

ANTI-INFLAMMATORY ACTIVITY OF *KIRGANELIA RETICULATA* (POIR). BAILL. ROOT BY CARRAGEENAN-INDUCED RAT PAW OEDEMA MODEL**RAJESH KUMAR SONI^{a*}, RAGHUVVEER IRCHHAIYA^a, VIHANGESH DIXIT^a, ZAHID AHMAD BHAT^b, HILAL AHMAD WANI^b, ASHIQ HUSSAIN NAJAR^b**^aInstitute of Pharmacy, Department of Pharmacognosy, ^bInstitute of Basic Science, Department of Chemistry, Bundelkhand University, Jhansi (U.P.) India. Email: rajeshsoni1987@gmail.com

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ABSTRACT

Objectives: To evaluate anti-inflammatory activity of ethanolic roots extract of *Kirganelia reticulata* (Poir). Baill. by carrageenan- induced rat paw oedema method.

Methods: Ethanolic roots extract of *K. reticulata* was investigated for anti-inflammatory activity by carrageenan induced right hind rat paw oedema in Wistar rats at the dose of 200 and 300mg/kg, p. o. (per orally).

Results: The ethanolic extract of *K. reticulata* root shows significant anti-inflammatory activity ($p < 0.05$ and $p < 0.01$) at the dose of 300mg/kg, p.o. $P < 0.001$ when compared to control.

Conclusion: The results obtained demonstrated that ethanolic roots extract of *K. reticulata* (Poir) Baill. has potential health benefits as it showed dose dependant anti-inflammatory activity.

Keywords: *Kirganelia reticulata*, Carrageenan, Anti-inflammatory, Rat paw oedema method.

INTRODUCTION

Inflammation is the protective mechanism of the local microcirculation to tissue injury which is caused by physical trauma, noxious stimuli by chemical agents, heat, antigen-antibody reaction and microbial effect[1]. It is a body defence reaction in order to eliminate or limit the spread of injurious agents[2]. It is known to be involved in the inflammatory reactions such as release of histamine, bradykinin, prostaglandins, fluid extravasations, cell migration, tissue break down and repair which are aimed at host defence and usually activated in most disease conditions [3]. The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophil and macrophages. This over production leads to tissue injury damaging the macromolecules and lipid peroxidation of membranes[4]. Nature has provided a complete store-house of remedies to cure all ailments of mankind[5]. Many herbal based remedies are belived to have a range of biomedical efficacies including treatment of inflammation, hyperlipemia, arteriosclerosis, osteoporosis and bone resorption etc[6].

Kirganelia reticulata Baill. is a large, straggling or climbing shrub growing from 8 to 10 ft in height. Synonymously, it is also named as *Phyllanthus reticulatus* Poir[7]. The plant is used for variety of ailments, including smallpox, syphilis, asthma, diarrhoea, bleeding from gums[8].

Although *K. reticulata* has traditionally been used in treatment of many types of pain and inflammatory conditions in Bangladesh, no scientific report is available to date to validate these folkloric uses. We now report anti-inflammatory activities of ethanolic extract of *K. reticulata* root.

MATERIALS AND METHODS**Sample collection and authentication**

The fresh root of *K. reticulata* were collected from Kukrail forest near CIMAP Lucknow, India in the month of September and authenticated by Dr. Tariq Hussain, Senior Principal Scientist & Head of Plant Diversity, Systematics and Herbarium Division NBRI Lucknow- (U.P), India. A voucher specimen has been deposited at Department of Systematics and Herbarium, NBRI Lucknow, (U.P), India. (Accession No. LWG-007).

Preparation of extracts

The air dried, coarse powdered root (200gm) material was extracted with ethanol using soxhlet apparatus and continuously extracted by

ethanol at the temperature 70 ± 2 °C. The extracts were pooled together and concentrated by rotary evaporator. The yield was 7.5% w/w. It was dried and used.

Drugs and chemicals

Carrageenan was procured from chemical store room of Institute of Pharmacy, Bundelkhand University Jhansi, India. All chemicals and drugs used in this study were of the high analytical grade.

Animals

Wistar rats of either sex (180-220gm) were taken from Institute of Pharmacy, Bundelkhand University Jhansi, India. The animals were housed under standard conditions of temperature [(25 ± 2) °C], relative humidity (55 ± 2) %, 12/12 h light/dark cycles and fed with standard pellets. All animal experiments were conducted with the permission from IAEC of the Bundelkhand University, India. (Reference number BU/Pharm/IAEC/12/026).

In-vivo Anti-inflammatory activity by Carrageenan- induced rat paw oedema model

The method of Winter *et al* [9]; was used to evaluate anti-inflammatory activity with some minor modifications. The rats were divided into five groups, each group containing 6 animals. Group I served as control and received 0.5% Tween 80 (1ml/kg, p.o.) as vehicle only. Group II of animals served as standard and were administered standard drug diclofenac sodium (100 mg/kg i.p.). Group III and IV received the ethanolic extract of root of *K. reticulata* respectively at the dose of 200 and 300mg/kg, p.o. The percent of inhibition of oedema volume was obtained by following equation [10]; see Table 2.

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

A mark was made on the right hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the column of the plethysmograph up to the mark to ensure a constant paw volume. 30 min after administration (as per experimental protocol), 0.1 ml of 1% carrageenan solution was administered beneath the plantar tissue of all the animals in the right hind paw (plantar region). The paw volume was measured at first 60 min and followed by every 60 min till the 180 min after administration of carrageenan of each group.

Table 2: Percentage (%) inhibition of paw oedema volume

Group	Treatment	Percentage (%) inhibition of paw volume		
		60 min	120 min	180 min
Group II	Diclofenac sodium (100mg/kg)	36.27%	60.34%	82.38 %
Group III	Root extract (200mg/kg)	20.68%	50.45%	70.20%
Group IV	Root extract (300mg/kg)	29.70%	56.58%	74.26%

Statistical analysis

All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed by multiple comparison test by **graph prism pad software (version 6.03)**. Comparison between control and drug treated groups were considered to be significant *P* values less than 0.05 were considered significant. All values are expressed as mean±SEM.

RESULTS

Evaluation of in- vivo anti- inflammatory study

In this experiment, results of carrageenan- induced right hind rat paw oedema was used as the animal model that ethanolic extract of *K. reticulata* significantly inhibited. Anti- inflammatory activity was

compared with diclofenac sodium (100 mg/kg, *i.p.*). The doses (200 and 300 mg/kg *p.o.*) showed comparable effects with diclofenac sodium (Table 1).

In bar diagram (Fig.1. (A) & (B) showed comparative studies between standard treated group I *i.e.* (Diclofenac sodium 100mg/kg, *i.p.*), ethanolic roots extract of *K. reticulata* (Poir) Bail at the dose of 200 & 300 mg/kg, *p.o.* treated group II & III. The effective significant result shows after treated with 300 mg/kg, *p.o.* of ethanolic roots extract in 180 min by right hind rat paw oedema method inducing carrageenan every 60 min. The results shows 300 mg/kg, *p.o.* more potent than 200 mg/kg, *p.o.* ethanolic roots extract. Whereas Fig. 1. (B) total average in change in height (mm) showed max. Effective dose of 300mg/kg, *p.o.* ethanolic roots extract.

Table 1: Effect of ethanolic extract of root of *K. reticulata* on anti – inflammatory activity on carrageenan rat oedema

Group	Treatment	Carrageenan induced rat paw oedema ^a Mean±SEM		
		60 min	120 min	180 min
Group I	Control (0.5% Tween 80)	38.24±2.5	53.51±3.2	68.27±3.8
Group II	Diclofenac sodium (100mg/kg)	24.37±2.7	21.22±2.1**	15.37±3.2***
Group III	Root extract (200mg/kg)	30.33±2.4**	26.51±3.8*	20.42±4.1***
Group IV	Root extract (300mg/kg)	26.88±2.3***	23.23±2.7**	17.57±3.3***

^aValues are Mean±SEM (n=6); paw volume is expressed in change of height (in mm) of Hg bath. One way ANOVA; **P* < 0.05 when compared to control, ** *P* < 0.01, *** *P* < 0.001 when compared to control.

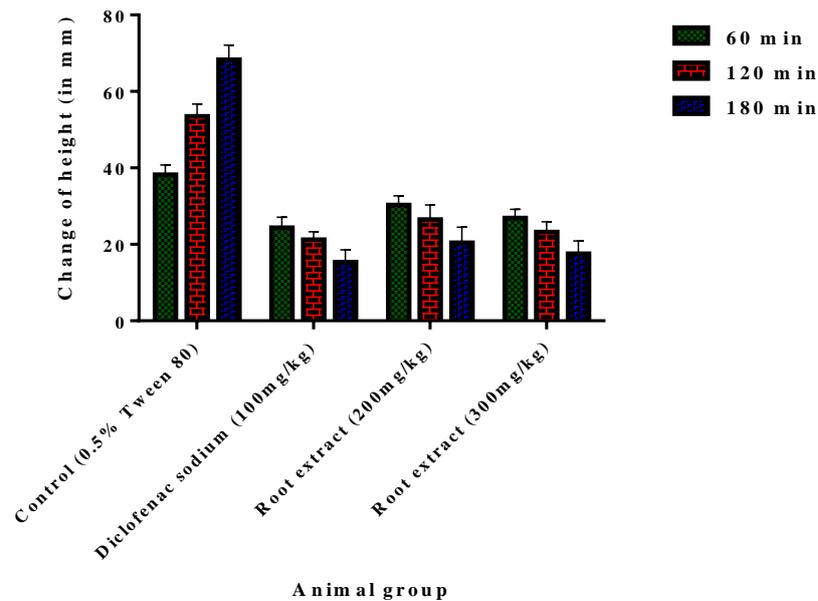


Fig. 1A: Bar diagram representation of data using one- way ANOVA^b (statistical software graph pad prism, version 6.03).

^bAll values are expressed as mean±SEM.

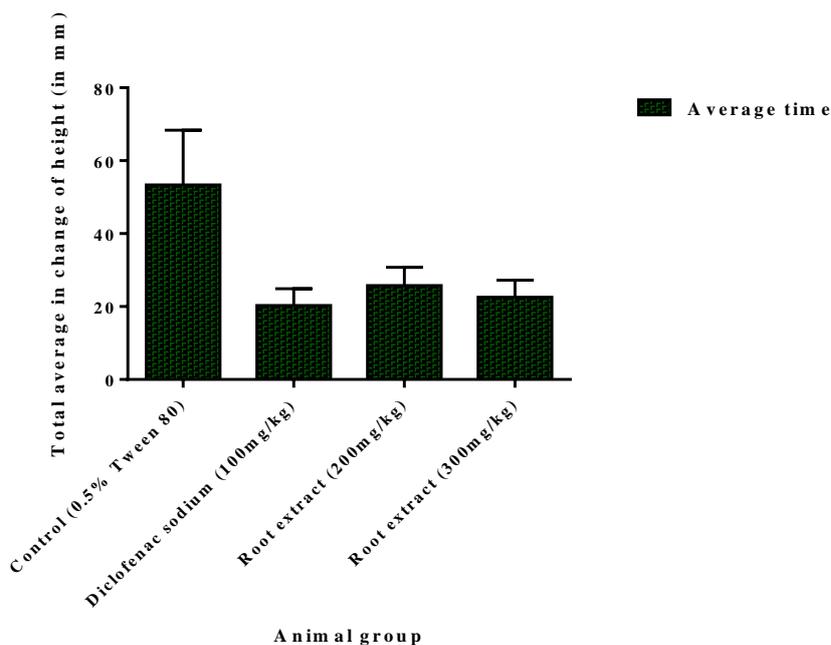


Fig. 1B: Bar diagram showing total average in change of height (mm).

In Fig. 2. The histogram shows the % inhibition of carrageenan induced paw oedema in rats. Histogram is plotted between doses of standard drug Diclofenac sodium (100mg/kg), extracts of root

(200mg/kg, 300mg/kg) in X axis and inhibition of paw oedema in Y axis. Data shows inhibition of paw oedema by root extract significantly decreases in 200mg/kg than 300mg/kg from Standard.

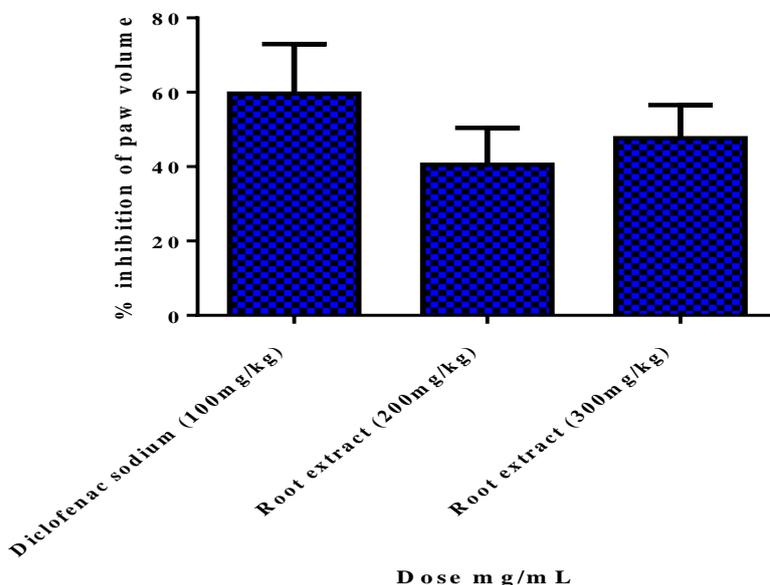


Fig. 2: Bar diagrammatic shows % inhibition of paw volume

DISCUSSION

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response [11].

Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin and prostaglandin [12,13]. There is good evidence that the early or first phase of transient permeability is due to the release of histamine and can thus be suppressed by antihistamines. The mediation of the delayed or second phase of

exudation is more controversial and complex, and has been attributed in part to kinins, prostaglandins, neutrophils, and lipoxygenase products of arachidonic acid metabolism. The probable mechanism of anti-inflammatory action of extract may be due to its influence on the second phase of inflammation, the cyclooxygenase pathway rather than the lipoxygenase pathway. This is evident by the maximal inhibition of inflammation at the end of the third hour after the challenge with carrageenan [14,15]. The anti-inflammatory effect of the ethanolic extract of *Kirganelia reticulata* root may be due to the inhibition of any inflammatory mediators like alkaloids and flavonoids [16] present in the extract.

CONCLUSION

The present study on ethanolic extract of root of *K. reticulata* has demonstrated that this plant has significant anti-inflammatory properties and it justifies the traditional use of this plant in the treatment of various types of inflammation.

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