



## Antinociceptive profile of the ethanolic extract of *andrographis paniculata* in mice

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### SUMMARY

The present study was conducted to evaluate the analgesic activity of ethanolic extract of *Andrographis paniculata* (AP) in mice. The analgesic investigations were carried out using the acetic acid-induced abdominal writhing and the hot-plate tests. It was demonstrated that intraperitoneal (i.p.) administration of the extract at a dose of 30, 100, 300, 500 mg/kg, produced significant inhibition of abdominal constriction induced with 0.6% (v/v) acetic acid in dose-dependent manner. It also demonstrated that the extract produced significant dose-dependent increase in the time of latency to a discomfort reaction in the hot-plate model. In addition, the analgesic effect of the ethanolic extract of AP was significantly reversed by a non-specific opioid receptor antagonist, naloxone. These results indicate that AP has an analgesic effect that was mediated through opioid receptors.

**Key words:** *Andrographis paniculata*; Ethanolic extract; Antinociceptive activity; Opioid receptor

### INTRODUCTION

*Andrographis paniculata* (AP) or locally known as "hempeđu bumi" is a herbaceous plant from the *Acanthaceae* family, widely used in folk medicine for the treatment of diabetes, fever and skin problems (Jain and Sharma, 1967; Goh *et al.*, 1988). It has been reported that the crude extract prepared from this plant and its chemical components produced a variety of pharmacological actions such as anti-

inflammatory (Thamlikitkul *et al.*, 1991; Gabrielian *et al.*, 2002; Shen *et al.*, 2002), anti-hypertensive (Zhang and Tan, 1996), anti-diabetic (Zhang and Tan, 2000), anti-bacterial (Leelarasamee *et al.*, 1990; Gabrielian *et al.*, 2002) and hepatoprotective activity (Handa and Sharma, 1990; Visen *et al.*, 1993). In the present study, we examined the antinociceptive effect of ethanolic extract of AP in the acetic acid-induced abdominal constriction and hot-plate tests.

### MATERIALS AND METHODS

#### Preparation of extract and drugs

The ethanolic extract of AP was obtained using the method as previously described (Somchit *et al.*, 2003). The stem bark and leaves of AP were oven

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dried at 40°C for 24 h. The dried material was then powdered and extracted using Soxlet apparatus with distilled ethanol as solvent. The resultant extract was freeze dried and kept at -20°C prior to use. The extract were dissolved in normal saline at desired concentration (30, 100, 300 and 500 mg/kg) just before use and administered intraperitoneally (i.p.) 30 min prior to the administration of an inducer. Acetic acid, acetylsalicylic acid, morphine hydrochloride, naloxone hydrochloride and all other drugs were purchased from Sigma Chemical Co. (St Louis, Mo). Fresh drug suspensions were dissolved in warm physiological saline (0.9% NaCl in distilled water).

#### Experimental animals

Adult male Balb/c mice weighing 20 - 30 g were used throughout the experiments. They were housed in standard cages (10 animals per cage) at temperature of 24 ± 2°C and at a 12:12 h light-dark cycle. Food and water were available *ad libitum*. Animals were acclimated to at least 1 week before the beginning of experiments. Experimental procedures were carried out in strict compliance with the Animal Ethics Committee rules and regulation followed in this institute. All experiments were performed in the morning according to guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

#### Acetic acid-induced writhing test

The analgesic property of AP extract was assessed using acetic acid-induced abdominal writhing test as described previously by Koster *et al.* (1959). The total number of writhing following i.p. administration of an irritant dose of 0.6% acetic acid (10 ml/kg) was recorded for 30 min, starting 5 min after the injection. The animals were pretreated with extract from AP (30, 100, 300, 500 mg/kg, i.p.) 30 min before acetic acid administration. Negative control animals received a similar volume of physiological saline (10 ml/kg, i.p.) and positive control animals

received acetylsalicylic acid (ASA; 100 mg/kg, i.p.). Antinociceptive activity was expressed as the percentage reduction or inhibition of the number of abdominal writhes using the following ratio: (control mean - treated mean) × 100/control mean.

#### Hot plate test

The hot plate test was used to measure the latency of the response as described by Hosseinzadeh *et al.* (2000) with minor modification. The temperature of the hot-plate (Model 7280, Ugo Basile, Italy) was maintained at 55.0 ± 0.2°C. Animals were placed into the Perspex cylinder on the heated surface, and the time between placement and licking of the paws or jumping was recorded as response latency. Latency period was recorded for negative control animals (normal physiological saline, 10 ml/kg, i.p.), positive control animals (pretreated with morphine, 5 mg/kg, i.p.), and animals pre-treated with the AP extract (30, 100, 300 and 500 mg/kg; i.p.). All substances were administered 30 min before the beginning of the experiment. Mice were selected 24 h before the experiment on their reactivity to the test. Only the animals showing a reaction time within the range of 5.0 - 8.0 were used. All mice were observed before and 30, 60, 120, 150, 180 and 210 min after substance administration. A latency period of 20 s was defined as complete analgesia and the measurement was terminated if the latency exceeded the latency period to avoid tissue injury.

#### Analysis of the analgesic mechanism of action of AP

In an attempt to investigate the participation of opioid system in the antinociceptive action of AP, the effect of non-selective opioid receptor antagonist, naloxone was examined in the acetic acid-induced abdominal writhing test, with slight modification. A separated groups of mice were pre-treated with naloxone (5 mg/kg, i.p), which was injected 15 min before the administration of the extract (300 mg/kg, i.p). As a basis of comparison, ASA (100 mg/kg) and morphine (5 mg/kg) were used in the abdominal writhing test and the hot-plate test, respectively.

### Statistical analysis

The results of statistical analysis are expressed as mean values  $\pm$  S.E.M, unless otherwise stated and compared by ANOVA followed by Dunnett's Test.  $P < 0.05$  being the criterion for statistical significance.

## RESULTS

### Abdominal writhing test

The antinociceptive effect of the AP extract (i.p.) on the abdominal writhes of mice induced by 0.6% acetic acid are summarised in Table 1. The i.p. administration of AP extract at doses of 30, 100, 300 and 500 mg/kg produced significant and dose-dependent reduction in the number of abdominal writhes with 33.0%, 49.3%, 53.1% and 81.0% of inhibition, respectively, as compared in relation to the respective control value. Acetylsalicylic acid

(ASA) exerted a significant inhibitory effect, inducing a inhibition of 78.0% at a dose of 100 mg/kg

### Hot plate test

Table 2.0 shows the effect of the extract on hot-plate test. All doses of the extract used significantly increased the response latency compared to control in dose-dependent fashion. The effect of morphine (5 mg/kg) was significantly higher than that of produced by the highest dose of the extract.

### Analgesic mechanism of action of AP

The antinociceptive effect of the AP was significantly reversed by pretreatment of animals with naloxone (5 mg/kg, i.p) when assessed in the acetic acid-induced abdominal writhing test (Table 1) and the hot-plate test (Table 2).

**Table 1.** Effect of ethanol extract of *A. paniculata* on acetic acid-induced writhing test in mice

Group	Dose (mg/kg; i.p.)	Mean of writhings (30 min)	Inhibition (%)
Control	-	33.6 $\pm$ 4.0	-
<i>A. paniculata</i>	30	22.5 $\pm$ 3.7*	33.0
	100	17.6 $\pm$ 3.0*	49.3
	300	15.8 $\pm$ 3.1*	53.1
	500	6.4 $\pm$ 1.6*	81.0
Acetylsalicylic acid	100	7.4 $\pm$ 2.8*	78.0
Naloxone (5 mg/kg, i.p.) + <i>A. Paniculata</i> , (300 mg/kg i.p.)		32.8 $\pm$ 3.9	2.4

Values are mean  $\pm$  S.E.M. \* $P < 0.05$  significantly different from mean value of control (ANOVA followed by Dunnett's test).

**Table 2.** Effect of ethanol extract of *A. paniculata* on hot-plate test in mice

Group	Dose (mg/kg)	n	Latency time (min) $\pm$ S.E.M.					
			0	30	60	120	180	210
Control	-	6	5.6 $\pm$ 0.4	5.4 $\pm$ 0.5	5.5 $\pm$ 0.5	5.9 $\pm$ 0.4	5.8 $\pm$ 0.3	5.7 $\pm$ 0.8
	30	6	6.1 $\pm$ 0.7	6.1 $\pm$ 1.0	5.7 $\pm$ 0.9	6.0 $\pm$ 1.0	5.9 $\pm$ 0.1	5.8 $\pm$ 0.1
<i>A. paniculata</i>	100	6	6.2 $\pm$ 0.5	6.8 $\pm$ 0.3*	7.5 $\pm$ 0.6*	7.1 $\pm$ 0.3*	6.6 $\pm$ 0.3*	6.5 $\pm$ 0.3*
	300	6	5.7 $\pm$ 0.3	7.8 $\pm$ 0.3*	8.1 $\pm$ 0.5*	7.4 $\pm$ 0.9*	7.3 $\pm$ 0.2*	6.7 $\pm$ 0.3*
	500	6	5.7 $\pm$ 0.2	8.8 $\pm$ 0.3*	9.4 $\pm$ 0.3*	8.9 $\pm$ 0.6*	8.1 $\pm$ 0.2*	8.2 $\pm$ 0.2*
	<i>A. paniculata</i> (300 mg/kg) + Naloxone (5 mg/kg)	6	5.4 $\pm$ 0.4	5.9 $\pm$ 0.5	5.7 $\pm$ 0.3	5.5 $\pm$ 0.8	5.8 $\pm$ 0.2	5.6 $\pm$ 0.5
Morphine	5	6	5.5 $\pm$ 0.4	10.2 $\pm$ 1.2*	12.4 $\pm$ 2.2*	11.0 $\pm$ 1.4*	9.8 $\pm$ 1.3*	8.9 $\pm$ 1.6*
Morphine (5 mg/kg) + naloxone (5 mg/kg)		6	5.7 $\pm$ 0.4	5.6 $\pm$ 0.4	5.8 $\pm$ 0.6	5.5 $\pm$ 1.1	5.6 $\pm$ 0.4	5.4 $\pm$ 1.3

Values are mean  $\pm$  S.E.M. \* $P < 0.05$  significantly different from mean value of control (ANOVA followed by Dunnett's test).

## DISCUSSION

The potential antinociceptive effect of the ethanolic extract of AP was investigated. The overall results of the present study clearly demonstrated that the ethanolic extract of AP, administered intraperitoneally to mice exhibited both peripheral and central analgesic properties, since it produced dose-dependent and pronounced antinociceptive activity when assessed on chemical- and thermal-induced nociception from the respective doses of 30 to 500 mg/kg.

In acetic acid-induced writhing test, the extract exhibited a significant dose-dependent inhibition as compared to the respective control value with strong inhibition was observed at the dose of 500 mg/kg. The peripheral analgesic action of the extract at this dose (81.0%) was also found to be comparable to 100 mg/kg (78.0%) of standard NSAIDs, ASA. The abdominal writhing test, however, has been reported to be a less selective analgesic model but widely used method for evaluation of peripheral analgesic effect (Gene *et al.*, 1998). It has been proposed that acetic acid acts indirectly by inducing the release of endogenous mediators such as PGE<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  in peritoneal fluid that stimulates the nociceptive neurones sensitive to centrally acting agents such as narcotics and non-steroidal anti-inflammatory drugs (NSAIDs) (Collier *et al.*, 1968).

The hot-plate test has been reported to be more specific for evaluation of centrally acting analgesics (Vogel and Vogel, 1997). Comparing values obtained for reaction time of animals treated with the extract and the control values both before and after treatment, it is clear that the extract caused a considerable prolongation of latency times in dose dependent manner. Morphine, used as reference drug, also produced a significant analgesic effect during all the observation times when compared with control values.

The present results also demonstrated that pretreatment of the animals with naloxone (5 mg/

kg, i.p) significantly inhibited the analgesic effect of AP on acetic acid-induced abdominal writhing as well as on the hot-plate tests. Since naloxone, a classical opioid receptor antagonist, was able to modify the analgesia induced by AP treatment, it is most likely that this effect could be due to direct agonist activities of opioidomimetic constituents in the extract and/or due to increase release of endogenous opioid peptides.

In conclusion, the ability of the extract, in present study, to inhibit abdominal writhes as well as increased the time of latency to a discomfort reactions in the hot-plate test, revealed the existence of peripheral and central analgesic property of the AP extract, which probably mediated through opioid mechanisms. The extract will, therefore, be of potential benefit in the management of pain. The actual mechanism underlying this analgesic effect remains unknown, but the extract obtained is endowed with an apparently opioimimetic analgesic property. Further work is obviously required to purify and identify the structure of the active principle(s) present in this extract, as well as to isolate pure substance(s) in order to determine their mechanism of action.

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