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THE ANALGESIC AND ANTICONVULSANT EFFECTS OF PIPERINE IN MICE

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Piperine, is the major active principal of black pepper. In traditional medicine, black pepper has been used as an analgesic, anti-inflammatory agent and in the treatment of epilepsy. This study was conducted to evaluate the *in vivo* analgesic and anticonvulsant effects of piperine in mice. The analgesic and anticonvulsant effects of piperine were studied in mice using acetic acid-induced writhing, tail flick assay, pentylenetetrazole (PTZ)- and picrotoxin (PIC)-induced seizures models. The intraperitoneal (i.p.) administration of piperine (30, 50 and 70 mg/kg) significantly inhibited ($P<0.01$) the acetic acid-induced writhing in mice, similar to the effect of indomethacin (20 mg/kg i.p.). In the tail flick assay, piperine (30 and 50 mg/kg, i.p.) and morphine (5 mg/kg, i.p.) caused a significant increase ($P<0.01$) in the reaction time of mice. Pre-treatment of animals with naloxone (5 mg/kg i.p.), reversed the analgesic effects of both piperine and morphine in the tail flick assay. Piperine (30, 50 and 70 mg/kg, i.p.) and standard drugs, valproic acid (200 mg/kg, i.p.), carbamazepine (30 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) significantly ($P<0.01$) delayed the onset of PTZ-and PIC-induced seizures in mice. These findings indicate that piperine exhibits analgesic and anticonvulsant effects possibly mediated *via* opioid and GABA-ergic pathways respectively. Moreover, piperine being the main constituent of black pepper, may be contributing factor in the medicinal uses of black pepper in pain and epilepsy.

K e y   w o r d s: *piperine, gamma aminobutyric acid, analgesic effect, anticonvulsant effect, central pathway, morphine, diazepam, tail flick assay, seizures*

INTRODUCTION

Piperine is the main pungent alkaloid present in the fruits of black pepper (*Piper nigrum*) and long pepper ; *Piper longum* (1). Black pepper is the most common culinary spice and considered an essential constituent of soups world-wide particularly in south Asia. In traditional medicine, black pepper has been used as an analgesic and anti-inflammatory agent and in the treatment of epilepsy and snake venom poisoning (2). In Chinese herbal medicine, black pepper has a long tradition in the treatment of epilepsy (3). The beneficial effects of black pepper in the management of pain and epilepsy may be attributed to its active constituent, piperine; however, its mechanism of action is still unclear. Piperine has been reported to have variety of pharmacological properties such as antipyretic, analgesic and anti-inflammatory (4) cytoprotective and antioxidant and neuroprotective effects (1, 3). Piperine is known to exhibit antidepressant and memory enhancing effects in animal experiments after chronic oral administration (5, 6). The anticonvulsant activity of piperine on seizures induced by excitatory amino acid receptor agonists has also been reported (7). In addition, its anti-inflammatory activities have been demonstrated in several animal models of inflammation (8-10). These studies indicate the diverse therapeutic potential of piperine in variety of ailments.

Constituents of the piper species have shown to have an *in vitro* inhibitory activity against 5-lipoxygenase and cyclooxygense-1 (11). These effects of piperine seem to be beneficial in inflammatory diseases accompanied by severe pain such as rheumatoid arthritis. Its antiarthritic efficacy was evaluated in animal models of experimental arthritis (10). However, the exact role of piperine in pain and convulsion is not clearly understood. Moreover, to the best of our knowledge, piperine has not been thoroughly investigated for its effect in the acetic acid-induced writhing, tail flick assay, pentylenetetrazole (PTZ) and PIC-induced seizures, the widely used classical *in vivo* experimental models of pain and convulsion. The current study was planned to evaluate the effects of piperine in the aforementioned animal models of pain and convulsions. Its effects was compared with standard analgesic and anticonvulsant drugs to validate the traditional uses of black pepper in epilepsy, pain and inflammatory conditions.

MATERIALS AND METHODS

Animals

Male mice (20–30 g) were obtained from the animal house facility of The College of Medicine, King Saud University, Riyadh, Saudi Arabia. All the experimental protocols were reviewed by institutional review board of Council of Medical Research, College of Medicine, King Saud University Riyadh and complied with the National Institutes of Health guidelines

for the care and use of laboratory animals. The animals were housed in plastic cages under standard condition with 12-h light:dark cycle with access to food and water *ad libitum*.

Chemicals

The following chemicals were used in the experiments; acetic acid, indomethacin, diazepam, naloxone, valproic acid, carbamazepine, petylenetetrazole, piperine and picrotoxin were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All chemicals used in the study were of analytical grade.

Experimental methods

Writhing test

Male mice (20–25 g, n=6) were used in this experiment according to the method of Koster *et al.* (12). Animals were randomly divided into three groups and the treatment to each group was given as follows:
Group I: Control (saline treated).
Group II: Pre-treated with indomethacin (reference drug).
Group III: Pre-treated with different doses of piperine (30, 50 and 70 mg/kg suspended in normal saline with 0.1% Tween 80).

Mice were given an injection of 0.7% v/v acetic acid solution (volume of injection 0.1 ml/10 g), 30 min after the administration of the piperine or indomethacin and placed individually into glass beakers followed by a 5-min lapse time. The number of writhes produced in these animals was counted for 20 min. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Control animals received normal saline (10 ml/kg,), and indomethacin (20 mg/kg,) was used as a positive control.

Tail-flick assay

The test was performed with the tail-flick model using analgesiometer (Ugo Basile, Comwerio VA, Italy). Each mouse was gently held with one hand and the distal half of its tail was positioned on the source of radiant heat The tail-flick response was elicited by applying radiant heat to the ventral surface of the tail. The time elapsed till the animal flicked its tail (reaction time) was determined as described elsewhere (13). Preliminary experiments were conducted to assess the pre-drug reaction time of animals (usual response 3–4 seconds). Mice with a reaction time of more than six seconds were not used in the test. In order to prevent tissue injury, a 20 seconds cut off latency was kept to prevent damage to the mouse tail. Animals were pre-treated with vehicle, test material (piperine 30 and 50 mg/kg) or morphine (5 mg/kg) 50 min before the assessment of tail flick latency. Naloxone (5 mg/kg, i.p.) was injected 30 min before the administration of morphine or piperine.

Pentylenetetrazole-induced seizures

Male mice (20–30 g) were randomly assigned into six groups and treated as follows:
Group 1 served as control and was administered vehicle (saline).
Group 2 was treated with standard anticonvulsants, valproic acid (200 mg/kg, i.p.), carbmazepine (30 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.).
Group 3 received different doses of piperine (30, 50 or 70 mg/kg i.p.).

Pentylenetetrazole (PTZ) was prepared daily by dissolving in normal saline. Test materials were administered in a volume not exceeding 10 ml/kg, 30 min before the seizure induction by PTZ. Animals were observed for myoclonic jerk latency and the occurrence of generalized tonic clonic seizures with loss of righting reflex up to 30 min after PTZ injection (14). The ability of a drug/extract to prevent the seizures or delay/prolong the latency or onset of the tonic hind-limb extensions was considered as an indication of anticonvulsant activity.

Picrotoxin-induced seizures

In brief, clonic-tonic seizures were induced in drug or vehicle pretreated male mice (20–30 g) by an i.p injection of 15 mg/kg picrotoxin. The animals were pretreated with different doses of piperine (30, 50 and 70 mg/kg i.p.) or diazepam (1 mg/kg), 30 minutes before the injection of picrotoxin. The control animals received only saline solution (10 ml/kg). After the picrotoxin injection, animals were placed in the testing chamber. The latencies to myoclonic jerks and clonic-tonic seizures were registered (15).

Statistical analysis

The results are presented as mean ±S.E.M. Comparison between experimental and control groups was performed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls multiple comparison test when significant difference was present. $P < 0.05$ was considered significant.

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

Treatment	Dose (mg/kg, i.p.)	Number of writhes	% inhibition
Control	Saline (10 ml/kg)	75 ± 6	-
Piperine	30	31 ± 4**	59
	50	22 ± 3***	71
	70	8 ± 2***	89
Indomethacin	20	25 ± 7 ***	67

Values represent mean ± S.E.M. of 6–15 observations. Animals in the control group received equal volume of saline. Piperine and indomethacin were administered 30 min before the acetic acid injection (i.p.). ** $P < 0.01$ and *** $P < 0.001$, compared to saline control.

RESULTS

Writhing test

As shown in *Table 1*, the intraperitoneal administration of different doses of piperine (30, 50 and 70 mg/ kg), caused significant inhibition (P<0.001) of the nociception induced by acetic acid with maximum effect being 89% at 70 mg/kg. The results were comparable to standard drug indomethacin that produced 67% inhibition of the acetic acid-induced constrictions at the dose of 20 mg/kg.

Tail flick assay

In the Tail flick assay piperine (30 and 50 mg/kg, i.p.) showed significant analgesic activity (P<0.01), similar to that produced by morphine (*Fig. 1*). Piperine at the dose of 50 mg/kg,

increased latency of reaction time from 3.7±0.3 (control) to 17.2±0.5 seconds (treated). Pre-treatment of the animals with naloxone (5 mg/kg,) abolished the analgesic effect of piperine (50 mg/kg, i.p.), similar to the analgesic effect of morphine (*Fig. 1*). Naloxone alone (50 mg/kg) had no significant effect on the reaction time of animals in the tail flick assay (data not shown). The reaction time of naloxone pre-treated animals was slightly increased compared to control animals (data not shown).

Pentylenetetrazole-induced seizures

As shown in *Fig. 2*, pentylenetetrazole (PTZ) alone (Control) at the dose of 70 mg/kg, induced convulsions in all the animals used in the study with latency of convulsion 160.7±12.5 seconds. Piperine (30, 50, 70 mg/kg, i.p.) protected animals from PTZ-induced seizures in a dose-dependent manner (*Fig. 2*), however, its efficacy was slightly declined at 70 mg/kg. The latency of
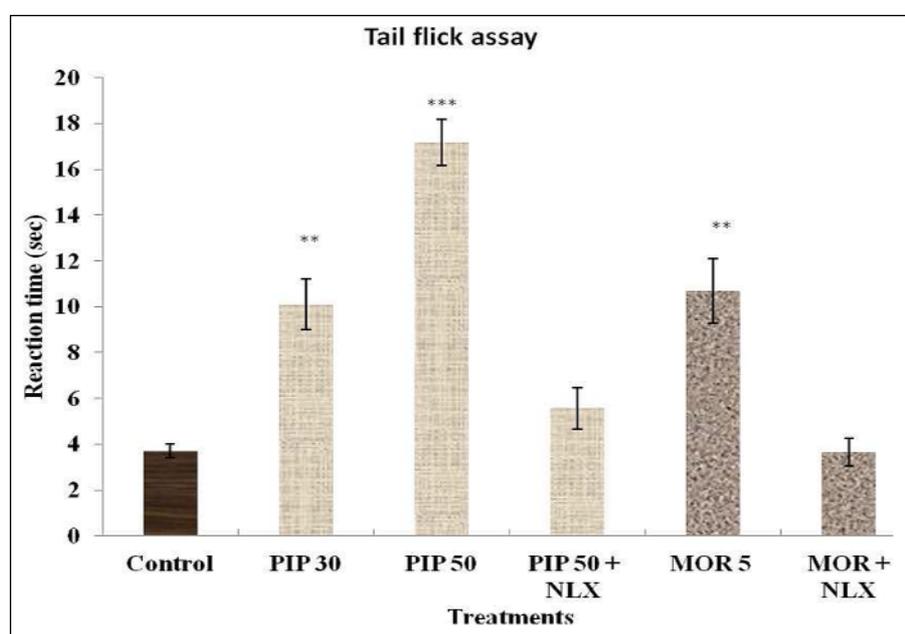
Fig. 1. Antinociceptive activity of piperine 30 and 50 mg/kg, i.p. (PIP30 and PIP50), morphine 5 mg/kg (MOR 5) in the absence and presence of naloxone (NLX 5 mg/kg, i.p.) in the tail flick assay. Values are expressed as mean ± S.E.M (n=6–15); ** P<0.01 and *** P<0.001 with respect to saline control.
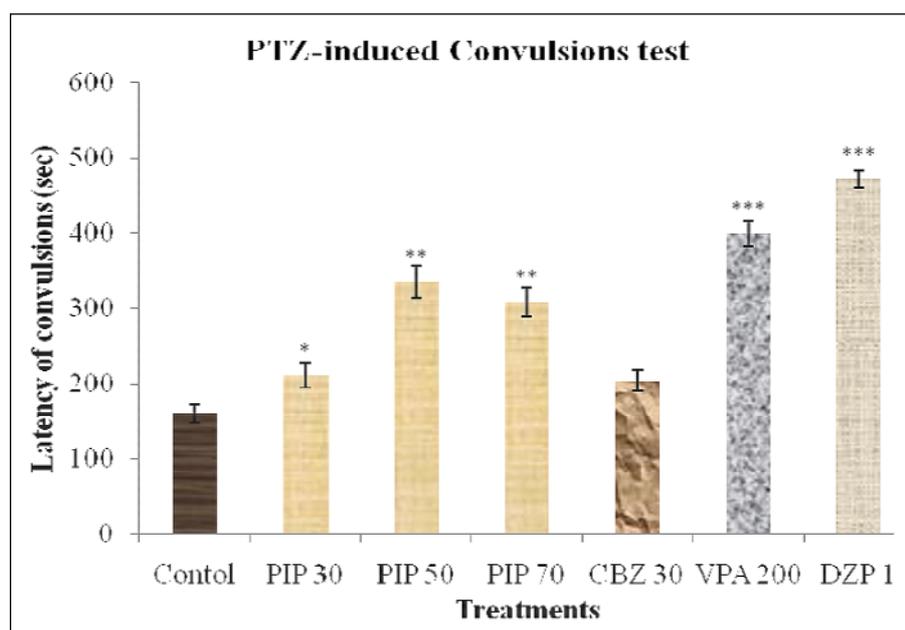
Fig. 2. Anticonvulsant effect of piperine 30, 50 and 70 mg/kg, i.p. (PIP30, PIP50 and PIP 70), carbamazepine 30 mg/kg i.p (CBZ 30), valproic acid 200 mg/kg, i.p. (VPA 200) and diazepam 1 mg/kg, i.p. (DZP 1) in the pentylenetetrazole (PTZ; 75 mg/kg, i.p.)-induced seizures in mice. Values are expressed as mean ± S.E.M. (n=6–15);* P<0.05, **p<0.01 and *** P<0.001 compared to control.
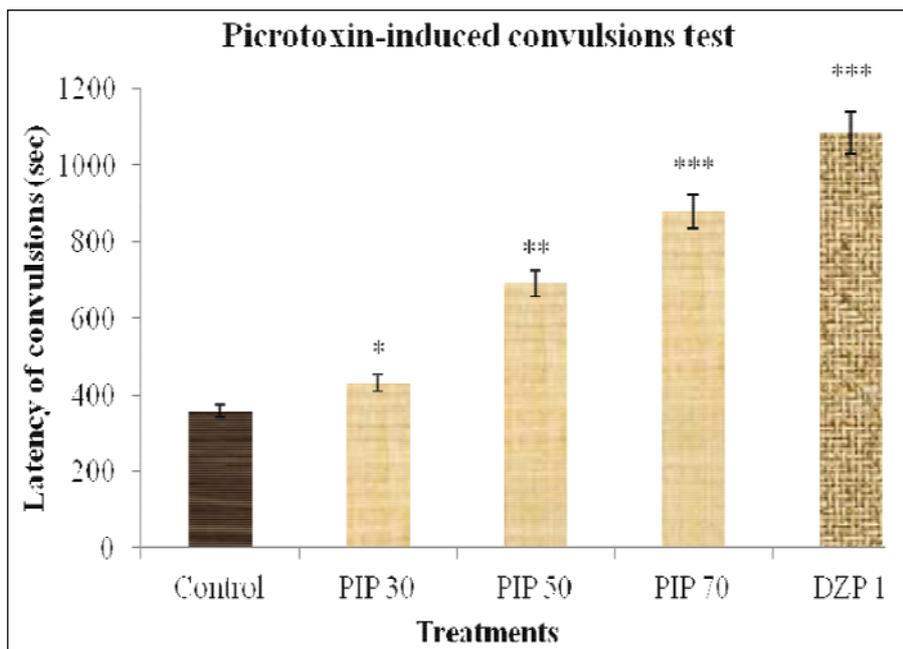
Fig. 3. Anticonvulsant effect of piperine 30, 50 and 70 mg/kg, i.p. (PIP30, PIP50 and PIP 70) and diazepam 1 mg/kg, i.p. (DZP 1) in the picrotoxin (15 mg/kg, i.p.)-induced seizures in mice. Values expressed as mean ± S.E.M. (n=6-15),* $P<0.05$, ** $P<0.01$ and *** $P<0.001$ compared to control.

PTZ-induced convulsion in piperine treated animals was significantly different compared to unprotected saline treated animals ($P<0.01$). Pretreatment of animals with the standard anticonvulsant drugs, carbamazepine (30 mg/kg, i.p.) and valproic acid (200 mg/kg, i.p.) delayed the onset of seizures and protected the animals against PTZ-induced seizures as expected.

Picrotoxin-induced seizures

Preliminary experiments were conducted to select the optimal dose of picrotoxin for the induction of seizure in mice. Pictrotoxin (15 mg/kg, i.p.) induced seizures in all animals with latency of convulsions 358.4±14.4 seconds. Piperine at the doses of 30, 50 and 70 mg/kg significantly increased the picrotoxin-induced latency of convulsion compared to control saline treated animals ($P<0.001$), *Fig. 3*. The increase in the latency of picrotoxin-induced convulsions by the highest tested dose of piperine (70 mg/kg) was 878.5±32 seconds compared to value of control group (358.4±14.2), which was highly significant ($P<0.001$), as shown in *Fig. 3*. Similarly, diazepam (1 mg/kg, i.p.) delayed the onset of picrotoxin-induced seizures in mice.

DISCUSSION

This study was conducted to evaluate the analgesic and anticonvulsant activities of piperine in mice. For the anti-nociceptive effects, the two widely used pain models, acetic acid-induced writhing and tail flick tests were employed. Acetic acid produces nociception by increasing the level of prostaglandins, serotonin, and histamine in peritoneal fluids, and this animal model is commonly used for screening analgesics acting through peripheral pathway (16, 17). In the present investigation, piperine inhibited acetic acid-induced writhing in mice similar to indomethacin suggesting that the analgesic activity of the piperine might be related to inhibition of the prostaglandin function (17). Commonly employed non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, indomethacin and aspirin have been shown to exhibit analgesic effect in acetic acid-induced writhing in mice (18, 19). Acetic acid-induced nociception is a non-selective model because it releases endogenous mediators (prostaglandins), which are capable of stimulating both the peripheral nociceptor(s) and neurons sensitive to NSAIDs, opioids, and other centrally acting drugs (20).

The analgesic effect of piperine was further investigated in tail flick assay, a test considered suitable for studying the central mechanism of analgesia. Piperine significantly increased the tail flick latency (reaction time) in mice similar to morphine, used as a centrally acting reference drug. Naloxone, an opioid antagonist, abolished the analgesic effect of piperine and morphine. These results suggest that the antinociceptive activity of piperine is likely to be mediated through opioid receptors.

Piperine attenuated the PTZ and picrotoxin-induced seizures in mice. In the PTZ-induced seizures model, piperine and reference anticonvulsant drugs, carbamazapine, valproic acid and diazepam delayed the onset of seizures. Pentylenetetrazole (PTZ) has been reported to produce seizures by inhibiting gamma aminobutyric acid (GABA) neurotransmission (21). GABA is the major inhibitory neurotransmitter substance in the brain, and is widely implicated in epilepsy. Enhancement of GABAergic neurotransmission has been shown to inhibit or attenuate seizures, while inhibition of GABAergic neurotransmission or activity is known to promote and facilitate seizures (22, 23).

In the picrotoxin-induced seizures model, piperine caused a significant increase in the latency of convulsion similar to diazepam, a standard anticovulsant drug. Postsynaptic GABAA-receptors are functionally linked to chloride ion channels to form GABA-chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission (24). Picrotoxin, a GABAA-receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABAA-receptors, thus preventing the entry of chloride ions into neurons and inhibiting GABA neurotransmission and activity in the brain (25). Barbiturates and benzodiazipnes (diazepam) are believed to enhance GABA-ergic neurotransmission by increasing chloride ion flux through the chloride-ion channels at GABAA-receptor sites (26). Therefore, it is likely that the observed protective effect of piperine against picrotoxin-induced seizures in mice may involve opening of the chloride-ion channels associated with GABAA-receptors and/or glutamate NMDA antagonist action (27, 28). These findings are consistent with earlier study reporting the inhibitory effect of

piperine on pilocarpine-induced convulsions in mice (29). However, the anticonvulsant effect of piperine reported in this study was achieved at much lower doses indicating that piperine displays variable potency in chemically induced seizure models.

Piperine has been reported for anti-depressant and anxiolytic properties being mediated *via* the serotonergic system (30). It has been shown that anti-depressant drugs affecting the serotonergic pathway have protective effect in the hippocampus (31). Serotonergic pathway also modulates the function of GABA (32). It is possible that the anticonvulsant effect of piperine may be due to the activation of serotonergic receptors located on interneurons resulting in an enhancement of the release of GABA (32, 33). Furthermore, based on earlier reports, it is likely that activation of GABA-ergic and/or opioid pathways contribute toward the observed analgesic and anticonvulsant effects of piperine (34).

In summary, the findings of the present study suggest the analgesic and anticonvulsant effects of piperine similar to reference drugs, indomethacin, valproic acid, carbamazepine and diazepam. The observed inhibitory effect of piperine in the tail flick assay, PTZ and picrotoxin-induced seizures, is possibly mediated by an interaction with opioid and/or GABAergic pathways. This study may support the folkloric use of black pepper in the management of epilepsy and pain (35).

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794

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