

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *JUSTICIA BETONICA* EXTRACT

K. Pydiraju¹, K. Ravi Shankar² and B. Rama Krishna³

¹Aditya Pharmacy College, Surampalem, Surampalem-533 437, Andhra Pradesh, India.

²Department of Pharmacology, Aditya College of Pharmacy, Surampalem, Surampalem-533 437, Andhra Pradesh, India.

³Glocal School of Pharmacy, Glocal University, Uttar Pradesh, India.

ABSTRACT

The present research study highlights the Analgesic and Anti-Inflammatory activities of *Justicia Betonica* extract. *Justicia Betonica* was evaluated for analgesic activity using Tail flick method, Eddy's hot plate method and Acetic acid induced writhing responses, Invitro anti-inflammatory activity by Protein Denaturation method and Human Red Blood Cells (HRBC) followed by Invivo anti-inflammatory method using Carragenen rat paw odema was carried out. The results obtained are found to be significant and compared to standard reference drugs.

Keywords: *Justicia Betonica*, Analgesic and Anti-Inflammatory activities.

INTRODUCTION

Since the time immemorial our traditional system of medicine and folkloric claiming several medicinal plants as whole or their parts are being used in all types of various ailments. The medicinal preparations available in the market are not effective or has developed resistance resulting in reoccurrence again^{1,2}. The literature survey on this medicinal plant *Justicia Betonica* not much pharmacological work has been carried out and the natives are using this plant has folkloric for treatment of various ailments. Hence the researcher made a sincere attempt to evaluate the Analgesic and Anti-Inflammatory activities on this medicinal plant root extract.

MATERIALS AND METHODS

Collection of Plant

The whole plant of the medicinal plant *Justicia Betonica* was collected from interior parts of Jeddangi forest region of East Godavari District, Andhra Pradesh and the plant was authenticated by taxonomist Prof. Dr. S.B.Padal.

Preparation of the Extract

The whole plant was dried under the shade coarsely powdered and was subjected to extraction process using soxhlet apparatus using ethyl alcohol for 72 hours. The solvent was evaporated and the crude extract was dried in a dessicator for few days and this extract powder was used for evaluation of anti bacterial and antioxidant activities.

Analgesic Activity Tail Flick Method^{3,7}

Procedure

In this method adult albino rats of either sex were selected. The basal reaction time to radiant heat by placing the tip of the tail on the radiant heat sources was recorded using stopwatch. The basal reaction time was observed at 0, 15, 30, 60, 120 mins, the analgesic effect of ethanolic leaf extract was assessed using this method.

In Tail flick method rats were treated with ethanolic extract of *Justicia Betonica* (150mg/kg and 300mg/kg) orally significantly inhibited nociception in rats.

The Extract of *Justicia Betonica* 150mg/kg body weight at 30 mins significantly inhibited pain reception by 46.77% and 300 mg/kg body

weight significantly inhibited pain perception at 30 min by 49.26% and these results were compared with standard reference drug Ibuprofen which significantly reduced the pain at 30 min time interval by 60%, all these results were tabulated in the table No.1.

Eddy's hot plate method ^{4,6,7}

Procedure

The eddy's hot plate was maintained between $55 \pm 1^{\circ}$ C. The animals were placed on the hot plate and the time taken for paw licking or jumping was recorded using stop watch. The reaction time was observed at 0, 15, 30, 60, 120 and 180 min. A cut off period of 10sec was observed to avoid damage to the paws. The percentage inhibition in reaction time (as index of analgesia) at each time interval was calculated (kulkarni S, Ket *al.*, 2005). The anti-nociceptive effect of *Justicia Betonica* extract was assessed using this method.

$$\text{Percentage inhibition in reaction time} = \frac{[R_t/R_c - 1] \times 100}{1}$$

Where

R_t: is reaction time in treated group

R_c : is reaction time in control group

In Eddy's Hot plate Method rats were treated with ethanolic root extract of *Justicia Betonica* with doses (150 mg/kg and 300mg/kg) orally significantly inhibited nociception in rats.

Justicia Betonica extract 150mg/kg at 30 min inhibited significant pain perception by 79.03% and 300mg/kg at 30 mins by 89.54% Whereas tramadol 5mg/kg significantly inhibited pain perception at 30 mins by 85%, all these results were tabulated in Table 2.

Acetic acid induced Writhing responses method ^{5,8}

Procedure

Intraperitoneally acetic acid (0.1ml of 1% solution) is injected and number of writhes displayed from 5-20min were recorded. All animals received 0.1ml acetic acid of 1% v/v i.p. The mice were placed individually in to glass beakers and 5 min allowed to elapse and number of writhes are recorded for each animal (Jackson C *et al.*). For scoring purpose, a writhe is indicated by stretching of abdomen with simultaneous stretching of atleast one hind limb. This was observed for 30min and change in number of writhings in test group compared with standard treated and control treated groups. The percentage inhibition was calculated by using the formula. Table No 3.

$$\text{Percentage inhibition} = [1 - (N_t / N_c)] \times 100$$

Where

N_t: is average number of writhings in treated group

N_c : is average number of writhings in control group.

Invitro anti-inflammatory activity

Protein Denaturation method

Procedure

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test samples of different concentrations (100µg/ml and 200µg/ml).

Test control solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water.

Product control solution (0.5 ml) consists of 0.45ml of distilled water and 0.05ml of test samples of different concentrations (100µg/ml and 200µg/ml).

Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of different concentrations (10µg/ml, 25µg/ml) of Diclofenac sodium.

All the above solutions were adjusted to P^H 6.3 using 1N hydrochloric acid .The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, 2.5ml of phosphate buffer was added to the above solutions. The absorbance was measured using UV Visible Spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated as results were tabulated in the table 4.

$$\% \text{ Inhibition of Protein Denaturation} = 100 - \left[\frac{\text{O.D of test solution} - \text{O.D of product control}}{\text{O.D of test control}} \right] \times 100$$

HRBC Membrane Stabilization method

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

HRBC Membrane stabilization is a versatile method used to evaluate invitro anti-inflammatory effect of *Justicia Betonica* extract. The root extract prevented hypotonicity induced membrane lysis (HRBC Membrane stabilization method) to an extent

of 42% and 66% at concentrations of 100µg and 200 µg/ml which is comparable to that of the standard drug Diclofenac sodium 60.50% and 68.50 % at concentrations of 10 µg /ml and 25 µg /ml respectively. The anti inflammatory activity of the extract is concentration dependent. The results were tabulated in the table 5.

In vivo Method

Carrageenan induced paw oedema in rats

Procedure

Carrageenan was used to induce oedema in this study. The animals were pretreated with ethanolic extract (150 mg/kg and 300 mg/kg) suspended in 2%acacia positive control animals received Diclofenac sodium (10mg/kg) *i.p.* negative control group received a similar volume of 2% acacia. After 30 min 0.1ml of 1%w/v suspension of Carrageenan in distilled water was injected subcutaneously on to the sub plantar region of the left hind paw of the animals. Measurement of paw size was carried out with plythesmometer. Paw sizes were measured immediately before and 1 hr after Carrageenan injection. Oedema inhibitory activity was calculated using the following formula.

$$\% \text{ inhibition} = (1 - v_t/v_c) 100$$

Whereas v_t = oedema volume of control animal
 V_c = oedema volume of treated animal

Carrageenan induced paw oedema model in rats is known to be very sensitive to cyclooxygenase inhibition and has been used to evaluate the effect of Non steroidal anti inflammatory agents which primarily inhibit the cyclooxygenase enzyme involved in prostaglandin synthesis.

The invivoanti inflammatory effect of *Justicia Betonica* extract in Carrageenan induced paw oedema in rats was evaluated and observed that the extract at a dose of 150mg/kg and 300mg/kg showed significant anti inflammatory activity and caused significant inhibition in the percentage increase carrageenan induced rat paw oedema and the results compared with standard drug Diclofenac sodium and all these results were tabulated in the table 6.

RESULTS AND DISCUSSION

Invivo Analgesic Activity

Tail flick method

In Tail flick method rats were treated with ethanolic extract of *Justicia Betonica* (150mg/kg and 300mg/kg) orally) significantly inhibited nociception in rats.

The Extract of *Justicia Betonica* 150mg/kg body weight at 30 mins significantly inhibited pain reception by 46.77% and 300 mg/kg body

weight significantly inhibited pain perception at 30 min by 49.26% and these results were compared with standard reference drug Ibuprofen which significantly reduced the pain at 30 min time interval by 60%.

Eddy's Hot Plate method

In Eddy's Hot plate Method rats were treated with ethanolic root extract of *Justicia Betonica* with doses (150 mg/kg and 300mg/kg) orally) significantly inhibited nociception in rats.

Justicia Betonica extract 150mg/kg at 30 min inhibited significant pain perception by 79.03% and 300mg/kg at 30 mins by 89.54% Whereas tramadol 5mg/kg significantly inhibited pain perception at 30 mins by 85%.

Acetic acid induced Writhing method

Intraperitoneal injection of low dose of acetic acid produces painful reaction which is characterized as writhing response. Constriction of abdomen turning of trunk or twisting of trunk and extension of hind legs are taken as reaction to chemically induced pain. The *Justicia Betonica* extract at a dose of 150mg/kg body weight reduced the writhing responses (30) and the percentage of inhibition was found to be 68.40% and at a dose of 300 mg/kg body weight the writhing responses were (14) and the percentage of inhibition is 87.22%. The Pentazocine 5 mg/kg which is a reference standard drug reduced the writhing responses (3) and the percentage of inhibition was 92.12%, based on these results the test drug extract inhibited the perception of pain.

Invitro Anti-inflammatory Activity

Protein Denaturation Method

Protein Denaturation Method is a very important invitroanti inflammatory method which is implemented to evaluate the anti inflammatory effect of *Justicia Betonica* extract. The root extract at a concentrations of 100 µg /ml and 200 µg /ml produced maximum percentage of inhibition which was observed as 40.50 and 65.0 respectively and the results were compared with standard anti inflammatory drug diclofenac sodium which produced inhibition of 72.5 and 80.5 at 10 µg /ml and 25 µg /ml concentrations respectively.

HRBC Membrane Stabilization method

HRBC Membrane stabilization is a versatile method used to evaluate invitroanti inflammatory effect of *Justicia Betonica* extract. The root extract prevented hypotonicity induced membrane lysis (HRBC Membrane stabilization method) to an extent of 42% and 66% at concentrations of 100µg and 200 µg/ml which is comparable to that of

the standard drug Diclofenac sodium 60.50% and 68.50 % at concentrations of 10 µg /ml and 25 µg /ml respectively. The anti inflammatory activity of the extract is concentration dependent.

Invivo Anti Inflammatory Activity Carrageenan induced paw oedema in rats

Carrageenan induced paw oedema model in rats is known to be very sensitive to cyclooxygenase inhibition and has been used to evaluate the effect of Non steroidal anti inflammatory agents which primarily inhibit the cyclooxygenase enzyme involved in prostaglandin synthesis. The invivoanti inflammatory effect of *Justicia Betonica* extract in Carrageenan induced paw oedema in rats was evaluated and observed that the extract at a dose of 150mg/kg and 300mg/kg showed significant anti inflammatory activity and caused significant inhibition in the percentage

increase carrageenan induced rat paw oedema and the results compared with standard drug Diclofenac sodium.

CONCLUSION

From the results of qualitative Phytochemical tests of selected medicinal plant *Justicia Betonica* shows the presence of flavanoids, phenolic compounds, glycosides and many other important Phytochemical constituents. These constituents are responsible for Analgesic and Anti-Inflammatory activities. Based on extensive research work carried out which indicates the folkloric usage of these herbal medicinal drugs possessing variety of biological activities that would be an emerging source for treating various diseases. Further these medicinal plant drugs are to be carried out further research in detail in the light of modern science.

Table 1: Effect of ethanolic extract of *Justicia Betonica* extract in rats using Tale Flick method

Treatment Group	0 Min		15 Min		30 Min		60 Min		120 Min	
	B.R.T	% inh	B.R.T	% inh	B.R.T	% inh	B.R.T	% inh	B.R.T	% inh
Group-I Control	5.5±0.65	--	6.3±0.30	--	7.2±0.90	--	6.5±0.03	--	4.7±0.70	--
Group-II Ibuprofen 50mg/kg	5.5±0.45	--	7.5±0.05	36.36	8.8±0.10	60.00	7±0.30	27.27	6.3±0.88	14.55
Group-III Extract of <i>Justicia Betonica</i> 150 mg/kg	6.5±0.50	--	8.56±0.05	31.69	9.54±0.03	46.77	7.95±0.05	22.31	7.29±0.04	12.15
Group-IV Extract of <i>Justicia Betonica</i> 300 mg/kg	5.4±0.65	--	7.43±0.03	37.59	8.06±0.05	49.26	6.78±0.06	25.56	6.47±0.07	19.81

All the values were expressed in mean ± SEM, n=3. *p<0.001 when compared with standard values

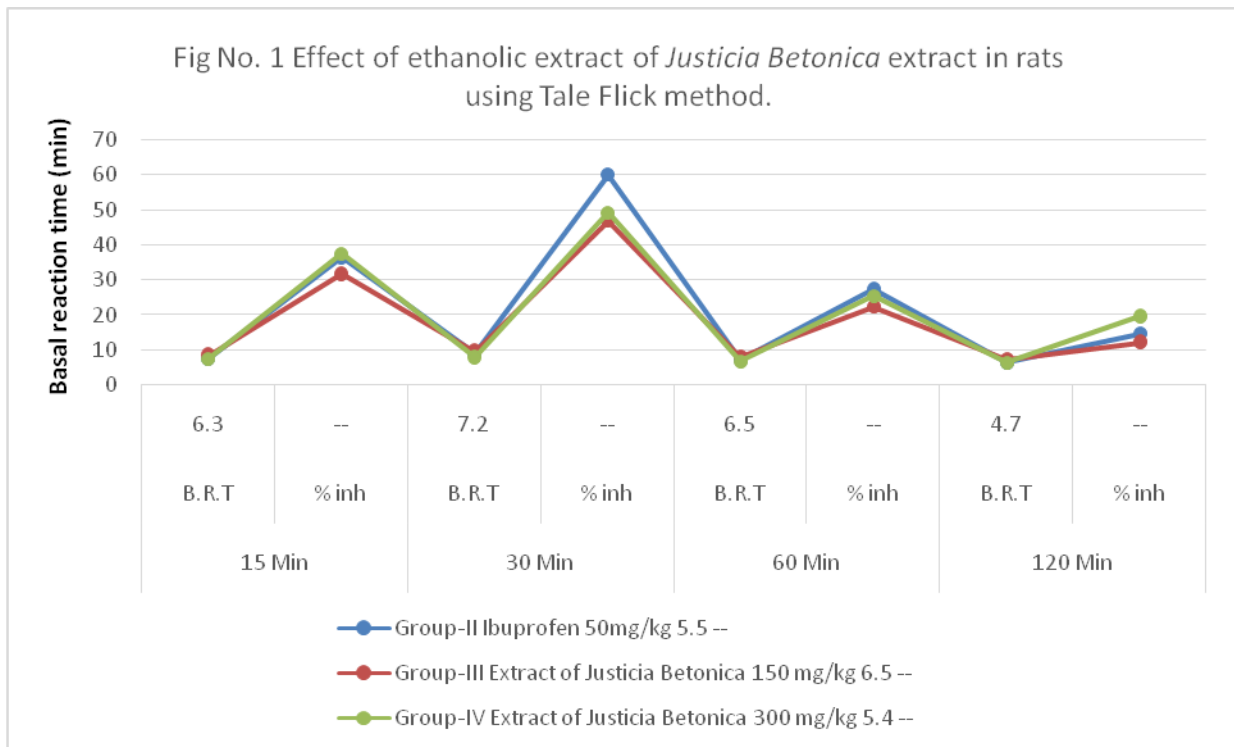


Fig. 1:

Table 2: Effect of ethanolic extract of *Justicia Betonica* extract in rats using Edd's Hot Plate Method

Treatment Group	0 Min		15 Min		30 Min		60 Min		120 Min	
	B.R.T	% inh	B.R.T	% inh	B.R.T	% inh	B.R.T	% inh	B.R.T	% inh
Group-I Control	6.5±0.06	--	7.3±0.20	--	8.0±0.13	--	8.5±0.15	--	7.2±0.24	--
Group-II Tramadol 5mg/kg	7.2±0.06	--	10.4±0.96	47.2	11.1±0.2	85	14±0.20	94	10.5±0.2	46
Group-III Extract of <i>Justicia Betonica</i> 150 mg/kg	6.2±0.20	--	8.62±0.04	39.03	11.1±0.30	79.03	11.92±0.02	92.26	10.39±0.06	67.58
Group-IV Extract of <i>Justicia Betonica</i> 300 mg/kg	6.5±0.15	--	9.32±0.04	43.38	12.32±0.03	89.54	11.81±0.02	81.69	9.21±0.05	41.69

All the values were expressed in mean ± SEM, n=3. *p<0.001 when compared with standard values

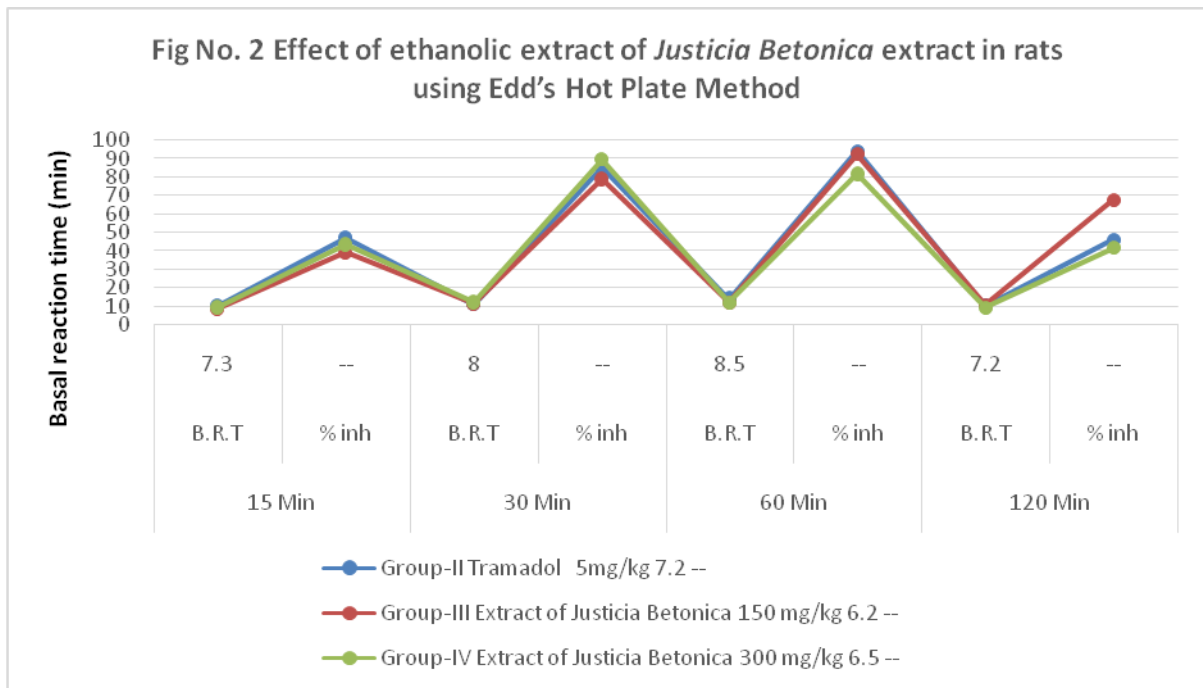


Fig. 2:

Table 3: Effect of *Justicia Betonica* extract in mice using Acetic acid of induced writhings test

Treatment	No. of Writhings	% of Inhibition
Group I Control	80.0±1.50	--
Group II Pentazocine (5mg / kg)	3.0± 0.20	92.12
Group III <i>Justicia Betonica</i> root extract 150 mg/kg	30 ±3.0	68.40
Group IV <i>Justicia Betonica</i> root extract 300 mg/kg	14±1.50	87.22

All the values are expressed in mean ± SEM, n=3

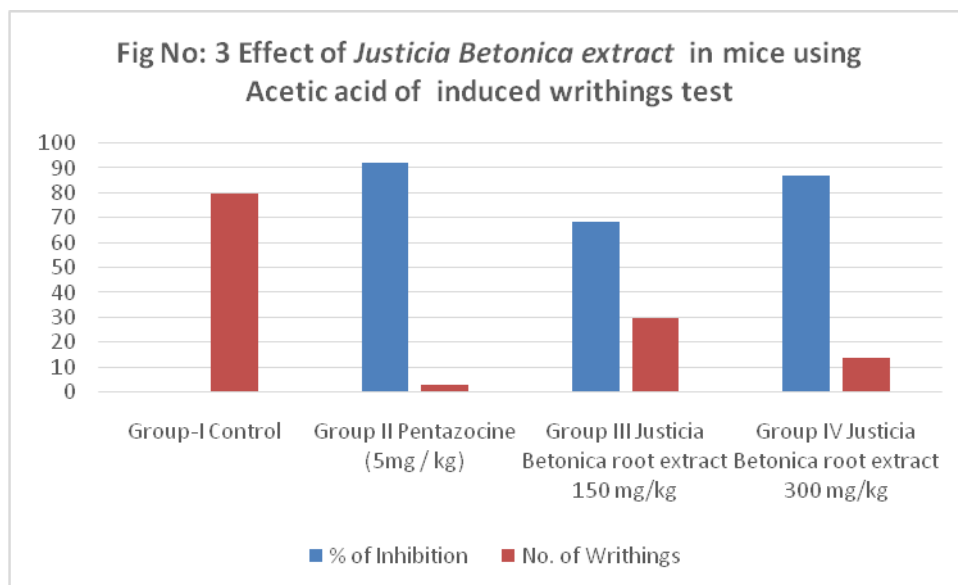


Fig. 3:

Table 4: In-vitro Anti Inflammatory effect of *Justicia Betonica* root extract by Protein Denaturation Method

S.No	Concentration	% of inhibition	
		<i>Justicia Betonica</i> extract	Diclofenac sodium
1	10 µg /ml	---	72.50
2	25 µg /ml	---	80.50
3	100 µg /ml	40.50	
4	200 µg /ml	65.00	

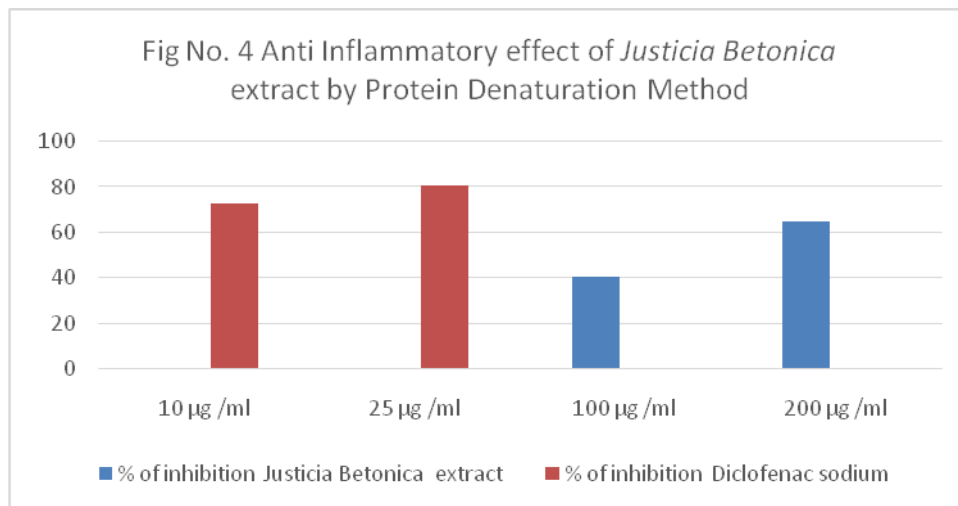


Fig. 4:

Table 5: Invitro Anti Inflammatory effect of *Justicia Betonica* extract

S.No	Concentration	% of Membrane lysis	
		<i>Justicia Betonica</i> root extract	Diclofenac sodium
1	10 µg /ml	---	60.50
2	25 µg /ml	---	68.50
3	100 µg /ml	42.00	
4	200 µg /ml	66.00	

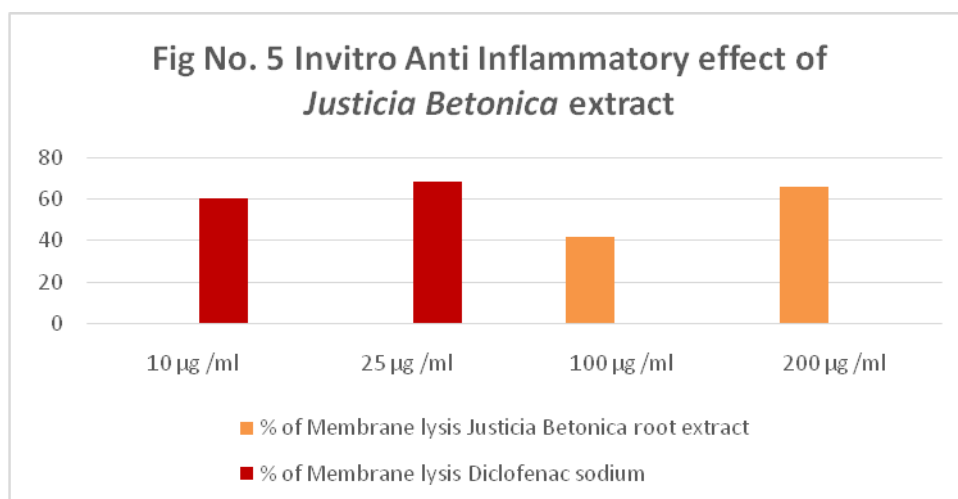
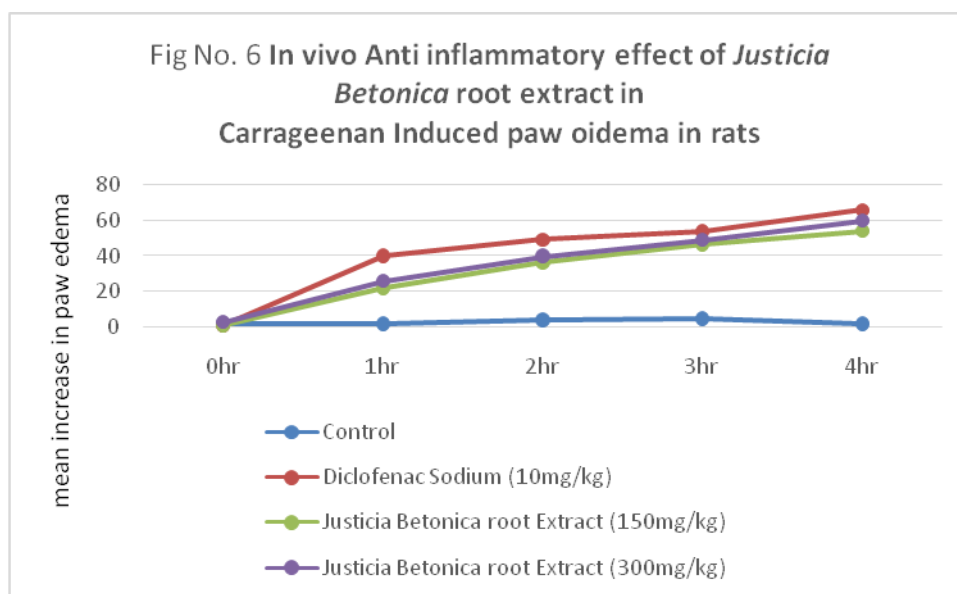


Fig. 5:

Table 6: In vivo Anti inflammatory effect of *Justicia Betonica* root extract in Carrageenan Induced paw odema in rats

Treatment	Mean increase in paw diameter mm				
	0hr	1 hr	2hrs	3 hrs	4 hrs
Control	0.34±0.02	0.50±0.02	0.88±0.04	1.10±0.15	0.84±0.02
Diclofenac Sodium (10mg/kg)	0.24±0.11	0.3±0.01 40	0.45±0.02 48.86	0.51±0.02 53.64	0.29±0.02 65.48
<i>Justicia Betonica</i> root Extract (150mg/kg)	0.28±0.01	0.61±0.05 22.00	0.56±0.05 36.36	0.59±0.06 46.36	0.39±0.03 53.57
<i>Justicia Betonica</i> root Extract (300mg/kg)	0.27±0.03	0.63±0.06 26.00	0.53±0.04 39.77	0.5±0.03 49.09	0.34±0.05 59.52

**Fig. 6:****REFERENCES**

1. Tapas Kumar K and Sunder PD. Evaluation of antibacterial antifungal and anthelmintic activity of *Murrayakoenigisprengr*. 2011;2(2):105-110.
2. Tagbota S and Towson S. Antiparasitic properties of medicinal and other naturally occurring products. *Advanced parasitology*. 2000;50:199-205.
3. Tapas kumar K and Sunder PD. Evaluation of antibacterial antifungal and anthelmintic activity of *Murrayakoenigisprengr*. 2011; 2(2):105-110
4. Tagbota S and Towson S. Antiparasitic properties of medicinal and other naturally occurring products. *Advanced parasitology*. 2000;50:199-205.
5. Janssen PAJ. The inhibitory effect of fentanyl and other morphine-like analgesic on the warm water induced tactile withdrawal reflex in rat. *Arzneimittelforschung*. 1963;13:505-507.
6. Kar B, Kumar RS, Karmakar I, Dola N, Bala A, Mazumder UK and Hard PK. Antioxidant and in vitro anti-inflammatory activities of *Mimusopselengi* leaves. *Asian Pacific Journal of Tropical Biomedicine*. 2012;2(2):S976-S980.
7. McCurdy CR and Scully SS. Analgesic substances derived from natural products (natureceuticals). *Life Sciences*. 2005;78(5):476-484.
8. Olajide OA, Makinde JM and Okpako Dt. Evaluation of the anti-inflammatory property of the extract of *Combretummicranthum*G.Don (Combretaceae), *Inflammopharmacology*. 2003; 11(3):293-298.
9. Purnima A, Koti BC, Trippeswamy AHM, Jaji MS, Swamy AV, Kurhe YV and Sadiq AJ. Antiinflammatory, analgesic and antipyretic activities of *Mimusopselengi* Linn. *Indian Journal*

- of Pharmaceutical sciences.
2010;72(4):480
10. Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH and Cunha FQ. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic in mice. *European Journal of Pharmacology*. 2000;387(1):111-118.