CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *ACACIA EHRENBERGIANA* (SALAM) LEAVES BY *IN-VIVO* AND *IN- VITRO* MODELS

Isra I Mohammad[1], Mazin Y Alsafi[1], Mohammed M. A. AL-mahdi[1], Tasnim O. Elsammani[1*], Mahmoud M. E. Mudawi [2,3]

[1] Department of Pharmacology, Faculty of Pharmacy, International University of Africa, Khartoum, Sudan.

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Northern Border University, Kingdom of Saudi Arabia.

[3] Department of Pharmacology, Faculty of Pharmacy, Omdurman Islamic University, Sudan.

Received: 12 October 2016 **Accepted:** 28 December 2016 **Published:** 31 January 2017

Abstract:

The aim *of this research is to study the anti-inflammatory activity of ethanolic extract of the leaves of Acacia eherbengiana (Hayne), using carrageenan induced paw edema model in rats, albumin denaturation and heat induced hemolysis.*

Methods: *a total of twelve animals of both sexes weighing (60 - 130 g) were randomly divided into three groups consisting of 4 animals each. Group I served as control and receive distilled water, while group II and III received the ethanolic extract of Acacia eherbengiana (300 mg\kg body weight) and diclofenac sodium (20mg\kg) respectively. Inflammation was induced by injecting carrageenan (1% w\v) in the sub-plantar tissues of the rat hind paw.*

In vitro anti-inflammatory activity of the ethanolic extract was evaluated by inhibition of protein denaturation and human RBCs membrane stabilization methods at 250, 500, 750 and 1000 µg/ml.

Results: *the ethanolic extract of Acacia eherbengiana (300 mg\kg body weight) produced 40% inhibition of paw edema volume while diclofenac sodium produced 40.6% inhibition. The percent of membrane stabilization of Acacia eherbengiana was found to be 57.9% while for diclofenac sodium was 55.8% and that of protein denaturation was found to be 84.6%, 42.8% for Acacia eherbengiana and diclofenac sodium respectively*

Conclusion:

The ethanolic extract of the Acacia Eherbengiana exhibited significant anti-inflammatory activity which was found to be time dependent.

Keywords: *Acacia eherbengiana , anti-inflammatory, carrageenan, albumin denaturation, heat induced hemolysis.*

Corresponding author:
Tasnim O. Elsammani,
Department of Pharmacology,
Faculty of Pharmacy,
International University of Africa,
Khartoum, Sudan.
E-mail: seema_skj@hotmail.com

QR code

Please cite this article in press as Tasnim O. Elsammani et al, **Evaluation of Anti-Inflammatory Activity of *Acacia Ehrenbergiana* (Salam) Leaves by *In-Vivo* and *In- Vitro* Models**, *Indo Am. J. P. Sci, 2017; 4(01).*

INTRODUCTION:

Inflammation is a normal, fundamental set biological response to an invasive pathogen such as chemical irritants or infective pathogens [1-3] that defends the host from infection and other invectives; it initiates pathogen killing as well as tissue healing processes and helps to restore homeostasis at infected or injured sites [4].

The past decade has witnessed a great interest and use of medicinal plant products [5]. Huge studies are made to confirm the use of the plant as potential therapeutic agents for various diseases in humans [6].

Medicinal plants are plants used as natural medicines. This practice has existed since prehistoric times. Historically, the medicinal value of plants was tested by trial and error [7]. For many of the medicinal plants of current attention, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy, and pharmacology [5]. The area of phytochemistry and medicinal plants have been characterized for their possible bioactive compounds [5].

The therapeutic properties of medicinal plants are conditioned by the presence in their organs of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds, which physiologically affect the bodies of humans and animals or which are biologically active in relation to the causative agents of various diseases [7].

Modern science has found medicinal plants act as an alternative source for treating several disorders since their usage is increasing gradually and approximately 72 000 plant species were estimated for having medicinal properties [8].

Acacia eherbengiana belong to family *Fabaceae* local name Salam originates in the North Sahel and the Southern and Central Sahara. Also found in Sahara, Arabian Peninsula, and East Africa. It used traditionally to alleviate the swelling and pain associated with rheumatoid arthritis.

The active ingredients of the plant possess Antioxidant[9] activity. The Ethanolic extract of leaves showed standard antibacterial for G+ve bacteria & G-ve and antifungal activity which was recorded in comparison to standard ampicillin (10mg/ml) and nystatin (10mg/ml) respectively in DW[10]. On the basis of these common uses of this plant in traditional folk medicine and its above reported activities in the literature, we have evaluated the anti-inflammatory effect of ethanolic extract of leaves of *Acaia eherbengiana*Materials and methods. Drugs and chemicals. All drugs, chemicals, solvent, and reagents used in this study were of the high analytical grade. Plant collection and identification.Fresh mature leaves were collected from fully-grown plants from Khojalab near Bahry city, Khartoum, Sudan in February and authenticated by a taxonomist at the department of

pharmacognosy, Khartoum. Then the plant material was dried at room temperature. Extract preparation The leaves of the plant material were crushed using pestle and mortar, then weighed and transferred to the soxhlet apparatus and the solvent (ethanol) was added.

The extraction was carried out for 22 hours till the solvent became colorless in the last siphon. The product was then transferred to the rotary evaporator to separate the solvent from extract and dried at room temperature for 7 days in a porcelain dish. After complete dryness extract was transferred to an airtight container.

Experimental Animals housing and Ethical considerations

Healthy adult Wistar Albino rats (130-160 g) of both sexes were obtained from National center for Research, Khartoum, Sudan.

Animals were housed in standard polypropylene cages to provide them with sufficient space, and to allow acclimatizing under laboratory conditions for a period of 7 days prior to the experiment.

Animals were maintained under standard condition (12: 12- hour's light/ dark cycle and at an ambient temperature at 25 ± 2°C, with 65 ± 5 % humidity).

The animals had free access to a standard commercial pellet diet (National Center for Research, Khartoum, Sudan) and water *ad libitum.*

All the studies and experimental protocols were approved by the Institutional Animal Ethical Committee (I.A.E.C) in the department of pharmacology, Faculty of pharmacy, International University of Africa, (**Registration No:** IUA, IAEC/Ph.016/01).

In-vivo Anti-inflammatory Activity using carrageenan-induced paw edema model

The test compound was suspended in 10 mL distilled water and administered at a dose of 300 mg/kg of body weight (i.p.), while 20 mg/kg, (i.p.) of Diclofenac sodium was administered as a reference standard drug. The control group

received 1 ml distilled water.

Carrageenan was prepared by adding 1 gm in 100 ml distilled water to prepare (1% Carrageenan solution) and administered at the sub-planter region of the right hind paw.

The animals were divided into three groups; Group I was served as control and received corn oil, Group II was treated as a positive control and received standard diclofenac, Groups III has received the test compound dose as described in test preparation at different time intervals.

The compounds were tested for anti-inflammatory activity by carrageenan-induced rat paw edema method described by Winter et al [11].

One hour after receiving the drug(s), acute inflammation was produced by sub-planter administration of 0.1 ml of 1% carrageenan in the right hind paw of the rats.

The average volume (Vo) of the right hind paw of each rat was measured from 3 readings immediately prior to the carrageenan injection and 1, 2, 3 and 4 h later using the Vernier caliper (with interval time =30mins).

The paw edema volume was determined in milliliters as the difference between the final and initial volumes, and the anti- inflammatory activity was determined as the percentage of inhibition of inflammation and it was calculated for each group at each time using following equation:

$$\% \text{ Inhibition} = \frac{[\text{Predrug reading} - \text{Postdrug reading}]}{\text{Predrug reading}} * 100$$

The percentage inhibition of paw volume for each test group is calculated, the results were shown in figures below.

In vitro determination of Anti-inflammatory Activity

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10ml) was collected and transferred to the heparinized centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline (Sadique et al) [12] and (Sakat et al) [13].

The Procedure

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample the only saline was added to the control test tube. Diclofenac sodium was used as a standard drug.

All the centrifuge tubes containing reaction mixture were incubated in water bath at 56^0C for 30min, and then heated to 51 ° C for 20 min, after cooling the samples the turbidity was measured at 660nm by using UV-Visible Spectrophotometer.

The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm.

The experiment was performed in triplicates for all the test samples.

Percent membrane stabilization activity was calculated by the following formula (Shinde et al) [14] and (Sakat et al) [13].

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of the Control} - \text{Absorbance of the Test}]}{\text{Absorbance of the Control}} * 100$$

Anti-denaturation activity

The anti-inflammatory activity of acacia extract was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al [15] and Sakat et al [13].

Test solution (1 ml) containing different concentrations of drug was mixed with (1 ml) of the reaction mixture which was consists of test samples and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl.

The compounds were incubated at 37 ºC for 20 min and then heated to 51 ° C for 20 min, after cooling the samples the turbidity was measured at 660nm by using UV-Visible Spectrophotometer.

The experiment was performed in triplicate and the mean absorbance was recorded and the Percentage inhibition of protein denaturation was calculated as discussed above.

Statistical Analysis

The values were expressed as mean ± S.E.M. The statistical analysis was carried out by means of the two-tailed unpaired t-test for the data having two groups and one-way analysis of variance (ANOVA) followed by multiple comparisons using the Dunnet's test for the data having more than two groups. Statistical evaluation was performed with Prism 5.0 computer program. The differences were considered to be significant $P \leq 0.05$.

RESULTS:

Percent of inhibition in paw edema versus time

The percent of inhibition of ethanolic extract and standard diclofenac were represented graphically in figure 1 and 2 respectively.

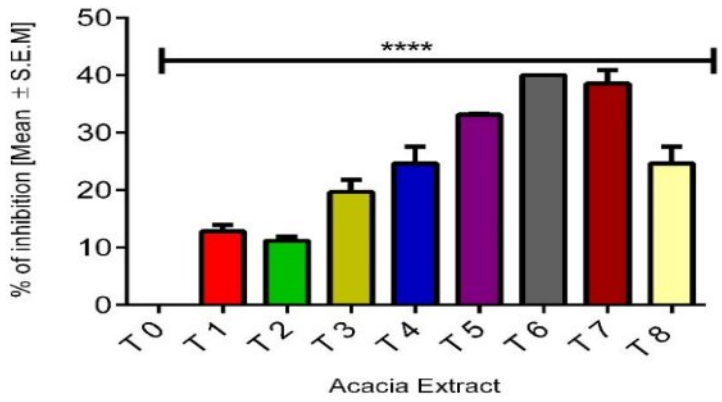

Fig 1: Bar diagram represents the effect of acacia extract using carrageenan induced paw edema model in rats, highest inhibition is 40% after 3 hours. Values are expressed as Mean ± S.E.M (n = 3). *P* value < 0.00001 **, T (0 to 8) represent the time interval =30 min**

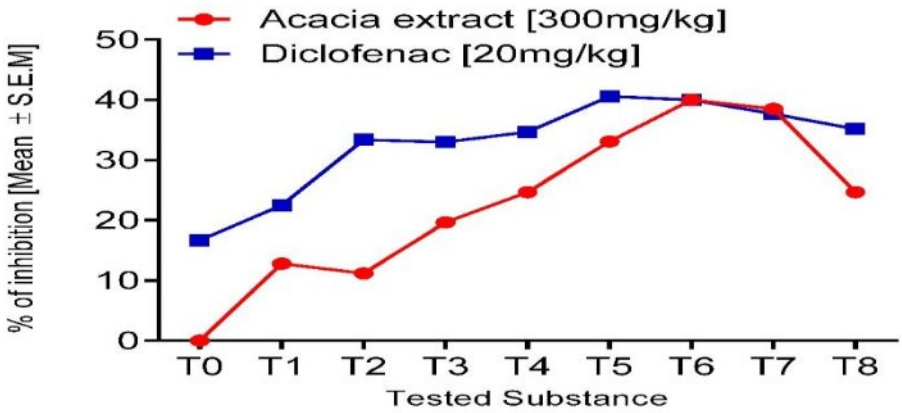

Fig 2: Curves represent the effects of acacia extract and diclofenac sodium using carrageenan induced paw edema model in rats. T0-T8 is time interval of the study (each time interval represent half an hour); Values are expressed as Mean ± S.E.M (n = 3).

Two side test, p-value=0.0740

In vitro membrane stabilization method

Table 1: Summary of the main Mean absorbance and percent of inhibition of Diclofenac and ethanolic acacia extract, using HRBC stabilization technique

Tested Substance	Mean Absorbance	Percent of Inhibition
Control	0.024	-
Diclofenac [1000µg/ml]	0.037	54.17
Diclofenac [750µg/ml]	0.036	50.00
Diclofenac [500µg/ml]	0.037	54.17
Diclofenac [250µg/ml]	0.036	50.00
Acacia Extract [1000µg/ml]	0.037	54.17
Acacia Extract [750µg/ml]	0.037	54.17
Acacia Extract [500µg/ml]	0.036	50.00
Acacia Extract [250µg/ml]	0.036	50.00

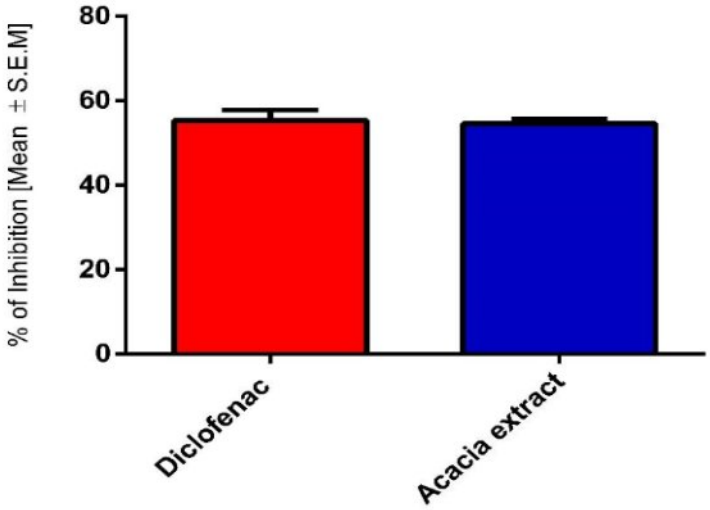

Fig 3: Column represents the effects of acacia extract and diclofenac sodium using H-RBC stabilization method. Column represents the effects of acacia extract and diclofenac sodium using H-RBC stabilization method. Values are expressed as Mean ± S.E.M (n = 3).

Two side test, p-value =0.7197

In vitro Albumin denaturation technique

Table 2: Summary of the main Mean absorbance and percent of inhibition of diclofenac and ethanolic acacia extract, using Albumin Denaturation technique

Tested Substance	Mean Absorbance	Percent of Inhibition
Control	0.011	-
Diclofenac [1000µg/ml]	0.014	27.27
Diclofenac [750µg/ml]	0.013	18.18
Diclofenac [500µg/ml]	0.015	36.36
Diclofenac [250µg/ml]	0.016	45.45
Acacia Extract [1000µg/ml]	0.020	81.82
Acacia Extract [750µg/ml]	0.020	81.82
Acacia Extract [500µg/ml]	0.019	72.73
Acacia Extract [250µg/ml]	0.020	81.82

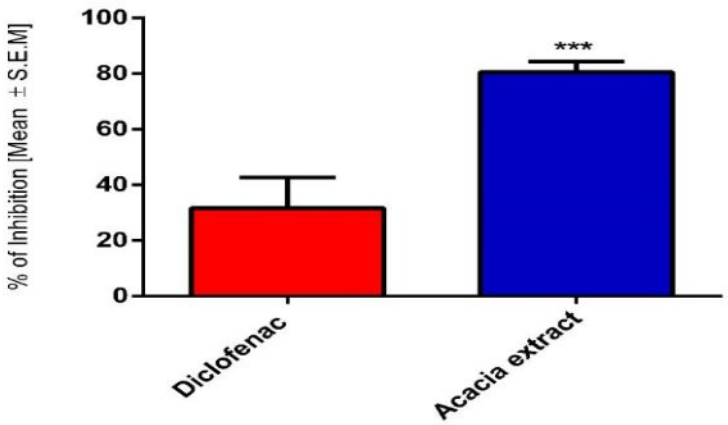

Figure 4: Column represents the effects of acacia extract and diclofenac sodium using inhibition of albumin denaturation technique. Values are expressed as Mean ± S.E.M (n = 3).
Two side test, p-value =0.0002 (***)

DISCUSSION:

The ethanolic extract of leaves of *Acacia ehrenbergiana* (Salam) showed no signs of toxicity in a dose of 300mg/ kg while in dose higher than 1.25 g /kg induced the following effects: muscle spasm, abnormal breathing, decrease activity and death. Therefore, the dose 300mg/kg was selected to be used in this study.

Carrageenan-induced inflammation is a useful model for the assessment of anti-inflammatory effect.

The ethanolic extract of leaves of *Acacia ehrenbergiana (300mg/kg)* produced significant inhibition in paw edema with maximum percent of inhibition 40% (p-value <0. 0001) after three hours of administration of carrageenan, while the standard anti-inflammatory drug diclofenac sodium (20mg/kg) produced 40.6% inhibition of paw edema. This results indicates that the ethanolic extract of leaves of *Acacia ehrenbergiana (300mg/kg)* possess anti-inflammatory activity almost similar to the anti-inflammatory effect of diclofenac sodium (20mg/kg).

Carrageenan induces inflammation in two phases*:* the edema produced in the initial phase, about 2h after carrageenan injection is related to the production of serotonin, histamine, bradykinin and cyclooxygenase products, while in the late phase about 3 hours is more complex and has been accredited in part to prostaglandins, neutrophil and lipoxygenase products of arachidonic acid metabolism [16].

In this study the ethanolic extract of leaves of *Acacia ehrenbergiana* produced the highest anti-inflammatory activity after 3 hours of carrageenan administration; therefore, the probable mechanism of action may be due to inhibition of the late phase of inflammation.

The anti-inflammatory activity of the ethanolic extract of leaves of *Acacia ehrenbergiana* observed in this study was confirmed by using an in vitro model of anti-inflammatory activity namely stabilization of H-RBC membrane test in which the maximum inhibition of heat-induced hemolysis produced by ethanolic extract of leaves of *Acacia ehrenbergiana* and Diclofenac sodium was 54.17% .Another confirmatory test used in this study was albumin denaturation test. However, denaturation of protein is one of the causes of inflammation. Therefore, production of autoantigens in inflammatory diseases may be due to *in vivo* denaturation of the protein.

The maximum percent of inhibition of protein denaturation produced by the ethanolic extract of leaves of Acacia ehrenbergiana was 81.82% while for Diclofenac sodium was 45.45%.

CONCLUSION:

The present investigation demonstrated that the ethanolic extract of *Acacia ehrenbergiana* exhibited significant anti-inflammatory activity in dose 300mg/kg, it was time dependent, and it rationalizes the traditional use of this plant in the treatment of various types of inflammation.

Recommendations

We intensely recommend, making biologically guided fractionation and isolating the active ingredients.

Deep molecular level pharmacodynamics studies will be required using advanced techniques to confirm the intracellular signaling mechanism.

Toxicological studies will be compulsory for c+hecking its safety and to be ready for clinical trials, suggesting its formulations in a suitable pharmaceutical dosage from.

REFERENCES:

1.Andersen, C.J., Bioactive Egg Components and Inflammation. Nutrients, 2015. 7(9): p. 7889-913.

2.Fox, L., S. Hegde, and J.E. Gumperz, Natural killer T cells: innate lymphocytes positioned as a bridge between acute and chronic inflammation? Microbes and Infection, 2010. 12(14): p. 1125-1133.

3.Greene, E.R., et al., Regulation of inflammation in cancer by eicosanoids. Prostaglandins Other Lipid Mediat, 2011. 96(1-4): p. 27-36.

4.Wright, H.L., et al., Neutrophil function in inflammation and inflammatory diseases. Rheumatology (Oxford), 2010. 49(9): p. 1618-31.

5.Briskin, D.P., Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant physiology, 2000. 124(2): p. 507-514.

6.Trujillo, J., et al., Renoprotective effect of the antioxidant curcumin: Recent findings(). Redox Biol, 2013. 1(1): p. 448-56.

7.El-Juhany, L.I. and A.A. Al-Harby, Status and Diversity of Ornamental Plants in King Saud University Campus at Riyadh, Saudi Arabia. Ame.-Euras. J. Agric. Environm. Sci, 2013. 13(4): p. 471-478.

8.Saranya, R., et al., Pharmacognosy of Enicostemma littorale: A review. Asian Pac J Trop Biomed, 2013. 3(1): p. 79-84.

9.Al-Mamary, M., A. Al-Meeri, and M. Al-Habori, Antioxidant activities and total phenolics of different types of honey. Nutrition research, 2002. 22(9): p. 1041-1047.

10.Almagboul, A., et al., Antimicrobial activity of certain Sudanese plants used in folkloric medicine.

Screening for antibacterial activity (V). Fitoterapia, 1988. 59: p. 57-62.
11.Winter, C.A., E.A. Risley, and G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Experimental Biology and Medicine, 1962. 111(3): p. 544-547.
12.Sadique, J., et al., The bio-activity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia, 1989. 60: p. 525-532.
13.Sakat, S., A.R. Juvekar, and M.N. Gambhire, In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharmacy and Pharmaceutical Sciences, 2010. 2(1): p. 146-155.
14.Shinde, U., et al., Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia, 1999. 70(3): p. 251-257.
15.Mizushima, Y. and M. Kobayashi, Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology, 1968. 20(3): p. 169-173.
16.Di Rosa, M., J. Giroud, and D. Willoughby, Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. The Journal of pathology, 1971. 104(1): p. 15-29.