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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY, ACTIVITIES OF *HEMEDESMUS INDICUS* ROOT EXTRACT

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ABSTRACT

The present research study highlights the Analgesic and Anti-Inflammatory activities of *Hemedesmus Indicus* root extract. *Hemedesmus Indicus* root 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: *Hemedesmus Indicus*, 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 *Hemedesmus Indicus* 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-Inflammatoryactivities on this medicinal plant root extract.

MATERIALS AND METHODS

Collection of Plant

The root of the medicinal plant *Hemedesmus Indicus* was collected from interior parts of Maredumilli forest region of East Godavari District, Andhra Pradeshand the plant was authenticated by taxonomist Prof. Dr. S.B.Padal.

Preparation of the Extract

The root of the 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 Analgesic and Anti-Inflammatory 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 observe 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 *Hemedesmus Indicus* (150mg/kg and 300mg/kg) orally) significantly inhibited nociception in rats.

The Extract of *Hemedesmus Indicus* 150mg/kg body weight at 30 mins significantly inhibited pain reception by 45.33% and 300

mg/kg body weight significantly inhibited pain perception at 30 min by 47.25% 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 ± 1^0 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,K*et al.,*2005). The anti-nociceptive effect of *Hemedesmus Indicus* extract was assessed using this method.

Percentage inhibition in reaction time = $[R_t/R_C-1]$ 100

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 *Hemedesmus Indicus* with doses (150 mg/kg and 300mg/kg) orally) significantly inhibited nociception in rats.

Hemedesmus Indicus extract 150mg/kg at 30 min inhibited significant pain perception by 76% and 300mg/kg at 30 mins by 88.33%.

Whereas tramadol the reference drug 5mg/kg significantly inhibited pain perception at 30 mins by 85.%, all these results were tabulated in Table No. 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 3).

Percentage inhibition= $[1- (N_t / N_C)]\times100$

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,

% Inhibition of Protein Denaturation =100 – [{(O.D of test solution – O.D of product control)/O.D of test control} × 100]

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium.

Protein Denaturation Method is a very important invitroanti inflammatory method which is implemented to evaluate the anti inflammatory effect of *Hemedesmus Indicus* root extract. The root extract at a concentrations of 100 µg /ml and 200 µg /ml produced maximum percentage of inhibition which was observed as 35.50 and 60.0 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. The results were tabulated in Table 4.

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 invitroanti inflammatory effect of *Hemedesmus Indicus* root extract. The root extract prevented hypotonicity induced membrane lysis (HRBC Membrane stabilization method) to an extent of 38% and 60% 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 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.

% 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 invivo anti inflammatory effect of *Hemedesmus Indicus* root extract in Carrageenan induced paw oedama 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 oedama 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

The Extract of *Hemedesmus Indicus* 150mg/kg body weight at 30 mins significantly inhibited pain reception by 45.33% and 300 mg/kg body weight significantly inhibited pain perception at 30 min by 47.25% 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 *Hemedesmus Indicus* with doses (150 mg/kg and 300mg/kg) orally) significantly inhibited nociception in rats.

Hemedesmus Indicus extract 150mg/kg at 30 min inhibited significant pain perception by 76% and 300mg/kg at 30 mins by 88.33%. Whereas tramadol the reference drug 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 *Hemedesmus Indicus* root extract at a dose of 150mg/kg body weight reduced the writhing responses (28) and the percentage of inhibition was found to be 65.50% and at a dose of 300 mg/kg body weight the writhing responses were (12) and the percentage of inhibition is 85.40%. 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

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Invivo Anti Inflammatory Activity

Carrageenan induced paw oedema in rats

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CONCLUSION

From the results of qualitative Phytochemical tests of selected medicinal plant *Hemedesmus Indicus* 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 processing 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 *Hemedesmus Indicus* root extract in rats using Tail 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	7+0.30	27.27	6.3+0.88	14.55
Group-III Extract of *Hemedesmus Indicus* 150 mg/kg	6.0±0.80	--	7.81±0.03	30.17	8.72±0.06	45.33	7.21+0.04	20.17	6.61+0.06	10.17
Group-IV Extract of *Hemedesmus Indicus* 300 mg/kg	5.1±0.75	--	7.01±0.05	37.45	7.51±0.04	47.25	6.36±0.07	24.71	5.92+0.05	16.08

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

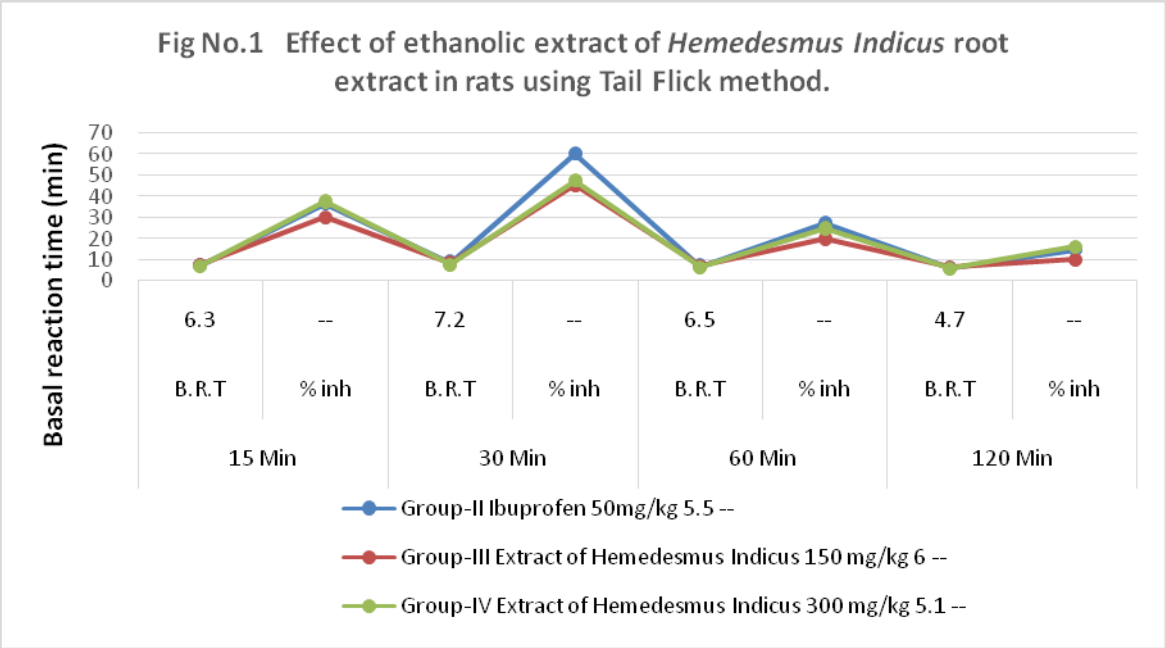

Fig. 1:

Table 2: Effect of ethanolic extract of *Hemedesmus Indicus* root extract in rats using Eddy'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 Tramadol5mg/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 *Hemedesmus Indicus* 150 mg/kg	5.8±0.20	--	8.4±0.02	36	10.8±0.20	76	11.21+0.28	92	10.0+0.02	65
Group-IV Extract of *Hemedesmus Indicus* 300 mg/kg	6.0±0.15	--	9.02±0.02	41.2	11.3±0.02	88.33	10.84±0.04	80.67	80.41+0.03	40.17

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

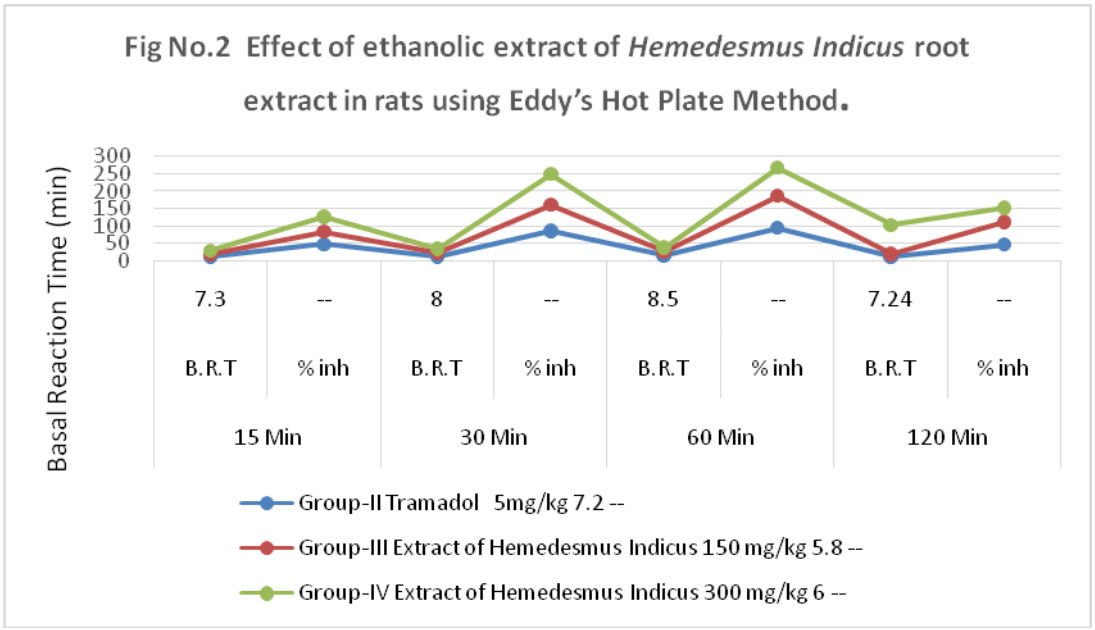

Fig. 2:

Table 3: Effect of *Hemedesmus Indicus root 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 *Hemedesmus Indicus* root extract 150 mg/kg	28.0 ±2.0	65.50
Group IV *Hemedesmus Indicus* root extract 300 mg/kg	12.0±1.50	85.40

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

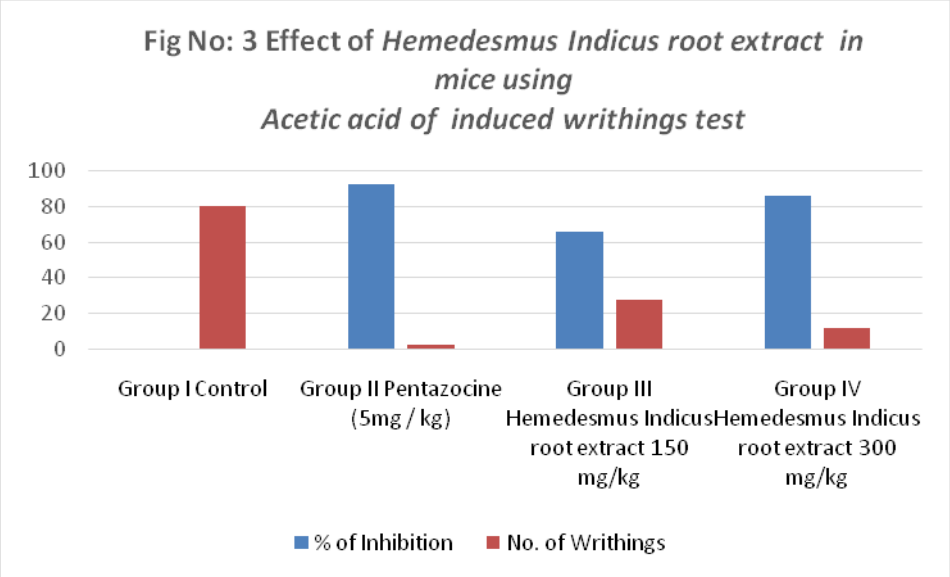

Fig. 3:

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

S.No	Concentration	% of inhibition	
		***Hemedesmus Indicus* root extract**	**Diclofenac sodium**
1	10 µg /ml	---	72.50
2	25 µg /ml	---	80.50
3	100 µg /ml	35.50	
4	200 µg /ml	60.00	

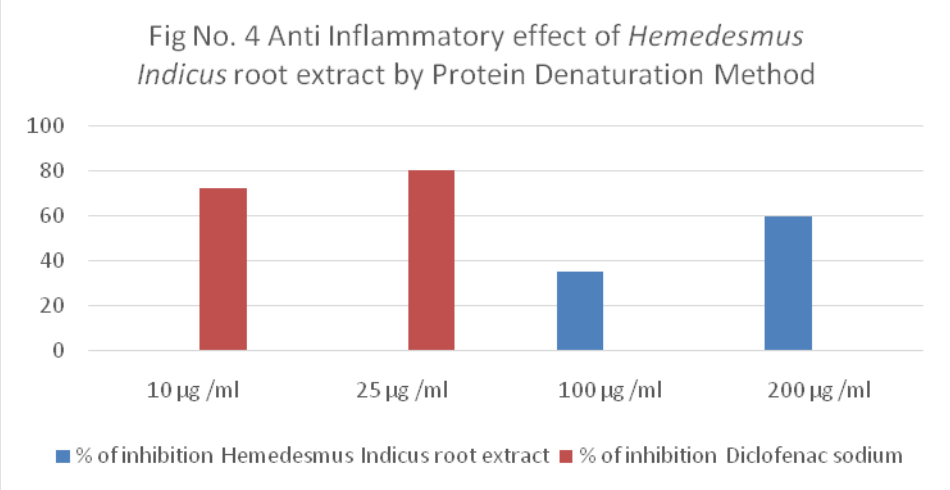

Fig. 4:

Table 5: Invitro Anti Inflmmatory effect of *Hemedesmus Indicus* root extract

S.No	Concentration	% of Membrane lysis	
		***Hemedesmus Indicus* root extract**	**Diclofenac sodium**
1	10 µg /ml	---	60.50
2	25 µg /ml	---	68.50
3	100 µg /ml	38.00	
4	200 µg /ml	60.00	

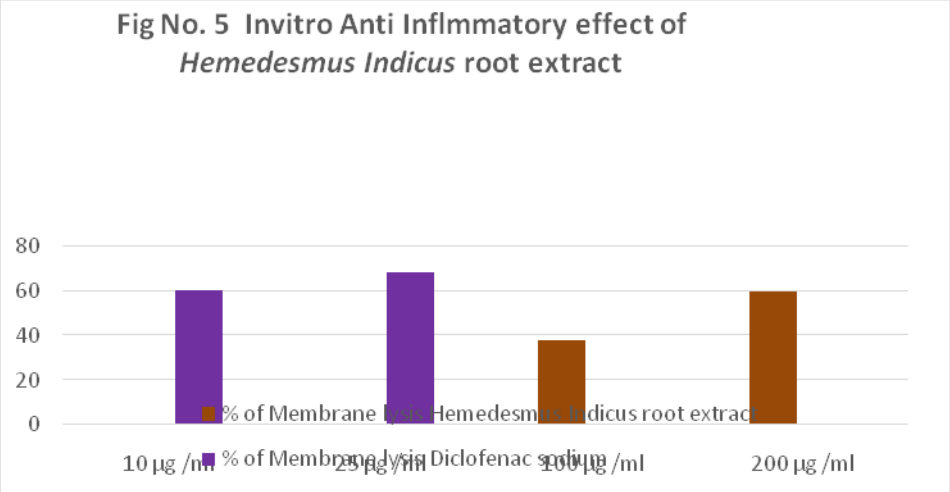

Fig. 5:

Table 6: In vivo Anti inflammatory effect of Hemedesmus Indicus 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
Hemedesmus Indicus root Extract (150mg/kg)	0.28±0.01	0.4±0.02 20.00	0.58+0.04 34.09	0.62±0.04 43.64	0.42±0.05 50.00
Hemedesmus Indicus root Extract (300mg/kg)	0.27±0.03	0.38±0.03 24.00	0.55+0.03 37.50	0.58±0.05 47.27	0.35±0.04 58.33

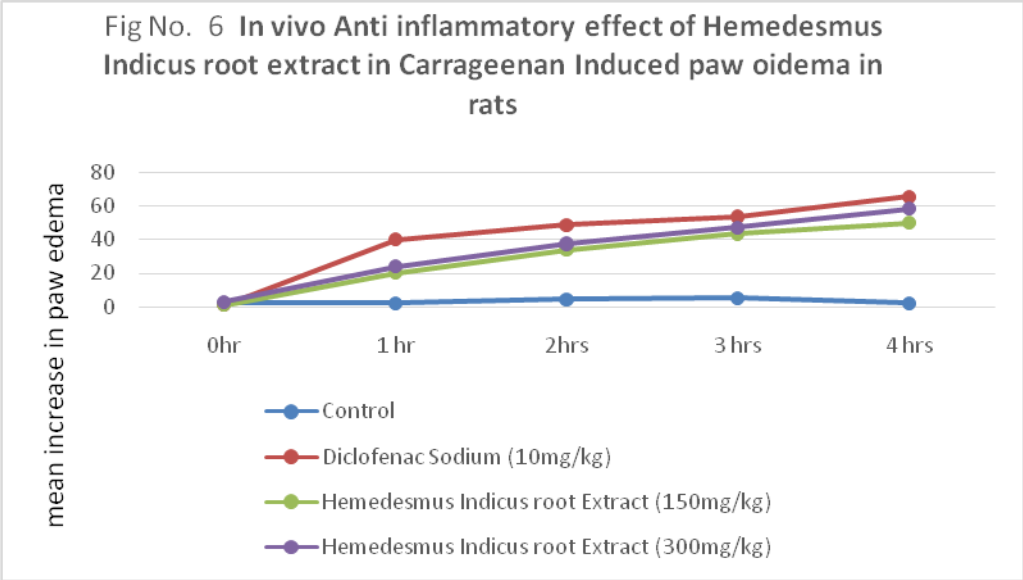

Fig. 6

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