

THE POTENCY OF PIPERINE AS ANTIINFLAMMATORY AND ANALGESIC IN RATS AND MICE

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ABSTRACT

The potency of piperine as anti inflammatory and analgesic were investigated. Piperine was evaluated for antiinflammatory effect by carrageen-induced rat paw edema. Sprague Dawley Rats were divided into the control groups were given saline perorally, piperine groups received doses of 2.5; 5 and 10 mg/kg piperine (peroral) respectively. This was followed by the administration of 1 % caragenin through the intraplantar route 30 min after administration piperine or saline. After the carrageenan injection was measurement of paw volume and prostaglandin production. The piperine in doses of 2.5; 5 and 10 mg/kg showed 5.4; 43.8 and 54.8 % inhibition of paw edema respectively at the end of three hours. The increase in prostaglandin (PGE2) levels after carrageenan injection was significantly prevented by preadministration of the piperine at 5 and 10 mg/kg but not piperine at 2.5 mg/kg. These results suggest that piperine has antiinflammatory properties may be attributed to inhibition of prostaglandin release. The analgesic activity of piperine was tested by acetic acid-induced writhing response and hot plate method in albino mice. The piperine in doses of 2.5; 5 and 10 mg/kg showed the percentage of protection from writhing was 1.4; 5.9 and 3.3 % respectively. However the piperine at 2.5; 5 and 10 mg/kg resulted no significantly analgesic effect in acetic acid-induced writhing response. In the hot plate model, the piperine at 2.5; 5 and 10 mg/kg resulted no significantly analgesic effect after 30 min, 1, 2 and 3 h of administration. These results suggest that piperine has not analgesic properties.

Keywords: *Writhing test, hot plate, carrageen, prostaglandin*

INTRODUCTION

Piperine is an alkaloid found naturally in plants belonging to the Piperaceae family, such as *Piper nigrum* L, commonly known as black pepper, and *Piper longum* L, commonly known as long pepper. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants. Piperine comprises 1 to 99% of these plants (Pei, 1983). It has putative anti-inflammatory activity and may have activity in promoting digestive processes. There is also preliminary evidence that it may have some anticonvulsant and anticarcinogenic properties (D'Hooge, 1996). On the other hand, there is also preliminary evidence that it might interfere with reproductive processes and have negative effects on sperm (Malini, 1999). Although this piperine has many useful claims, the mechanism of its medicinal effects are not understood. The objectives of this study were to evaluate the effect of piperine as analgesic on the hot-plate and acetic acid-induced writhing tests in mice, and as antiinflammatory on carrageen-induced foot paw edema in rats.

The carrageenan paw inflammation model has long been used to evaluate the anti-inflammatory activity. Previous studies showed that PGE2 production was induced by carrageenan in the foot pad edema could be blocked by a selective COX inhibitor (Seibert et al., 1994; Portanova et al., 1996). These results suggest a crucial role of PGE2 in the initiation of acute inflammation. While the hot-plate and acetic acid-induced writhing tests have used to evaluate the analgesic activity (Winter et al., 1962). There is a good correlation between efficacy in this model and activity in humans (Otterness and Bliven, 1985). We have used this model to investigate the analgesic and antiinflammatory activities of piperine in mice and rats.

MATERIAL AND METHODS

Chemicals

Acetic acid, piperine, carrageenin and other standard laboratory chemicals were obtained from Sigma Chemicals, Dorset, England. The monoclonal anti-PGE2 antibody 2B5 was generated as an ascites fluid in syngeneic BALB/c mice and goat anti rabbit IgG-alkaline phosphatase from Jackson Immuno Research (West Grove, PA).

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Animals

Adult male ICR Balb/c mice (20-25 g) were used for all the analgesic experiments. Adult male Sprague-Dawley rats (200-250 g) were used to study the antiinflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$), with access to food and water ad libitum. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals (Zimmermann, 1983).

Acetic acid-induced writhing in mice

The analgesic activity of piperine was assessed using writhing test (abdominal constriction test) (Collier et al., 1968). Acetic acid solution (10 ml/kg, 0.6%) was injected intraperitoneally, and the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 0.5 h beginning 5 min after acetic acid injection. The piperine was administered (0, 2.5, 5 and 10 mg/kg, perorally.) 0.5 h before the acetic acid injection.

Analgesic activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pretreated with the piperine using the ratio = $(\text{Control mean} - \text{Treated mean}) \times 100 / \text{Control mean}$.

Hot plate test in mice

The hot-plate test was performed to measure response latencies according to the method previously described (Seibert et al., 1994; Hosseinzadeh et al., 2000). The hot-plate (Model 7280, Ugo Basile, Italy) was maintained at $55.0 \pm 0.2^{\circ}\text{C}$ and the animals were placed into the Perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and 30, 60, 120 and 150 min after administration of the piperine (0, 2.5, 5 and 10 mg/kg, perorally.). A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Carrageenin-induced paw inflammation in rats

The carrageenan-induced paw inflammation model has been described previously (Seibert et al., 1994). Sprague Dawley rats were divided into the control groups were given saline perorally, piperine groups received 2.5; 5 and 10 mg/kg piperine (peroral) respectively. This was followed by the administration of 1 % caragenin through the intraplantar route 30 min after administration piperine or saline. The paw volume was measured by using plethysmometrically (Ugo Basile, Italy) at '0' and '3' hours after the carrageenan injection (Carter, 1991; Zakaria et al., 2001). The difference between the two readings was taken as the volume of edema and the percentage antiinflammatory activity was calculated as the percentage inhibition of rat paw edema between control animals and mice pretreated with the piperine using the ratio = $(\text{Control mean} - \text{Treated mean}) \times 100 / \text{Control mean}$.

Measurement of prostaglandin production in rat paw edema.

At selected times after dosing, rats were euthanized and their hind feet removed. A volume of 0.1 ml saline containing 10 μM indomethacin was injected into the paw to aid the removal of eicosanoid-containing fluid and prevent further PG production in paw tissue. Paw pads were incised with a scalpel, suspended off the bottom of polypropylene tubes with an Eppendorf pipet tip to facilitate fluid exudation and centrifuged at 1800 g for 15 min. The volume of exudate from each paw was measured. PGE2 levels in paw exudates were quantitated with a competitive binding ELISA using Dynatech (Mnich, 1995).

Statistical analysis

Numerical results are expressed as mean \pm SD, unless otherwise stated. One-way analysis of variance (ANOVA) was used for statistical comparison; $p < 0.05$ being the criterion for statistical significance. The significant treatment means were further subjected to LSD test.

RESULTS

Effect of piperine on the acetic acid-induced writhing

Table 1. Effect of piperine on acetic acid-induced writhing in mice

Group	No. of writhing (mean \pm SEM)	Percentage inhibition
Control	42.1 \pm 4.2 ^a	-
Piperine 2.5 mg/kg BW	41.5 \pm 3.9 ^a	1.4
Piperine 5 mg/kg BW	39.6 \pm 5.5 ^a	5.9
Piperine 10 mg/kg BW	40.7 \pm 4.7 ^a	3.3

a-c significantly different at $p < 0.05$, in the same column.

As shown in Table 1, piperine (2.5; 5 and 10 mg/kg, peroral.) showed no significant dose-dependent reduction in the number of writhing with approximately 1.4 %; 5.9 % and 3.3 % of inhibition respectively.

Piperine at 2.5; 5 and 10 mg/kg had mild analgesic effect was no significantly different from the control ($p < 0.05$).

Effect of piperine on hot-plate test

Table 2. Effect of piperine on hot plate reaction time in mice

Groups	Hot plate reaction time (min)				
	Pre	30	60	120	180
Control	6.7 \pm 0.5 ^a	7.3 \pm 0.6 ^a	7.6 \pm 0.5 ^a	7.2 \pm 0.8 ^a	7.8 \pm 0.6 ^a
Piperine 2.5 mg/kg BW	7.3 \pm 0.8 ^a	6.9 \pm 0.5 ^a	7.3 \pm 0.4 ^a	7.6 \pm 0.7 ^a	7.9 \pm 0.7 ^a
Piperine 5 mg/kg BW	6.6 \pm 0.5 ^a	7.1 \pm 0.8 ^a	7.8 \pm 0.2 ^a	6.9 \pm 0.9 ^a	8.1 \pm 0.6 ^a
Piperine 10 mg/kg BW	6.9 \pm 0.3 ^a	7.5 \pm 0.6 ^a	7.5 \pm 0.7 ^a	7.8 \pm 0.6 ^a	7.8 \pm 0.8 ^a

a-c significantly different at $p < 0.05$, in the same column.

Table 2 shows the time course of the analgesic produced by of piperine (2.5; 5 and 10 mg/kg). Administration of piperine at 2.5; 5 and 10 mg/kg peroral resulted no

significantly analgesic effect after 30 min, 1, 2 and 3 h of administration in the hot-plate test.

Effect of piperine on carrageenin -induced inflammation

Table 3 . Effect of piperine on carrageenin -induced inflammation

Group	Increase in paw volume (mean \pm SEM) in ml	Percentage inhibition
Control	0.73 \pm 0.05 ^a	-
Piperine 2.5 mg/kg BW	0.69 \pm 0.09 ^a	5.4
Piperine 5 mg/kg BW	0.41 \pm 0.04 ^b	43.8
Piperine 10 mg/kg BW	0.33 \pm 0.02 ^c	54.8

a-c significantly different at $p < 0.05$, in the same column.

The results of antiinflammatory activity are shown in Table 3. In the acute inflammation model, the piperine in doses of 2.5; 5 and 10 mg/kg, p.o. produced dose-dependent inhibition of rat paw edema. Piperine in doses of 2.5; 5 and 10 mg/kg showed 5.4; 43.8 and 54.8

% inhibition of paw edema respectively at the end of three hours. The antiinflammatory activity of piperine at 20 and 40 mg/kg but not 2.5 mg/kg was significantly different from the control ($p < 0.05$).

Effect of piperine on PGE2 production in inflammatory

Table 4 . Effect of piperine on prostaglandin production in carrageenin -induced inflammation

Group	PGE ₂ level in paw edema (mean \pm SEM) in ng/ml
Control	5.8 \pm 0.7 ^a
Piperine 2.5 mg/kg BW	6.1 \pm 0.6 ^a
Piperine 5 mg/kg BW	4.7 \pm 0.3 ^b
Piperine 10 mg/kg BW	3.9 \pm 0.4 ^c

a-c significantly different at $p < 0.05$, in the same column.

To study more directly the role of PGE₂ in established inflammation, we measured PGE₂ production at the site of inflammation. The data presented in Table 4 demonstrated that measurement of PGE₂ levels from the paw exudate fluid indicated that the reversal of inflammatory was accompanied by a concomitant reduction in PGE₂ levels in affected paws. Piperine at concentration of 0; 2.5; 5 and 10 mg/kg, p.o. induced dose-dependent inhibition of PGE₂ production were 5.8 \pm 0.7 ; 6.1 \pm 0.6; 4.7 \pm 0.3; 3.9 \pm 0.4 ng/ml, respectively. Piperine at concentration of 5 and 10

mg/kg mg/kg, p.o. significantly inhibited carrageenan-induced paw PG production

DISCUSSION

The hot-plate and acetic acid-induced writhing tests are one of the most common tests for evaluating the analgesic efficacy of drugs in rodents. Administration of piperine showed analgesic activity in the hot-plate and acetic acid-induced writhing tests (Table 1 and 2). The piperine (2.5; 5 and 10 mg/kg, po.) showed no

significantly suppressed the acetic acid-induced writhing response and resulted no significantly dose-dependent prolongation of the response latency in the hot-plate test. These results indicate that piperine has not analgesic properties.

The carrageenan-induced edema in the rat hind paw most widely used for the screening of new anti-inflammatory agents (Somchit and Nur, 2003). Carrageenan is the phlogistic agent of choice for testing antiinflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. Carrageenan-induced edema is mediated through the release prostaglandin and slow reacting substances which peak at 3 h (Somchit and Nur, 2003). The increase in the paw volume following carrageenan administration in the control. In the carrageenan-induced paw edema, the piperine induced dose-dependent reduction of paw edema in rat. The piperine in doses of 5 and 10 mg/kg, p.o. produced significant inhibition of paw edema as compared to the control. Measurement of PGE2 levels from the paw exudate fluid indicated that the reversal of inflammatory was accompanied by a concomitant reduction in PGE2 levels in affected paws. These results agree closely with the effect of piperine as anti inflammatory and consistent with NSAIDs are widely used for the treatment of inflammation and they are thought to act via inhibition of the synthesis of PGs (Smith and Willis, 1971; Seibert et al., 1994). These results indicate that piperine possesses inhibition of prostaglandin release mediated anti-inflammatory properties. In conclusion, this study demonstrated that piperine has only the anti-inflammatory effect which may be attributed to inhibition of prostaglandin release.

REFERENCES

- Carter RB, 1991. Differentiating analgesic and non-analgesic drug activities on rat hotplate: Effect of behavioral end-point. *Pain*. 47, pp. 211-20.
- Collier HDJ, Dinnin LC, Johnson CA, Schneider C, 1968. The abdominal response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol*. 32, pp. 295-310.
- D'Hooge R, Pei YQ, Raes A et al., 1996. Anticonvulsant activity of piperine on seizures induced by excitatory amino acid receptor agonists. *Arzneimittelforschung* 46, pp. 557-560.
- Hosseinzadeh H, Ramezani M, Salmani G. 2000. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. *J Ethnopharmacol*. 73, pp. 379-85.
- Malini T, Manimaran RR, Arunakaran J et al., 1999. Effects of piperine on testis of albino rats. *J Ethnopharmacol*. 64, pp. 219-225.
- Mnich, SJ, Veenhuizen, AW, Monahan JB, Sheehan KC, Lynch KR, Isakson PC and Portanova JP, 1995. Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E2. *J. Immunol* 155, pp. 4437-4444.
- Otterness IG and Bliven ML, 1985. Laboratory models for testing nonsteroidal anti-inflammatory drugs. In *Non-steroidal Anti-inflammatory Drugs*, ed. by JG Lombardino, Wiley, New York, pp. 111-252.
- Pei YQ, 1983. A review of pharmacology and clinical use of piperine and its derivatives. *Epilepsia*. 24, pp. 177-182.
- Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K, Gregory SA and Isakson PC, 1996. Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. *J. Exp. Med* 184, pp. 883-891.
- Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L and Isakson PC, 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc. Natl. Acad. Sci. U.S.A.* 91: 12013-12017.
- Smith JB and Willis AL, 1971. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature (Lond.) New Biol* 231, pp. 235-239.
- Somchit MN and Nur SMH, 2003. Antiinflammatory property of ethanolic and aqueous extracts of *Zingiber zerumbet*. *Indian J Pharmacol* 35, pp. 181-3.
- Winter CA, Risley EA and Nuss GW, 1962. Carrageenan-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med* 111, pp. 544-552.
- Zakaria MNM, Islam MW, Radhakrishnan R, Chen HB, Kamil M, Al-Gifri AN, 2001. Antinociceptive and antiinflammatory properties of *Caralluma arabica*. *J Ethnopharmacol*. 76, pp. 155-8.
- Zimmermann M. 1983. Ethical guidelines for investigation of experimental pain in conscious animals. *Pain* 16, pp. 109-10.