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Pharmacological Study on Piperine

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Abstract □ Systematic pharmacological studies on piperine have revealed that this compound elicited diverse pharmacological activities; CNS depressant activity characterized by antagonism against electroshock seizure and by muscle relaxant activity in mice; antipyretic activity in typhoid vaccinated rabbits; analgesic activity as evaluated by tail-clip pressure and writhing syndrome in mice; antiinflammatory activity in carrageenin-induced edema in rats.

Keywords □ Piperine, CNS depressant activity, Antipyretic activity, Analgesic activity, Antiinflammatory activity.

Piperine is an alkaloid, the piperidine amide of piperic acid. It is a major pungent principle of various *Piper* species such as black pepper and long pepper which are commonly used for a condiment and employed in folkloric medicine for treatment of asthma, insomnia and abdominal disorders.[1,2] It is also present in the leaves of *Rhododendron fauriae* (Ericaceae).[3] Piperine was recently found to possess central nervous system (CNS) depressant properties.[4,5] In this paper, results of systematic pharmacological studies on piperine are described.

EXPERIMENTAL METHODS

Materials

Piperine was isolated from the fruits of *Piper nigrum*, purified and characterized by spectral analysis in our laboratory. Reference drugs, such as aminopyrine, acetaminophen, hydrocortisone and phenytoin were obtained commercially. Typhoid vaccine was a gift from Dong Shin Pharmaceutical Co.

Animals

Experiments were performed using male albino mice (dd strain) weighing 17~25g, male Sprague-Dawley rats weighing 150~200g and male New Zealand White rabbits weighing 2~3kg were used. The animals were allowed lab chows and tap water *ad. lib.* throughout the experiments. Animals received all drugs suspended in 0.5% sodium carboxymethylcellulose solution.

Maximal Electroshock Seizure Test

Electroshock seizure was induced in mice via corneal electrodes (100V) delivered for 0.3 sec. according to the method of Toman *et al.*[6] The drugs were given i.p. 30 min prior to giving the shock. The seizure typically consists of a short period of initial tonic flexion and a prolonged period of tonic extension followed by terminal clonus. Failure to extend the hind limbs to an angle with the trunk greater than 90° is defined as protection.

The ED_{50}, a dose of the drugs which antagonized 50% of mice to the seizure was calculated by the probit method.

Measurement of Pinna and Corneal Reflex

The reflexes were evaluated according to the method of Goodsell *et al.*[7] Mice (seven animals per group) were given piperine and 15, 30 and 45 min after treatment, pinna and corneal

reflex were tested by touching with a fine pig hair on the cornea and conjunctiva of the eyes in the case of corneal reflex and on the external auditory meatus of the ears in the case of pinna reflex and the disappearance of the reflexes were noted.

Measurement of Hypothermic Effect

Normal untreated mice (5~10 mice per group) were used. After treatment of drugs the rectal temperature was measured with a thermister thermometer at the stated intervals.

Measurement of Antipyretic Effect

The experiment was performed with 3 male New Zealand White rabbits per group showing 39°C of body temperature which were maintained in constant temperature (20±1°) and humidity (60~70%) for 10 days before experiment. 75 min after the injection of typhoid vaccine (0.5 ml/kg, DS-typhoid), piperine was given orally and the rectal temperature was measured with a thermister thermometer at the stated intervals.

Measurement of Writhing Syndrome

The experiment was performed with 10 male albino mice per group according to the method of Whittle.[8] Graded doses of drugs were orally administered one hr prior to induction of writhing which was evoked by i.p. injection of 0.1 ml of 0.7% acetic acid-saline solution. The writhing syndrome was counted for 10 min from 10 min after the injection of the irritant. The ED_{50} was calculated by the probit method.

Measurement of Tail Clip Pressure

The experiment was performed with 10 male albino mice per group according to the method of Bianchi *et al.*[9] Tail clip pressure was induced with an aortic clip (450g) one hr after oral administration of graded doses of drugs. The mice tolerated more than 6 sec were considered to be positive. The ED_{50} was calculated by the probit method.

Carrageenin-induced Edema Test

Carrageenin-induced edema test was performed using male Sprague-Dawley rats according to the method of Winter *et al.*[10] Six or seven male rats per group were given drugs orally 1 hr prior to the injection of 0.1 ml of 1% carrageenin into the plantar surface of the right hind paw. The edema volume was measured with plethysmometer at the stated intervals for 3.5 hr.

RESULTS

CNS Depressant Activity

Piperine exhibited a significant protection against electroshock seizure at relatively low dose range. The ED_{50} value was calculated to be 15.1mg/kg i.p. which was almost equipotent to the reference drug, phenytoin (Table I).

The results of the time course effect of piperine on pinna and corneal reflex are indicated in Table II. At a dose range of 50~100mg/kg i.p., piperine caused a marked paralysis in pinna reflex at 30 min and 45 min after treatment of the sample. Piperine, however, showed only weak paralysis of corneal reflex throughout the experimental period.

Antipyretic Activity

Fig. 1 shows the time course and dose-response

Table I: Effect of piperine on maximal electroshock seizure in mice.

Compound	Route	ED_{50} (mg/kg)
Piperine	*i.p.*	15.1 (8.4~27.3)
Phenytoin	*i.p.*	26.3(15.9~43.4)

Figures in parentheses indicate the 95% confidence limits.

Table II: Effect of piperine on pinna and corneal reflex in mice.

Compound	Dose (mg/kg, *i.p.*)	No. of mice	No. paralyzed					
			Corneal reflex			Pinna reflex		
			15	30 (min)	45	15	30 (min)	45
Piperine	50	7	0	0	1	0	6	5
	100	7	1	1	1	7	7	5
	300	7	5	3(2)*	1(3)	7	6(1)	4(3)

* Figures in parentheses indicate number of mice died.

of hypothermic effect of piperine which was estimated in normal mice. Piperine, with a single oral administration of 30mg/kg, caused a rapid and profound drop in body temperature. Rectal temperature fell by 5°C at 1.5hr after drug treatment and the effect persisted for upto 4hr. The rectal temperature returned to normal within 24hr. This hypothermic effect was rather stronger than that of reference drug, acetaminophen which was given at a dose of 400mg/kg. In agreement with the hypothermic property, piperine showed a strong antipyretic activity in typhoid vaccinated rabbits. When given orally in the dose range of 10~30mg/kg 75 min after i.v. injection of typhoid vaccine, piperine was shown to abate the fever induced by the vaccine the rectal temperature reaching nomal level in 4hr. (Fig. 2).

Analgesic Activity

The inhibitory potency of piperine against acetic acid-induced writhing syndrome as expressed by its ED_{50} value(3.7mg/kg) was approximately 70 times as potent as acetaminophen(Table III). The potency of toleration against tail clip pressure as expressed by ED_{50} value(104.7mg/kg) was approximately 1.5 times as potent as aminopyrine (Table III).

Antiinflammatory Activity

It was found that piperine had a strong antiedemic activity as indicated in Table IV. At a dose of 50mg/kg orally, piperine showed a significant inhibition against increase in edema volume at 2.5 and 3.5 hr after the injection of the irritant. The antiedemic effect was stronger than that of hydrocortisone at the same dose level.

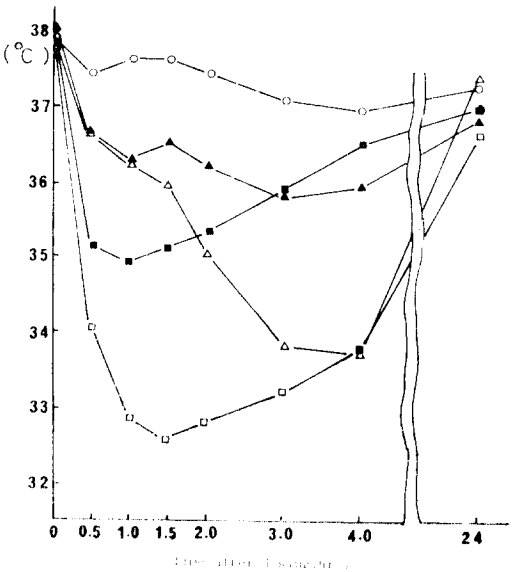

Fig. 1: Hypothermic effect of piperine in mice.
○—○, Control
■—■, Piperine, 10mg/kg, p.o.
□—□, Piperine, 30mg/kg, p.o.
▲—▲, Acetaminophen, 200mg/kg, p.o.
△—△, Acetaminophen, 400mg/kg, p.o.

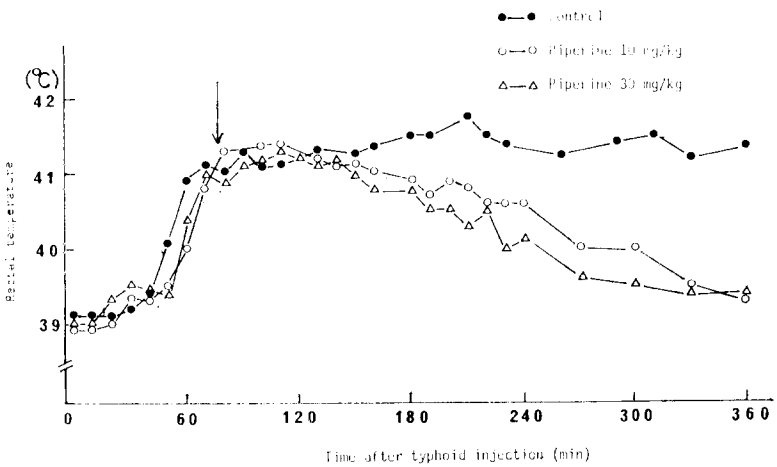

Fig. 2: Antipyretic activity of piperine in rabbit.
Piperine was given orally 75min (arrow) after the injection of typhoid vaccine.

Table III: Analgesic activity of piperine in mice.

Compound	Writhing method ED_{50}, mg/kg, *p.o.*	Tail-clip method ED_{50}, mg/kg, *p.o.*
Piperine	3.7(2.5～ 5.5)	104.7(52.4～209.4)
Acetaminophen	258.8(172.5～388.2)	—
Aminopyrine	—	158.1(98.8～253.0)

Figures in parentheses indicate the 95% confidence limits.

Table IV: Effect of piperine on carrageenin induced edema in rats.

Compound	Dose(mg/kg, *p.o.*)	No. of rats	Increase % in paw volume			
			0.5	1.5	2.5	3.5hr
Control	—	7	28.4±3.7[a)]	39.7+3.5	48.6±5.4	67.6±4.1
Piperine	50	7	23.8±3.9	35.5±3.7	27.3±3.4**	28.9±3.4**
	150	6	19.3±4.5	26.2±5.1*	22.6±5.8**	17.4±5.7**
Hydrocortisone	50	6	32.8±3.6	38.5±5.9	37.2±4.0	42.8±4.4**

Significantly different from the control; *$p<0.05$ **$p<0.01$
a) Mean±S.E

DISCUSSION

We have previously demonstrated that piperine had a CNS depressant activity since it showed a synergistic effect on hexobarbital induced narcosis, and antagonistic effect on chemoshocks induced by strychnine and pentetrazole as well as a heavy muscular incoordination in mice.[11-13] Pei also recently reported that piperine antagonized convulsion induced by electroshock and pentetrazole.

Piperine was shown to have a strong protection against maximal electroshock seizure and cause paralysis of pinna and corneal reflex in the present experiment. From these results, it is confirmed that piperine possessed not only anticonvulsant but also centrally acting muscle relaxant properties.

Singh *et al.*[14], however, postulated that piperine caused a significant increase in spontaneous motor activity, antagonized pentobarbital narcosis in mice and produced clonic and tonic convulsions in frogs at a dose of 10mg/kg. It was, moreover, reported that increase in the dose of piperine successively produced clonic and tonic convulsions terminating in the death of the animals. We did not, however, observe any convulsions in frogs (data not shown) and in mice when treated with piperine in 10~100mg/kg dose range.

Furthermore, LD_{50} values of piperine presented by them[14] were quite different from ours[11]. LD_{50} values for i.p. and p.o. administration were approximately 9.2 times and 29 times lower than those we obtained at the same experimental conditions, respectively. Judging from the experimental data thus far obtained, it can be convinced that Singh's sample could not be piperine.

It was found that piperine, besides CNS depressant activity, had various pharmacological activities such as antipyretic, analgesic and antiinflammatory activities through the present investigation.

In the separate experiments, we also found that repeated administration of pharmacologically effective doses of piperine caused a significant induction of hepatic mixed function oxidase system.[15]

Pipeine is a main component (6~9%) of black pepper which is not only used for therapeutic purposes in herbal drug prescriptions but also consistently offered as a condiment or an additive of various foods.

Piperine has been used clinically as an antiepileptic agent in China.[5] Therefore, untoward side effects may arise due to diverse pharmacological activities of piperine if large amounts of herbal drugs or foods containing piperine are taken within a day for a long period of time.

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