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Research Article

INVESTIGATION OF ANALGESIC AND ANTI-INFLAMMATORY POTENTIAL OF ETHANOLIC EXTRACT OF FLEMINGIA *WIGHTIANA*

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ABSTRACT

Analgesic potential of ethanolic extract of *Flemingia wightiana* was investigated on male swiss albino mice by hot plate, tail flick, tail immersion, tail clip and acetic acid induced writhing methods. Anti-inflammatory effect was tested on wistar rats using carragenan, dextran, histamine and cotton pellets as inducing agents. Results of EEFW were compared with the efficacy of standard analgesics and anti-inflammatory drugs. Alkaloids, glycosides, flavanoids, steroids etc were found in the extract in appreciable quantities, which may plausibly responsible for the activities studied.

Keywords: *Flemingia wightiana,* antiinflammatory, analgesic, flavanoids, glycosides.

INTRODUCTION

Inflammation and pain is the condition results as responsive reaction of vascularized living tissue to local injury [(1)]. The inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischemia, antigen–antibody interactions and thermal or other physical injury. Each type of stimulus provokes a characteristic pattern of response that represents a relatively minor variation. The response usually is accompanied by some familiar clinical signs such as erythema, edema, hyperalgesia and pain. A large number of NSAID's as potential analgesics and anti-inflammatory agents are used in the market. However, on chronic usage majority of NSAIDs produces acute adverse reactions on GIT, liver and kidneys, hence necessitated Scientists across the world to search for safer herbal alternatives with analgesic and anti-inflammatory effects. Many herbal formulations were introduced into market with greater patient compliance.

The plant *Flemingia wightiana* (Family: Fabaceae) is an erect shrub. Young shoots are covered with yellow, soft tomentose hairs. The leaf is trifoliate, the leaflets are obloanceolate, oblong. Abaxial side pubesecular; the flowers are in axillary raeames. Fruits are legume with red glauds; seeds are two in each pod, lalack. In the current research, an effort was made to study the analgesic and anti-inflammatory potential of ethanolic extract of *Flemingia wightiana* by following standard pharmacological screening methods. Preliminary phytochemical investigation was carried out on the extracts in order to derive plausible scientific evidence to substantiate its pharmacological potential.

MATERIALS AND METHODS

Plant material

Ariel parts of plant of *Flemingia wightiana* was collected during April 2009 from the Tirumala hills, Andhra Pradesh, India. The plant was authenticated by Dr.Madhava chetty, Taxonomist, S.V. University, Tirupathi, India. The collected plant was cleaned immediately and shade-dried for a week, powdered mechanically, sieved (10/44) and stored in airtight containers.

Extraction

5000 grams of the powdered drug was accurately weighed and extracted using analytical grade solvents starting with Highly non polar petroleum ether (60 – 80°C) to successively increasing the polarity viz. chloroform, acetone, ethyl acetate and ethanol (95%) following soxhlation method. All the extracts were concentrated by using rota–vacuum evaporator (Buchi type, Mumbai, India) until a semisolid extract is obtained, dried at less than 50°C, comminuted in a ball mill and preserved in air tight containers kept in desiccators prior to its studies.

Preliminary phytochemical investigation

A preliminary phytochemical investigation was carried out for all the extracts obtained from the *Flemingia wightiana* [(2)] using analytical grade chemicals, solvents and reagents. The respective yields and the preliminary phytochemical investigation results were given in Table 1.

Pharmacological Screening

Toxicity studies

The method described by Lorke [(3)] was followed to determine LD_{50} - using ethanolic extract of *Flemingia wightiana* (EEFW) at different doses. Male swiss albino mice weighing 20 – 25gms were used in the study. They were acclimatized for one week by individually housing in polypropylene cages lined with husk renewed every 24 h. in well-ventilated rooms at 22±2°C under 12:12 h light and dark cycle in hygienic condition. They were and fed with standard laboratory pellet diet (Hindustan lever) and water *ad libitum* before initiation of the experiment. The chemicals, solvents and reagents used in the experiments were of analytical grade.

The mice were divided into three groups (20 in each group) namely I, II and III. A suspension of 2% v/v aq. Tween 80 was prepared freshly by using aliquot quantity of powdered EEFW prior to its use. The suspension was administered to group II and III animals respectively starting from the dose of 0.1gms/kg and increasing the dose every alternate days upto 3.2 gms/kg, p.o. Group I animals was administered with 2% v/v aq. Tween 80 (10 ml/kg, p.o.). A similar study was also conducted by administering same doses intraperitonially. Body weight before and after administration as per schedule was noted. They were observed in the open field for 72 hrs and any changes in skin, fur, eyes, mucous membranes, behavior pattern were observed. Sign of tremors, convulsions, salivation, diarrhoea, coma and number of deaths were recorded. The results were given in Table 2.

Analgesic activity

Analgesic activity of ethanolic extract of *Flemingia wightiana* (EEFW) at doses 100,200 and 400 mg/kg, p.o was studied by five different methods. The results were given in Table 3.

a. Hot plate method

The study was carried out according to the method of Eddy [(4)]. Mice that showed nociceptive responses within 10 sec, when placed on a Eddy's hot plate (Techno, Lucknow, India) maintained at 55 ± 0.5 °C were selected for study. The mice so selected were then grouped into five (6 in each group) namely I, II, III, IV and V. The group I was treated with 2% v/v, aq. Tween 80, 10 ml / kg p.o which served as control and the II, III and IV groups were treated with the EEFW 100, 200 and 400 mg/kg, p.o respectively and group V was treated with

morphine 2 mg/kg s.c. After 30 minutes of the above treatment each mouse was placed gently on the hot plate maintained at 55 ± 0.5 °C and the reaction time was noted. The reaction time was taken as the time interval between the animals placed on the plate till the moment it began to lick its forepaws or jump. Four consecutive trials after a gap of 5 minutes were done and the mean value was calculated.

b. Tail Immersion Method

The study was performed according to the method of Luiz et al [5]. The animals were treated and grouped similarly as described in hot plate method. Each mouse was held in position in suitable restrainer with the tail extending out. After 30 minutes of the above treatment each mouse 3-4 cm length of the tail was marked and immersed in the water bath thermostatically maintained at 51°C. The withdrawal time (in seconds) of the tail from hot water was noted as the reaction time/tail flick latency. Four consecutive trials after a gap of 5 minutes were done and the mean values were calculated.

c. Tail flick method

The method described by Kulkarni [6] was followed in this experiment. The animals were treated and grouped similarly as described in hot plate method except group V was treated with aspirin 20 mg/kg, p.o. After 30 minutes of the above treatment the basal reaction time for each mouse was noted by placing the tip (last-1 - 3cm) of the tail on the radiant heat source of the analgesiometer (Techno, Lucknow, India) and the time of withdrawal of tail from the heat source (Flicking response) shown within 5 -6 sec were selected for study. A cutoff period of 10 - 12 sec was observed to prevent the damage to the tail. Four consecutive trials after a gap of 5 minutes were done and the mean value was taken.

d. Caudal compression (Tail clip method)

The method followed in this study was as described by Bianchi and Franceschini [7]**.** The animals were treated and grouped similarly as described in Tail flick method. The pressure exerted by the clip was so adjusted that it was just sufficient to respond in all mice. All the mice were screened by applying a metal artery clip to the base of the tail with its jaw sheathed with thin rubber tubing. The animals that did not attempt to dislodge the clip within 4-5 seconds were discarded for the experiment. The grouping and treatment of animals was as followed in hot plate method. The time to dislodge the clip of each mouse was noted. Four consecutive trials after a gap of 5 minutes were done and the mean value was taken.

e. Acetic acid induced writhing test

The method described by Koster *et al* [8] was followed in this study. The animals were treated and grouped similarly as described in Tail flick method. Thirty minutes after the above treatment each mouse was injected 10 ml / kg of 0.7 % aqueous acetic acid intraperitoneally. Each mouse was placed in a plastic transparent observation cage and number of abdominal constriction was cumulatively counted from 5 to 15 minutes. Results were expressed as percent inhibition of analgesia.

$$\%\text{Inhibition} = \frac{\text{Mean no. of writhes in control group} - \text{in test group}}{\text{Mean no of writhes in control group}} \times 100$$

Anti inflammatory activity

Anti-inflammatory activity of ethanolic extract of *Flemingia wightiana* (EEFW) at doses 100, 200 and 400 mg/kg, p.o was studied by four different methods. The results were given in Table 4.

a. Carrageenan – induced rat paw edema

The study was conducted according to the method of Winter *et al* [9]. Male albino Wistar rats weighing 100 – 250 g were housed in wire netted cages in a controlled room temperature 22 ± 1ºC, relative humidity 60 – 70 % and with 12 h light and dark cycle. The animals were maintained with pellet diet and water *ad libitum.* The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. The chemicals, solvents and reagents used in the experiments were of analytical grade. Five groups of six animals each were used for the experiment. Group I of animals were administered with 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extact of *Flemingia wightiana* (EEFW) 100, 200 and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was treated with Indomethacin 20 mg/kg, p.o. One hour after oral administration, edema was induced by subplantar injection (left hind paw) of 0.1ml of 1% freshly prepared suspension of carragenan (Sigma Chemical Co., USA) in normal saline to all the animals. The volume of the injected and the contra lateral paws were measured at 3 hour after induction of inflammation using Plethysmometer. The percent inhibition of inflammation were calculated by using formula

Percentage of inhibition inflammation= (A-B/A) X100

Where A and B denote mean increase in paw volume of control and drug treated animals respectively.

b. Histamine induced rat paw edema

In this model edema was induced by subplantar injection (hind paw) of 0.05ml of 1% w/v, freshly prepared solution of histamine to all animals, which were grouped and treated similarly as followed in carragenan induced rat paw edema method. The volume of the injected and the contra lateral paws were measured 3 h after induction of inflammation using Plethysmometer according to the method described by Winter *et al* [9] .

c. Dextran induced rat paw edema

In this model edema was induced by subplantar injection of 0.05 ml of freshly prepared 1% w/v solution of dextran into the right hind paw of the rats, which were grouped and treated similarly as followed in carragenan induced rat paw edema method [10].

d. Chronic test

Four groups of six animals each were used for the experiment. The rats were anaesthetized under ether anesthesia and 10 mg of sterile cotton pellets were inserted into the axilla of each rat. Group I animals was given 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extract of *Flemingia wightiana*(EEFW) 100,200 and 400 mg/kg p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was given with the standard drug Indomethacin (20 mg/kg, p.o). The treatment was continued for seven consecutive days from the day of cotton pellets implantation. The animals were anaesthetized again on 8th day and the cotton pellets were surgically removed, freed from extraneous tissue; incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the cotton pellets was taken as a measure of granuloma formation [11].

Statistical analysis

All results were expressed as the mean ± SEM. The results were analyzed for statistical significance by one way ANOVA test using computerized GraphPad InStat version 3.05, Graph pad software Inc., San Diego, U.S.A.

RESULTS AND DISCUSSION

Results in Table 1 suggest that the plant contain several chemical constituents such as glycosides and flavanoids in appreciable quantities.

Results of the toxicity studies as represented in Table 2 suggest that upon oral administration, no clinical toxicity signs or death has been noted in the animals for the doses up to 3.2gms /kg,. In case of intra-peritoneal administration of EEFW, no clinical toxicity signs or death has been noted in the animals for the doses upto 1.6 gms/kg, but at dose of 3.2 gm/kg EEFW exhibited 25% mortality. Hence it can be concluded that the test drug is safe to administer in animals at the doses upto 3.2gms/ kg, orally and upto 1.6 gms /kg intraperitoneally. Further, no remarkable change in body weight, skin, fur, eyes, mucous membranes and behavioural pattern were observed. No sign of tremors, convulsions, salivation, diarrhoea and coma were seen in all the cases.

Table 1: Percent yield of extracts and preliminary phytochemical studies

	Inference				
	Pet. ether extract	**Chloroform extract**	**Acetone extract**	**Ethyl acetate extract**	**Ethanolic extract**
% Yield (w/w)	1.6	2.5	4.9	6.4	16.9
Alkaloids	Absent	Absent	Absent	Absent	Present
Amino acids	Absent	Absent	Absent	Absent	Present
Flavonoids	Absent	Present	Present	Present	Present
Anthraquinone glycosides	Absent	Absent	Absent	Absent	Present
Triterpenoids	Present	Present	Absent	Absent	Present
Steroids	Present	Present	Absent	Absent	Present
Reducing sugar	Present	Present	Present	Present	Present
Gums	Absent	Absent	Absent	Present	Present
Tannins	Absent	Absent	Present	Present	Present
Saponins	Present	Present	Present	Present	Present

Table 2: Toxicity studies of EEFW in mice

Route of administration	Treatment	Dose mg/kg,	No of animals	No of survival	No of death	LD_{50}
Peroral	Control	10 ml/kg	20	20	0	
		100	20	20	0	
		200	20	20	0	
		400	20	20	0	> 3.2 gms/kg, p.o
	SPE	800	20	20	0	
		1600	20	20	0	
		3200	20	20	0	
Intraperitonial	Control	10 ml/kg	20	20	0	
		100	20	20	0	
		200	20	20	0	
		400	20	20	0	> 3.2 gms/kg, i.p.
	SPE	800	20	20	0	
		1600	20	20	0	
		3200	20	18	2	

Table 3: Analgesic activity studies of EEFW on male swiss albino mice

	Group I	**Group II**		**Group III**		**Group IV**		**Group IV**	
Material administered	2%Tween 80	EEFW						Morphine	
Dose Route of administration	10 ml/kg Oral	100 mg/kg		200 mg/kg		400 mg/kg		2 mg/kg Subcutaneous	
Method	Reaction time in sec. (mean ± SEM)	Reaction time in sec. (mean ± SEM)	% Inhibition	Reaction time in sec. (mean ± SEM)	% Inhibition	Reaction time in sec. (mean ± SEM)	% Inhibition	Reaction time in sec. (mean ± SEM)	% Inhibition
Hot plate	6.4 ± 0.8	7.8 ± 0.3*	21.88	9.2 ± 0.2*	43.75	11.1 ± 0.4*	73.44	11.80 ± 0.32*	84.38
Tail Immersion	2.2±0.1	4.2±0.17**	90.91	4.9±0.2**	122.73	5.0±0.2**	127.27	5.6±0.311**	154.55
Tail flick	3.3 ± 0.3	4.9 ± 0.19*	48.48	5.6 ± 0.2*	69.70	5.8 ± 0.3*	75.76	6.16 ± 0.42*	86.67
Tail clip	5.1 ± 0.4	6.6 ± 0.24*	29.41	7.3 ± 0.6*	43.14	8.7± 0.6*	70.59	9.16 ± 0.41*	79.61
	Material administered							Aspirin 20 mg/kg p.o.	
Acetic acid induced writhing	53.3 ± 3.5	25.9 ± 2.44*	51.41	21.5 ± 2.1*	59.66	16.9 ± 3.2*	68.29	12.76 ± 3.46*	76.06

Figures in parentheses indicate the percentage inhibition of pain compared to control. N= 6 *p< 0.001,**p<0.05>0.02: Student's t-test.

Table 3 and Fig 1 represent the results of analgesic activity studies by five different methods. Several tests (acute and sub -acute) which differ with respect to stimulus quality, intensity and duration, were employed in evaluating the analgesic effect of the EEFW to ascertain the analgesic properties of a substance using behavioural nociceptive tests [12]. In the hot plate method, the test drug EEFW showed 21.88 & 43.75% and 73.44% of inhibition at the doses of 100,200 and 400 mg/kg respectively, whereas the percent inhibition for morphine was 84.38. The effect of test drug EEFW on tail flick test was observed as 48.48%, 67.7% and 75.76% for 100,200 and 400mg/kg respectively whereas morphine showed 86.67% of inhibition under similar conditions. In tail clip method, the test drug EEFW showed 29.41, 43.14% and 70.59% inhibition at the doses of 100,200 and 400 mg/kg respectively.

In Tail immersion method, the withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The tail flick latency of EEFW at 100 mg/kg at 60 minutes was comparable with that of standard drug morphine 2 mg/kg s.c. The drug under investigation (EEFW) showed a dose – dependent inhibition of pain in all the four acute pain models studied. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The test drug EEFW showed significant effect in various acute (phasic) pain models, namely, hot plate, tail flick and tail clip tests suggest that the effect on these pain models may act via centrally mediated pain control.

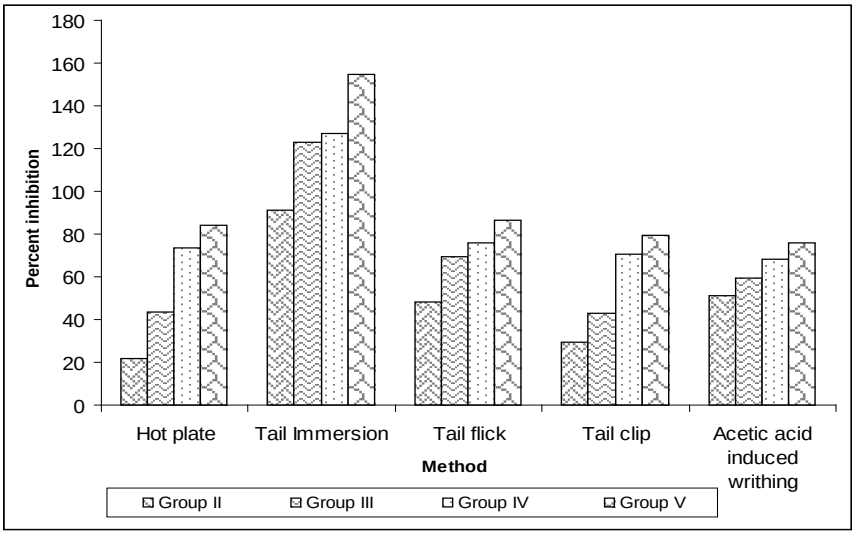

Fig. 1: Analgesic activity studies of EEFW on male swiss albino mice

The abdominal writhing response induced by acetic acid is sensitive process to establish peripherally acting analgesics. Local peritoneal receptors are responsible for abdominal writhing action. Intraperitoneal administration of acetic acid causes an increase in of PGE_2 and $PGF_2\alpha$ and produce analgesia by inducing capillary permeability and liberating endogenous substances like serotonin, histamine, prostaglandins, bradykinin, and substance P that sensitize pain nerve endings. It has been suggested that acetic acid stimulates the valinoid receptors and bradykinin B_2 receptors in the pathway comprising sensory afferent C-fibers [13]. Therefore, the observed activity may be due to interfering the synthesis or release of endogenous substances or desensitization of nerve fiber which carry pain sensation. In Acetic acid induced writhing assay the test drug EEFW at the doses of 100, 200 and 400 mg/kg p.o. exhibited 51.41, 59.66 & 68.29% of inhibition respectively. The commercial drug Aspirin at the dose of 100 mg/kg p.o. exhibited 76.06% inhibition under similar experimental conditions. The results suggest that EEFW also possess significant peripherally mediated analgesic effect. Hence it can be concluded that the EEFW possesses analgesic properties, which are mediated via peripheral and central inhibitory mechanisms.

The results of anti-inflammatory studies for four different models were summarized in Table 4 and Fig 2. Most of the investigators reported that inhibition of carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents [14]. The sub planter injection of carrageenan (1% w/v) developed edema of high intensity and persisted for 3 h after injection in the control groups. The oral administration of EEFW at the doses of 100, 200 and 400 mg/kg p.o. showed significant and dose dependent inhibition (31.86, 43.76 and 50.09% respectively).

Table 4: Anti-inflammatory activity studies of EEFW on male albino Wistar rats

	Group I	**Group II**		**Group III**		**Group IV**		**Group V**	
Material administered	2%Tween 80	EEFW						Indomethacin	
Dose	10 ml/kg	100 mg/kg		200 mg/kg		400 mg/kg		20 mg/kg	
Route of administration	Oral								
Inducing agent	Paw volume after 3 hours (mean ± SEM)	Paw volume after 3 hours (mean ± SEM)	% Inhibition	Paw volume after 3 hours (mean ± SEM)	% Inhibition	Paw volume after 3 hours (mean ± SEM)	% Inhibition	Paw volume after 3 hours (mean ± SEM)	% Inhibition
Carrageenan	53.7 ± 3.91	36.59 ± 2.34 **	31.86	30.2 ± 2.57 *	43.76	26.8 ± 1.84 *	50.09	23.8 ± 2.02 *	55.68
Histamine	47.3 ± 2.56	32.9 ± 1.34*	30.44	28.2 ± 1.24*	40.38	24.9 ± 1.3*	47.36	24.7 ± 0.9*	47.78
Dextran	41.70 ± 2.34	31.28 ± 1.23**	24.99	29.55 ± 1.16**	29.14	26.5 ± 1.6**	36.45	26.60 ± 1.54*	36.21
	Weight of granuloma formation (mg)	Weight of granuloma formation (mg)		Weight of granuloma formation (mg)		Weight of granuloma formation (mg)		Weight of granuloma formation (mg)	
Cotton wool	80.16 ± 5.78	51.6 ± 4.48*	35.63	39.57 ± 5.61*	50.64	33.26 ± 3.21*	58.51	31.16 ± 4.49*	61.13

p - Value was calculated by comparing with the control by students t-test, **p*< 0.001,***p*<0.05>0.02:N=6

The commercial anti-inflammatory drug, Indomethacin showed 55.68% of inhibition at the dose of 20mg/kg p.o. The development of carrageenan induced oedema is bi-phasic. The first phase is attributed to the release of histamine, serotonin and kinins, whereas, the second phase is related to the release of prostaglandins [9, 15]. The inhibitory action of the drug (EEFW) on carrageenan induced paw edema in rats may be mediated through either any of the mediators alone or in combination. Hence EEFW was further investigated against paw edema induced by individual agents like Histamine and Dextran and showed a maximum inhibition of and 47.36, 36.45%

respectively at the dose of 400 mg/kg. The drug EEFW also exhibited significant anti–inflammatory effect in the cotton pellet induced granuloma test (58.51% for 400mg/kg, p.o.). This reflected its efficacy to a high extent to reduce an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation [16, 17]. It was observed that the gain in weight of the pellets was linear with the time. This linearity was continued for eight days and then leveled off. There fore, seven days was chosen as a convenient duration for the experiments followed by one day incubation at 37°C [9]. Results suggest that the EEFW at doses of 100, 200 and 400 mg/kg p.o. significantly reduced the edema produced by several inducers and are comparable with many standard drugs suggested in each model. It has been reported by many researchers that flavanoids inhibit eicosanoids synthesis by inhibiting both cyclooxygenase and lipoygenase activities [18, 19], as well as hamper the non enzymatic peroxidation of polyunsaturated fatty acids required for the activation of these oxygenases [20]. Quercetin and other flavonoids inhibit leukotrienes synthesis and histamine, prostaglandins release, as well as acts as superoxide scavengers [21].

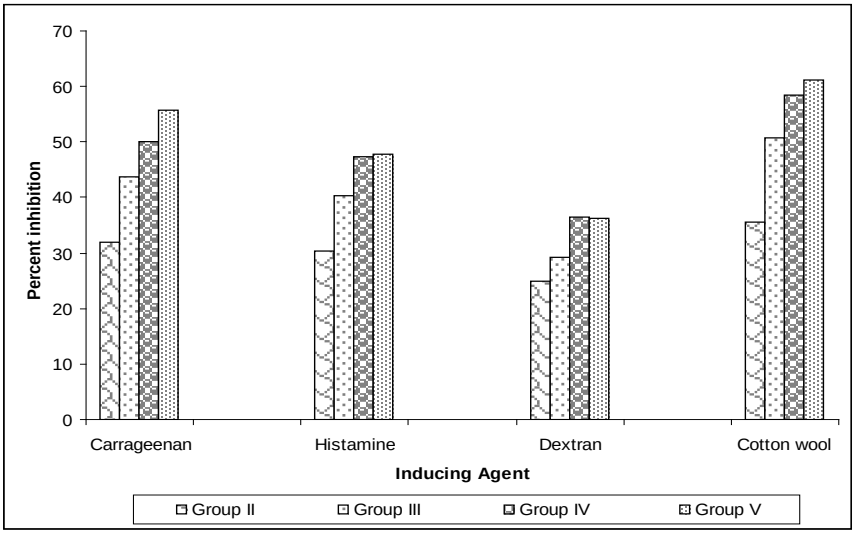

Fig. 2: Anti-inflammatory activity studies of EEFW on male albino Wistar rats

CONCLUSION

Ethanolic extract of *Flemingia wightiana* was systematically evaluated for its analgesic and anti-inflammatory potential by following standard pharmacological screening methods. Results suggested that the EEFW found to possess comparable efficacy with that of standard analgesics and anti-inflammatory drugs. *Flemingia wightiana,* an abundantly available shrub in Tirumala hills is certainly a nature's treasure for mankind for prevention and treatment of inflammation associated with pain.

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