Original Article

The analgesic and acute anti-inflammatory effect of the ethanolic extract of the leaves of Paederia foetida (EEPF) on experimental animal models

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Abstract

Objective: To study the analgesic and acute anti-inflammatory effect of the ethanolic extract of the leaves of Paederia foetida (EEPF) on experimental animal models. ***Materials and Methods:*** Fresh leaves were collected, air-dried, powdered, and percolated in 95% ethanol. Acute toxicity test was done according to OECD guidelines. Healthy albino rats (150-200 gm) of either sex were taken and divided into five groups with six animals in each group for central analgesic activity by tail flick method. Peripheral analgesic activity by 1% glacial acetic acid induced writhing test by taking albino mice (20-30 gm) of either sex which were divided into three groups with six animals in each. EEPF was used in a dose of 500 mg/kg bodyweight subcutaneously for tail-flick method and orally for writhing test. In tail-flick method the basal reaction time was taken and a cut-off period of 10 sec was observed. Reaction time were recorded at predrug, 15, 30, 60, 90, 120, 150 and 180 minutes after administration of drug . The standard drug used for central analgesic activity was pethidine 5 mg/kg bw, naloxone 1mg/kg as antagonist and naloxone 1mg/kg bw with 500mg/kg bw of EEPF to study the central mechanism of action. The standard drug used for peripheral analgesic activity was aspirin 100mg/kg bw orally. A control group was maintained in all the models. For anti-inflammatory study, three groups of animals of either sex (n = 6), weighing 150-200g of the species Rattus norvegicus were taken for the study. Group A was taken as control (Normal salie, 10 mL/kg body weight), Group B as test group (EEPF 500 mg/kg body weight), and Group C as standard (Aspirin 100 mg/kg body weight). The animals were studied for acute inflammation by Carrageenan-induced rat paw edema. Statistical analysis was done by one-way analysis of variance followed by multiple comparison tests. ***Results:*** EEPF significantly increased the reaction time in tail-flick method ($p<0.05$) whereas the combination of naloxone and EEPF decreased the reaction time indicating that naloxone inhibits the analgesic effect of EEPF. In 1% glacial acetic acid induced writhing EEPF reduced writhing significantly In acute inflammation, there was significant inhibition of paw edema in Groups B, C in comparison with Group A ($P < 0.05$). ***Conclusion:*** The ethanolic extract of Paederia foetida has significant analgesic and anti-inflammatory activity.

Keywords: Anti-inflammatory, analgesia, carrageenan, Paederia foetida.

Introduction

Pain is an unpleasant sensation localized to a part of the body. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distention, or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system.[1]

The inflammatory process is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents (eg, infections, antibodies, or physical injuries)[2]. The inflammatory response of the host is critical for interruption and resolution of the infectious process but also is often responsible for the signs and symptoms of disease. It involves a complex series of host responses, such as the complement, kinin, and coagulation pathways. An inability to kill or contain the microbe usually results in further damage due to progression of inflammation and infection.[3]

Paederia foetida belongs to the family Rubiaceae. In Assamese it is called Bhedailota and is a distinct part

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of Assamese cuisine. It is believed to be a laxative or bowel-function regulator. It is traditionally used in Rheumatic disease, as emetic, as astringent.[4]. We have found that its leaves contain flavonoids which was confirmed by test on our own laboratory. There is not much information regarding its anti-inflammatory and analgesic activity. Considering this, the present study has been undertaken to evaluate the anti-inflammatory and analgesic activity of the leaves of Paederia foetida on experimental animal models.

Collection, identification, and extraction of plant materials

Fresh tender leaves of Paederia foetida, approximately 1 kg collected during April-May, 2010, were used for the study. The plant was authenticated by Dr. M. Islam, Professor of Life Science, Dibrugarh University, Assam, India. The plant material was air-dried at room temperature. The dried leaves were ground to fine powder and stored in air tight container.

Preparation of the extract

An amount of 250 g of the dry powder obtained was soaked in 95% ethanol for 24 h in percolator. After 24 h it was allowed to percolate slowly and the extract was collected in Petri dishes5. The extract was concentrated in vacuum using rotary flash evaporator. There was a net yield of 22.6 g of concentrated extract (9.12%).

Animals

The experiments were carried out in healthy albino rats of the species Rattus norvegicus of either sex weighing 150-200 gm and healthy albino mice of the species Mus Musculus of either sex weighing 20-30 gm. The animals were procured from the Central Animal House, Assam Medical College & Hospital, Dibrugarh, Assam. The study was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines and the study was approved by the Institutional Animal Ethics Committee. They were fed with standard diet and water ad libitum was provided.

Acute toxicity studies

Acute oral toxicity test for the ethanolic extract of leaves of Paederia foetida was carried out as per OECD guidelines 425.6 One arbitrary dose 500 mg/kg was selected for the study, as the extract was found safe even at doses more than 2000 mg/kg without any sign of toxicity or mortality.

Method

For Analgesic studies:

THE FOLLOWING TWO METHODS WERE USED FOR THE EXPERIMENT---

Analgesia by central action:--The central analgesic activity was tested by tail flick method in Albino rats [7]. Healthy albino rats (150-200 gm) of either sex and were taken and divided into five groups with six animals in each group for central analgesic activity. The tail flick latencies (reaction time) of the animals were assessed by analgesiometer (Elite). The basal reaction time was taken by placing the tip (last 2cm) of the tail on the radiant heat source and a cut-off period of 10 sec was observed to prevent damage to the tail. Reaction time were recorded at predrug , 15, 30, 60, 90, 120, 150 and 180 minutes after administration of drug . EEPF was used in a dose of 500 mg/kg bw subcutaneously .The standard drug used for central analgesic activity was pethidine 5 mg/kg bw i.p 8, naloxone 1mg/kg as antagonist and naloxone 1mg/kg bw with 500mg/kg bw of EEPF to study the central mechanism of action.

Group A- Control, vehicle normal saline (NS) **10** ml/kg s.c

- Group B- EEPF 500 mg/kg s.c
- Group C- Naloxone 1 mg/kg s.c
- Group D- EEPF 500 mg/kg s.c + Naloxone 1 mg/kg
- Group E- Pethidine 5 mg/kg i.p

Analgesia for peripheral action-The peripheral analgesic activity was tested by glacial acetic acid-induced writhing test in Albino mice 9. Healthy albino mice(20-30 gm) of either sex which were divided into three groups .One hour after administration of drugs, induction of writhing was done in mice by giving intraperitoneal injection of acetic acid at the dose of 10ml/kg bw. The number of writhing responses were counted and recorded for 20 min. EEPF was used in a dose of 500 mg/kg bw orally The standard drug used for peripheral analgesic activity was aspirin 100mg/kg bw orally. Aspirin was taken as standard drug at the dose of 100mg/kg p.o [10]

- Group A- Control, normal saline 10 ml/kg per orally.
- Group B-. EEPF 500 mg/kg per orally
- Group C- Aspirin 100 mg/kg per orally

- For Anti-inflammatory studies:

Healthy albino rats of both sex weighing 150-200gm were taken. The animals were fasted overnight and water was given ad libitum during the experiment. The animals were divided into 3groups 6 animals each and treated as follows:

- Group A: Control -Normal saline-10 ml/kg body weight, orally,
- Group B: EEPF - 500mg/kg body weight, orally.
- Group C: Aspirin -100mg/kg body weight , orally

All the drugs were administered orally and the volume of medicaments kept constant at 10 mL/kg body weight of the animals.

The anti-inflammatory activity of ethanolic extract of leaves of Paederia foetida(EEPF) against acute inflammation was tested by carrageenan-induced rat paw edema method. Acute inflammation was produced by sub plantar injection of 0.1 mL of freshly prepared 1% carrageenan suspension in normal saline in the left hind paw of rats in each group. The animals were treated with normal saline, EEPF and aspirin in the respective groups,[1] h before carrageenan injection. The paw volume was measured plethysmometrically as described by Kulkarni.11 just before carrageenan injection, that is, at "0" h and then at 1st, 2nd, 3rd, and 4th h after carrageenan injection. Increase in paw edema was measured as the difference between the paw volume at "0" h and paw volume at the respective hour. The percentage inhibition of the rat paw edema was calculated after each hour of carrageenan injection up to 4 h by the formula described by Sudjarwo Agus.[12].

$$\text{Inhibition} = \frac{(\text{Control Mean - treated mean})}{\text{Control Mean}} X\ 100$$

Statistical analysis

For all the above methods, the results were expressed as mean ± standard error of mean. Statistical analysis was done using one-way ANOVA followed by Dunnet's multiple comparison test. P values < 0.05 was considered significant.

Results

Acute oral toxicity tests found the LD-50 of the leaves extract of P. foetida (EEPF) to be more than 2000 mg/kg.

The ethanolic extract of Paederia foetida showed significant central analgesic activity as compared to control ($p<0.01$; Table I) as evidenced by significant increase in the latency time. Significant peripheral analgesic action was also observed with EEPF and aspirin as compared to control ($p<0.01$; Table II) as evidenced by inhibition of abdominal writhes produced by acetic acid. There was also significant ($p<0.05$;Table III) reduction in carrageenan induced paw edema by EEPG and aspirin.

Results And Observation

Table I: Assessment of central analgesic action of ethanolic extract of *P.foetida* by tail flick method

Groups	Drug dose (ml/kg)	Pre-drug reaction time (sec)	15min	30min	60min	90min	120min	150 min	180min
Group A	Normal saline 10 ml/kg s.c	3.6+/-0.09	3.55+/-0.06	3.6+/-0.13	3.66+/-0.16	3.51+/-0.15	3.66+/-0.11	3.7 +/-0.16	3.75+/-0.12
Group B	EEPF 500 mg/kg s.c	3.3+/-0.09	3.1+/-0.07	3.3+/-0.03	3.5+/-0.08	3.8+/-0.10	4.2+/-0.11	3.7 +/-0.05	3.5+/-0.05
Group C	Naloxone 1mg/kg s.c	3.3+/-0.04	3.1+/-0.06	3.0+/-0.06	2.9+/-0.07	2.6+/-0.13	2.6+/-0.13	2.7+/-0.13	3.0+/-0.05

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GroupD	EEPF 500 mg/kg + naloxone 1mg/kg	3.5+/-0.05	3.5+/-0.03	3.0+/-0.07	3.0+/-0.07	4.0+/-0.11	3.1+/-0.05	2.9+/-0.08	3.2+/-0.07
Group E	Pethidine 5 mg/kg i.p	3.7+/-0.15	4.1+/-0.15	5.0+/-0.08	5.05+/-0.09	6.86+/-0.11	5.6+/-0.17	4.75+/-0.18	4.15+/-0.09
One way ANOVA	F	3.57	24.10	108.1	170.9	196.3	90.51	38.98	28.92
	df	25,4	25,4	25,4	25,4	25,4	25,4	25,4	25,4
	P	>0.01	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

N=6, ANOVA followed by Dunnet"s test, Data are (mean +/-SEM)

Table II: Assessment of peripheral analgesic action of ethanolic extract of *P.foetida* by glacial acetic acid induced writhing test in Albino mice.

Groups	Drug dose(mg/kg) p.o	Number of writhing movements (mean +/-SEM)	Percentage of protection
Group A	Normal saline10ml/kg	69.5+/-0.56	-
Group B	EEPF500mg/kg	22+/-0.68[a,b]	68.35
Group C	Aspirin 100mg/kg	7+/-0.26[a]	89.98
One way ANOVA	F	3757	
	df	15.2	
	P	<0.0001	

N=3, a---p<0.01 when compared to the control, b---p<0.05 when compared to the standard, ANOVA followed by Dunnets test.

Table III: Anti-inflammatory activity of the ethanolic extract of Parderia foetida(EEPF) leaves on carrageenan induced rat paw edema in albino rats

Group	Drug dose p.o.	Mean increase in paw volume (Mean ± SEM) (mL)(% Inhibition within parentheses)			
		1 hr	2 hr	3 hr	4 hr
A(control)	10ml/kg	0.22±0.01	0.27±0.02	0.52±0.01	0.33±0.01
B(EEPF)	500mg/kg	0.13±0.02a (40.90)	0.12±0.01a (55.55)	0.21±0.02a (59.61)	0.14±0.04a (57.57)
C(aspirin)	100mg/kg	0.10±0.01a (54.54)	0.11±0.05a (59.25)	0.18±0.01a (65.38)	0.13±0.03a (60.60)
One-way ANOVA	F	283	380.6	160.9	330.9
	Df	17,2	17,2	17,2	17,2
	P value	<0.05	<0.05	<0.05	<0.05

EEPF, Paederia foetida; SEM, standard error of mean; ANOVA, analysis of variance, n = 6 in each group; a→P < 0.05 when compared with control; Values are expressed as MEAN±SEM. One way ANOVA followed by Dunnett´s multiple comparison test is done.

Discussion

Our study showed that ethanolic extracts of the leaves of Paederia foetida produced significant analgesia, both centrally and peripherally. The extract (500 mg/kg s.c) and pethidine showed significant increase in the reaction time. Pre-treatment with naloxone significantly decreased the reaction time producing hyperalgesia while combined treatment of EEPF (500 mg/kg s.c) and naloxone (1 mg/kg s.c) produced significant decrease in the reaction time as compared to EEPF alone. Naloxone is a competitive antagonist at all types of opioid receptors. It also blocks the actions of endogenous opioid peptides[13].In the face of a variety of physical (pain) or psychological stressors, an increased release of a variety of opioid peptides occurs[14].This indicates the involovement of endogenous opioid peptides in mediation of analgesic activity of Paederia foetida

which seems to be its probable central mechanism of action. However, since there is only partial reduction in analgesic activity after naloxone, some other non-opioid mechanisms may also be involoved. The extract (500 mg/kg orally) and aspirin (100 mg/kg orally) significantly reduced the number of writhes induced by acetic acid. Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, prostaglandins, bradykinin and substance P which stimulate pain nerve endings. Local peripheral receptors are postulated to be partly involved in the abdominal constriction (writhing response). The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins[15]. Standard NSAIDs like aspirin offers relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process. Prostaglandins elicit pain by direct stimulation of sensory nerve endings to other pain provoking stimuli[16]. Therefore, it is likely that EEPF suppresses the formation of these substances or antagonize the action of these substances which may serve as it peripheral mechanism of analgesic activity. Carrageenan induced paw edema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-edematous effect of natural products. Development of edema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome[17]. Our study showed that the EEPF (500 mg/kg) and aspirin (100 mg/kg) produced significant reduction of the carrageenan induced paw edema suggesting its anti-inflammatory activity. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception[18]. Therefore, flavonoids present in the leaves of Paederia foetida may be responsible for its analgesic and anti-inflammatory activities.

The present study demonstrated that the ethanolic extracts of Paederia foetida showed significant analgesic and anti-inflammatory activity thereby establishing its traditional use in inflammatory and painful conditions. However further studies and development of more purified product of leaves of Paederia foetida are required for proper clinical use.

Conclusion:

The present study demonstrated that the ethanolic extract of the leaves of Paederia foetida showed significant anti-inflammatory activity against acute inflammation and both central and peripheral analgesic activity. However, further studies are required to establish and elaborate the molecular mechanism for proper clinical utility. Also, the development of more purified products of Paederia foetida for the treatment of various inflammatory diseases should be encouraged.

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References

1. Fields HL, Martin JB. Pain: Pathophysiology aand Management. In: Kasper DL,Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL(eds). Harrison's Principles of internal medicine, 17th ed. New York, McGraw Hill, 2008: 81-86.

2. Burke A, Smith E, Garret A, FitzGerald . Analgesic - antipyretic agents, Pharmacotherapy of gout. In: Brunton Laurence L, Lazo John S, Parker Keith L, editors. Goodman and Gillman's The Pharmacological basis of therapeutics. 11th ed. New York: McGraw Hill Publishing; 2006: 671-715.

3. Pier GB. Molecular mechanisms of microbial pathogenesis. In: Braunwald K, Hauser F, Jameson L, editors. Harrison's Principles of Internal Medicine. 17th ed. Vol. 1. USA: McGraw-Hill Publishing; 2008: 753-760.

4. Patwari B. In: A glossary of medicinal plants of Assam and Meghalaya. 1st ed. Guwahati, India. M.N. Printers, 1992:95

5. Clark WE. Extracta Liquida Pharmaceutical Formulations: The Chemist and Druggist Book. In: Le G, editor. 11th ed. London: 1950:183

6. OECD Guidelines for Testing of Chemicals [Internet] France: OECD Publishing; 2006. Organization for Economic Cooperation and Development (OECD) pp. 1-27. July 11. Section 4,Health Effects: Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Available from:http://www.oecdbookshop.org/oecd/index.asp?lang=en [Adopted 2006 Mar 23, Cited 2010 aug11]

7. D'Armour FE, Smith DL. A method for determining loss of pain sensation . *J Pharmacol Exp Ther* 1941; **72**: 74-79.

8. Ghosh MN. Guide to drug doses in laboratory animals. In: Fundamentals of experimental pharmacology. 4th ed.Kolkata , India, Ghosh and others, 2008: 243-256.

9. Witkin LB, Hebner CF, Gaddi F, O'Keefe E, Spilaletta P, Plummer AJ. Pharmacology of 2-amino-indanehydrochloride (SU-8629). A potent non-narcotic analgesic. *J Pharmacol Exp Ther.* 1961; **133**: 400-408.

10. Ghosh MN. Toxicity studies. In: Fundamentals of experimental pharmacology. 4th ed. Kolkata, India, Ghosh and others, 2008:176-183.

11. Kulkarni SK, Experiment on intact preparations: Handbook of Experimental Pharmacology; 3rd Revised and Enlarged ed. Delhi, Vallabh Prakashan; 2005: 128-131.

12. Agus S. The Potency of Piperine As Antiinflammatory and Analgesic in rats and mice. *Folia Med Indonesiana.* 2005; **41**:190-194.

13. Tripathi KD. Opioid analgesics and antagonists. In: Essentialsof medical pharmacology. 6th ed. New Delhi, India, JaypeeBrothers Medical Publishers (P) Ltd, 2008: 453-474.

14. Yaksh TL, Wallace MS. Opioids, Analgesia and Pain. In: Brunton L, Chabner B, Knollman B(eds). Goodman and Gillman's The pharmacological basis of Therapeutics. 12th ed. McGrawHill, 2011: 481-515.

15. Bhukya B,Anreddy R N R, William C M, Gottumukkala K M. Analgesic and anti-inflamamatory activities of leaf extract of Kydia calycina Roxb. *Bangladesh J Pharmacol* 2009 ;**4** : 101-104.

16. Kanodia L, Das S. A comparative study of analgesic propertyof whole plant and fruit extracts of Frageria vesca inexperimental animal models. Bangladesh J Pharmacol. 2009;4: 35-38.

17. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin oedema in rats. *J Pharmacol Exp Ther.* 1969;**166**:96-103

18. Khatale PN, Manikrao AM, Vijabaskar M, Shivkumar T, Sabale PM. Analgesic and anti-inflammatory activities of ethanolic extracts of Pseudoarthria Viscida (L) Roots. Pharmacologyonline; 1 : 1153-1159.