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Analgesic Activity Study of Polygonum Glabrum Willd in Rodents

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ABSTRACT

Investigations of herbal medicines may provide a novel drug for the treatment of pain, without addiction liability and gastric discomfort caused by opioids and nonsteroidal anti-inflammatory agents respectively. In the current study, an aqueous extract of Polygonum glabrum (PG) was evaluated for its putative analgesic activity in rodents. Hence the effects of the PG extract were evaluated through validated animal models of pain including acetic acid-induced writhing test, tail flick latent period, hot plate reaction time and formalin-induced paw licking. Aspirin (25 mg/kg, I.P.), pentazocine (10 mg/kg, I.P.) and indomethacin (5 mg/kg, I.P.) were administered to rodents as reference drugs.PG extract at doses of 12.5, 25, 50 and 100 mg/kg, i.p administered 30 min before the study has significantly decreased the number of writhing in acetic acid-induced writhing test and significantly increased the latent period and reaction time in the tail flick latent period and hot plate reaction time in rodents respectively. In acetic acid-induced writhing test, analgesic effect at doses 25, 50 and 100 mg/kg of PG were more significant than aspirin. In tail flick latent period, the effect of doses 50 and 100 mg/kg of PG were more significant than pentazocine. The confirmation test for pre-clinical analgesic activity of PG has performed by formalin-induced paw licking in rats. The observed analgesic activities of PG are mediated by both central and peripheral mechanisms.

Keywords: Analgesic, Herbal medicines, Polygonum glabrum,

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INTRODUCTION

The name *Polygonum* derived from the Greek words polys, "many," and gonu, "knee or joint," hence "many joints" because of the thickened joints on the stem. The species name *glabrum* is based on the smooth or hairless appearance of the leaves. *Polygonum glabrum* (PG) is known as dense flower knotweed due to its morphological appearance of the flower and stem. PG is a perennial plant belonging to the *Polygonaceae* family. The genus *Polygonum* encompasses 150 species, of which 79 are known to occur in India [1]. PG is distributed in Asia, Africa and North America. Indian PG is perennial herb growing up to a height of 150 cm distributed mainly in the eastern area at altitude up to 1900 m, especially near liberal water source.

A good number of species *Polygonum* have been used traditionally from a long time for a number of ailments in the indigenous system of medicine. The medicinal properties attributed to the species of *Polygonum* are demulcent and pectoral, astringent and tonic, diuretic, emetic, purgative, febrifuge, vesicant, vulnerary, insecticide and anthelmintic [2]. Besides it also possess antiviral [3] and antibacterial [4] properties.

Polygonum glabrum has been mentioned in Ayurveda and is known as *Rasna,* though its clinical use does not appear to include nervous disorders. It has been studied for its anti-inflammatory [5] and antidepressant [6] properties. The findings show that *Polygonum glabrum* extract is clinically effective as anti-inflammatory drug and works by the mechanism of action similar to that of NSAIDs. PG also has been researched for anthelmintic activity [7], which showed activity against *Hymenolepis nana* var. *fraternal.* PG contains several compounds of biological interest, including the sesquiterpenes, a broad spectrum of flavanoids [8] and polyphenols [9]. Although investigations continue, these are considered to be primarily responsible for PG's activity. The focus of this study was on evaluation of PG extract for analgesic activity.

MATERIALS AND METHODS

Animals

Adult Charles Foster albino rats (200±20g) and Wister mice (25±5g), of either sex, were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University and were randomly distributed into different experimental groups. The rats were housed in groups of six in polypropylene cages at an ambient temperature of 25ºC±1ºC and 45-55%RH, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water *ad libitum* unless stated otherwise. Experiments were conducted between 09.00 and 14.00 h. Animals were acclimatized for at least one week before using them for experiments and exposed only once to every experiment. "Principles of laboratory animal care" (NIH publication number 85-23, revised 1985) guidelines were followed.

Drug treatments

The plant *Polygonum glabrum* (PG) was collected during August from the Ayurvedic Garden of Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University. Authentication was performed by [5]. The air dried leaves of PG (500g) were extracted with 4L of hot water for 2 h. The procedure was repeated twice. The extract was filtered and then evaporated to dryness. The yield of extract was quantified (15.05%) and the material obtained was protected from light and stored under refrigeration at 0-4ºC until its use. Extract was solubilised in distilled water before experimental studies, and administered intraperitoneally in the doses of 12.5, 25, 50 and 100mg/kg 30 min before experiments. Pentazocine (Ranbaxy, India) (10mg/kg, i.p), Aspirin (Reckitt Benckiser, India) (25mg/kg, i.p) and Indomethacin (Ranbaxy, India) (5mg/kg, i.p) were used as the standard analgesic agent and were administered to one group of animals, 30min before experiments for comparison.

Acetic acid-induced writhing test

30min after receiving (i.p) injection of the plant extract, reference substance or solvent to groups of 10mice, each mouse was given intraperitoneally 0.7% aqueous solution of acetic acid (10ml/kg body weight). Immediately after the algic compound injection, each animal was placed in a transparent observation cage and the number of writhes per mouse was counted for 30minutes. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs [10]. The percentage of inhibition was calculated using the following ratio:
(Control mean – treated mean) × 100/control mean.

Tail flick latent period

The technique used was described by [11], using Techno analgesiometer. The rat was placed in a rat holder, with its tail coming out through a slot in the lid. The tail was kept on the bridge of the analgesiometer, called jacket with an electrically heated nichrome wire underneath. The tail received radiant heat from the wire, heated by passing current 6mA. Through the water jacket, cold water was continuously passed, so that the bridge did not get heated and tail could be conveniently placed over the bridge. The time taken for the withdrawal of the tail after switching on the current was taken as the latent period, in sec, of "tail flick" response. This latent period was considered as the index of nociception. The cut-off time for determination of latent period was taken as 30sec to avoid injury to the skin [12]. Three tail flick latencies were used for statistical analysis.

Hot plate reaction time in mice

Mice were screened by placing them on a hot plate maintained at 55±1ºC and recording the reaction time in seconds for forepaw licking or jumping [13]. Only mice which reacted within 15sec and which did not show large variation when tested on four separate occasions, each 15min apart, were taken for the test. The time for forepaw licking or jumping on the

heated plate of the analgesiometer maintains at 55ºC was taken as the reaction time. Prior to treatment, the reaction time of each mouse (licking of the forepaws or jumping response) was done at 0- and 10-min interval. The average of the two readings was obtained as the initial reaction time (*Tb*). The reaction time (*Ta*) following the administration of the extract, Pentazocine and distilled water was measured at 0.5, 1, 2, and 3h after latency period of 30min. The following calculation was:

Percentage analgesic activity = $Ta\text{-}Tb/Tb \times 100$

Formalin-induced paw licking in rat

The formalin-induced paw licking was studied in rats using the method of [14]. In this method, 100µl of 3% formalin was injected into the subcutaneous tissue on the plantar surface of the left hind paw of rats 30min after i.p administration of the extracts, Pentazocine, Indomethacin and distilled water. In each rat the time spent on licking the injected paw was observed as soon as the injection was given (i.e. early phase, 0-5min post-injection) and in the late phase (20-30 min post-injection) after injection. The mean of the time spent on licking the injected paw in each group was determined.

Statistical analysis

The data were expressed as mean ± S.E.M. for each treatment group. Statistical significance between groups was performed by one way analysis of variance (ANOVA) followed by inter group comparisons through Dunnett's test. *p* values less than 0.05 were considered statistically significant.

RESULTS

Acetic acid induced writhing response in mice: PG extract showed significant and dose dependent decrease in writhing response induced by acetic acid. Aspirin as well as Pentazocine also showed decrease in writhing response. But protection of Pentazocine against the algesic agent was comparatively less when compared to PG extract and aspirin. The results are summarized in Table-1.Tail flick latent period: PG extract has shown a significant and dose dependent analgesic activity by tail flick method. Potency of activity at the doses 50 and 100mg/kg were more than that of Pentazocine. PG also has shown long duration of action at 50 and 100mg/kg doses when compared to Pentazocine. The results are shown in Table-2.

Hot plate reaction time in mice: PG extract showed significant analgesic activity at all dose level except 12.5mg/kg when compared to vehicle and Pentazocine. The results are summarized in Table-3. Formalin-induced paw licking in rats: PG extract and Pentazocine decreased the time spent on licking (licking time) in the early phase after formalin injection. Similarly in the late phase, extract as well as Indomethacin reduced the licking time. But the dose 12.5mg/kg was not enough to show the analgesic activity in the inflammatory phase. The results are shown in Table-4.

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Table 1: Effect of PG on acetic acid induced writhing in mice

Treatment	Dose (mg/kg)	Number of writhing	Inhibition (%)
Distilled water	10ml/kg	86.5±1.7	-
P.glabrum	12.5	66.3±6.9*	23.35
P.glabrum	25	27.8±4.7***	67.86
P.glabrum	50	19.9±3.3***	76.99
P.glabru	100	17.0±2.9***	80.35
Pentazocine	10	67.2±2.9***	22.31
Aspirin	25	48.4±3.0***	44.05

*and *** indicate difference with vehicle treated group at p<0.05 and 0.001 respectively. n=10 in each group.

Table 2: Effect of PG on tail flick latent period in rats

Treatment	**Dose (mg/kg)**	**Latency period (h)**					
		0	**0.5**	**1**	**2**	**3**	**4**
Distilled water	10ml/kg	10.33±0.35	10.71±0.26	10.41±0.27	10.74±0.19	10.61±0.38	10.42±0.41
P.glabrum	12.5	10.57±0.51	15.38±1.07**	19.29±1.22***	15.57±0.83***	12.80±0.59*	11.19±0.38
P.glabrum	25	10.91±0.60	18.78±0.69***	20.72±0.45***	15.99±0.54***	13.73±0.22***	11.75±0.38*
P.glabrum	50	10.65±0.21	22.10±0.90***	28.38±0.61***	23.82±0.61***	19.17±0.38***	13.49±0.28***
P.glabrum	100	10.47±0.24	24.26±1.68***	29.29±0.64***	27.74±0.93***	14.60±0.42***	12.95±0.24***
Pentazocine	10	10.88±0.21	20.92±0.44***	24.24±0.37***	19.76±0.62***	15.66±0.65***	12.69±0.28**

*, ** and *** indicate difference with vehicle treated group at p<0.05, 0.01 and 0.001 respectively. n=6 in each group.

Table 3: Effect of PG on hot plate reaction time in mice

Treatment	**Dose (mg/kg)**	**Latency period (h)**				
		0	**0.5**	**1**	**2**	**3**
Distilled water	10ml/kg	3.57±0.14	3.48±0.15(-2.52)	3.28±0.11(-8.12)	3.67±0.20(2.80)	3.32±0.15(-7.0)
P.glabrum	12.5	3.86±0.36	4.06±0.30(5.18)	4.61±0.52(19.43)*	4.23±0.36(9.58)	3.46±0.55(-10.36)
P.glabrum	25	3.24±0.34	4.43±0.23(36.72)**	5.85±0.26(80.56)***	4.57±0.21(41.05)**	3.47±0.25(7.10)
P.glabrum	50	3.78±0.45	5.91±0.80(56.35)**	7.32±0.12(93.65)***	5.11±0.63(35.19)*	3.89±0.21(2.91)*
P.glabrum	100	3.28±0.29	6.89±0.76(110.06)***	9.49±0.59(189.33)***	5.42±0.65(65.24)*	4.11±0.16(25.30)**
Pentazocine	10	3.35±0.54	5.86±0.26(74.93)***	7.84±0.55(134.03)***	5.89±0.36(75.82)***	4.23±0.46(26.27)

*, ** and *** indicate difference with vehicle treated group at p<0.05, 0.01 and 0.001 respectively. n=10 in each group. Percentages of protection are in parentheses.

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Table 4: Effect of PG on formalin-induced paw licking in rats

Treatment	Dose (mg/kg)	Licking time (s)	
		Early phase	Late phase
Distilled water	10ml/kg	94.50±7.71	59.00±6.27
P.glabrum	12.5	65.67±6.98*	44.83±5.57
P.glabrum	25	41.19±2.97***	30.16±2.12**
P.glabrum	50	28.50±3.06***	24.50±2.49***
P.glabrum	100	21.00±2.06***	19.02±2.36***
Pentazocine	10	35.33±4.12***	47.17±5.13
Indomethacin	5	71.83±5.08*	35.33±2.12**

*, ** and *** indicate difference with vehicle treated group at $p<0.05$, 0.01 and 0.001 respectively. n=6 in each group

DISCUSSION

Pain is a symptom of many diseases requiring treatment with analgesics [15]. Severe pain due to cancer metastases needs the use of strong analgesics, means opioid drugs. The addiction liability of opioids led to intensive research for compounds without this side effect. Many approaches have been used to differentiate the various actions of strong analgesics by developing animal models not only for analgesic activity but also for addiction liability. Several types of opioid receptors have been identified in the brain allowing *in vitro* binding tests. However, the *in vitro* test can only partially substitute for animal experiments involving pain. Pain is a common phenomenon in all animals, at least in vertebral animals, similar to that felt by man. Analgesic effects in animals are comparable with the therapeutic effects in man. Needless to say, that in every instance painful stimuli to animals must be restricted as much as possible. Painful stimuli can consist of direct stimulation of the efferent sensory nerves or stimulation of pain receptors by various means such as heat or pressure. The role of endogenous peptides such as enkephalins and endorphins gives more insight into brain processes and the action of central analgesics.

Pain can also be elicited by inflammation. Progress has been made in elucidating the role of various endogenous substances such as prostaglandins and peptides in the inflammatory process. Most of the so called non-steroidal anti-inflammatory agents have also analgesic activity. Antipyretic analgesics causing analgesia by blocking impulse generation at pain receptors in the periphery while the narcotic analgesics block synaptic transmission of impulses signalling pain in the central nervous system were differentiated by [16]. An old but excellent survey on methods being used to test compounds for analgesic activity has been provided by [17]. Today, the classification into central and peripheral analgesics is definitively too simplified [18] but provides a guide for differentiation by pharmacological methods.

PG has got very good analgesic activity in rodents in all four analgesics models and found to be produced both central and peripheral analgesia. The extract was found to significantly increase the tail flick reaction time in rats. Originally tail flick method was developed by [19] and [20] for quantitative measurement of pain threshold in man against

radiation and for evaluation of analgesic opiates. Later on, the procedure has been used by many authors to evaluate analgesic activity in animal experiments by measuring drug induced changes in the sensitivity of mice or rats to heat stress applied on their tails. This test is very useful for discriminating between centrally acting morphine-like analgesics and non-opiate analgesics.

Hot plate method is originally described by [21]. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance [22]. Mixed opiate agonists antagonists can be evaluated if the temperature of the hot plate is lowered to 49.5ºC [23], [24]. It is known that centrally acting analgesic drugs elevate the pain threshold of rodents towards heat. The above findings indicate that PG may be centrally acting.

In order to distinguish between the central and peripheral analgesic action of PG, acetic acid induced writhing response in mice was used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test, the animals react with characteristic stretching behaviour, called writhing. It was found that, PG significantly inhibited the acetic acid induced writhing response similar to that of aspirin. The abdominal constriction is related to the sensitization of nociceptive receptor to prostaglandins. It is therefore possible that PG exert an analgesic effect probably by inhibiting synthesis or action of prostaglandins and leukotrienes.

Conformation of analgesic activity of PG has been done by formalin-induced paw licking method. The formalin test in rats has been proposed as a chronic pain model which is sensitive to centrally active analgesic agents by [25]. The formalin test was selected because of several advantages including the ability to mimic human clinical pain conditions, sensitivity to mild analgesics, production of tonic stimulus and sensitivity to non-steroidal anti-inflammatory drugs [26], [27], [28], [29]. According to this method, drugs acting through central mechanism inhibit the early response called neurogenic phase where as those acting peripherally are good effective in the late phase known as inflammatory phase. The extract inhibited early and late phases of the formalin-induced pain indicate that PG is a drug which acts both centrally and peripherally.

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