy in inflammatory and arthritic disorder could possible because of its excellent anti-inflammatory and antioxidant potential. This review gives an insight of the frequently used in-vitro assays to test anti- inflammatory activity of herbal extracts. Although some workers have relied only one assay to evaluate the in-vitro anti- inflammatory properties of herbal extracts, most of the workers have preferred to use more than one assay at the same time. We suggest to reduce the animal use in vitro assays as animal ethical issue is important as human welfare. Most of the workers have used either nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, diclofenac and acetyl salicylic acid is positive reference.

Acknowlegment

The authors wish to acknowledge Dr. Patil M. S Principle Sahyadri college of pharmacy and Asso. Prof Tamboli A. Head of department of pharmaceutical chemistry, for the facilities provided to complete this research work.

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