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Piperine, the potential functional food for mood and cognitive disorders

Jintanaporn Wattanathorn [a,*], Pennapa Chonpathompikunlert [b], Supaporn Muchimapura [a], Aroonsri Priprem [c], Orathai Tankamnerdthai [a]

[a] Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Physiology and Graduate School (Neuroscience Program), Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[c] Department of Pharmaceutical Technologies, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

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ABSTRACT

The effect of piperine, the main alkaloid from piper nigrum, on the central nervous system is not clearly known until now. In the present study, male Wistar rats were administered piperine at various doses ranging from 5, 10 and 20 mg/kg BW once daily for 4 weeks and the animals were determined the neuropharmacological activity after single, 1, 2, 3 and 4 weeks of treatment. The results showed that piperine at all dosage range used in this study possessed anti-depression like activity and cognitive enhancing effect at all treatment duration. Therefore, piperine may be served as the potential functional food to improve brain function. However, further investigations about precise underlying mechanism are still required.

1. Introduction

In recent years, it has been reported that more than 450 million people worldwide suffer from the mental and brain disorders (ICMR, 2001). To date, the efficacy of the drugs for these conditions are very limited so the need for newer, better-tolerated and more efficacious treatments is remaining high. Therefore, herbal therapies and functional food have been increasing their importance as alternative/complementary strategies to prevent against these conditions.

Piperine (1-piperoylpiperidine), a nitrogenous pungent substance, is an alkaloid presents in the fruits of black pepper (*Piper nigrum*), long pepper (*Piper longum*) and other piper species (family: Piperaceae). These plants are commonly used worldwide as household spices such as food additives and condiments. In addition, they are used as important ingredients for various medicinal purposes in traditional medicine in many Asian countries. Since piperine has been recognized as a main alkaloid in these plants, numerous studies have focused on investigating the pharmacological activities of piperine. Recent pharmacological studies have shown that piperine possesses anti-inflammatory and analgesic effect (Gupta et al., 2000), anticonvulsant (D'Hooge et al., 1996) and anti-ulcer (Bai and Xu, 2000) activities, anti-depressant effect (Lee et al., 2005), cytoprotective effect and antioxidant activity (Selvendiran et al., 2003).

With regard to numerous effects of piperine on the nervous system just mentioned, the effects of subchronic administration of piperine on the mood and cognitive disordered have been considered. Unfortunately, the supported document is not available until now, therefore, the current study is set up to investigate this issue.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (180–220 gm, 8 weeks old) were obtained from National Animal Center, Salaya, Nakhon Pathom, and were housed in group of 5 per cage in standard metal cages at 22 ± 2 °C on 10:14 h light–dark cycle. All animals were given access to food and water ad libitum. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC).

The experimental protocols were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs

Diazepam (2 mg/tablet), and fluoxetine (20 mg/tablet) (Government Pharmaceutical Organization), donepezil hydrochloride (Aricept 5 mg/tablet) (Pfizer pharmaceuticals Inc.) were used as standard drugs in this study. They were dissolved in propylene glycol and administered via oral route. Diazepam at dose of 2 mg/kg BW was used as positive control to test anxiolytic activity whereas fluoxetine was treated at dose of 10 mg/kg BW and used as positive control for anti-depression activity evaluation. In addition to both drugs, donepezil hydrochloride was administered at doses of 1 mg/kg BW and used as positive control for the determination of cognitive function in this study.

Piperine was obtained from Sigma Chemical Co., MO.

Abbreviations: mg, milligram; kg, kilogram; BW, body weight; cm, centimeter; C, celsius; NE, Northeast; NW, Northwest; SE, Southeast; SW, Southwest; N, North; E, East; W, West; S, South.

* Corresponding author. Tel.: +66 43 348394; fax: +66 43 343715.
E-mail address: jintanapornw@yahoo.com (J. Wattanathorn).

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2.3. Experimental protocol

In all part of this study, rats were divided into 6 groups of 8 each as described following except in the determination of stereotype behaviors.

Group 1: Naïve intact control.
Group 2: Vehicle treated group. Rats were treated with propylene glycol which served as vehicle in this study.
Group 3: Positive control-treated group. Rats were treated with various drugs including diazepam, fluoxetine and donepezil hydrochloride as positive control in the determination of anxiolytic, anti-depressant and cognitive function respectively.
Group 4–Group 6: Piperine 5, Piperine 10, Piperine 20 treated groups. Rats in these groups were orally treated with piperine at various doses ranging from 5, 10 and 20 mg/kg BW once daily for 4 weeks respectively. The doses used in this study were selected based on previous study about the effect of piperine on the central nervous system (Li et al., 2007; D'Hooge et al., 1996). It was found that piperine at dosage range between 2.5 and 86 mg/kg BW produced the effect on the central nervous system such as anti-stress and anti-convulsion.

The animals in all groups were assessed all behavioral tasks except that in the assessment of spontaneous locomotor behaviors, there was no positive control-treated group.

2.4. Behaviors evaluation

The rats were divided into various groups as mentioned earlier. The behavioral profiles were assessed both after the single dose and repetitive administration of the substance as following: 1, 2, 3 and 4 weeks of treatment. All animals were submitted to the following behavior tasks (a) elevated plus maze (b) spontaneous locomotor behavior (c) forced swimming test (d) cognitive function. Diazepam (2 mg/kg BW), fluoxetine (10 mg/kg BW) and donepezil hydrochloride (1 mg/kg BW) were used as reference drugs for administration to rats belonging to positive control group for the evaluation of anxiolytic, depression and cognitive enhancing activities, respectively.

2.4.1. Elevated plus maze test

The elevated plus maze for rat consisted of open arms (50 × 10 cm) and two enclosed arms (50 × 10 cm) with 40 cm high walls, extending from a central platform (10 × 10 cm). The arms were connected with a central square, 10 × 10 cm, to give the apparatus a plus sign appearance. The maze was raised to a height of 50 cm above floor. The maze floor and walls were constructed from dark opaque wood. Each rat was placed on the center of the platform facing an enclosed arm. Animals were tested individually and only once for 5 min according to the following parameters: number of entries in the open and closed arms, and time of performance in each of them (Lister, 1987). The time of performance measures the time spent by the animal in the opened and closed arms. The maze was cleaned following each trial to remove any residue or odors. Each rat was assessed individually 30 min after the treatment.

2.4.2. Forced swimming test

In order to assess the anti-depressant activity of plant extract, the modified Porsolt test (Porsolt et al., 1978) was conducted. In the first trial, the rats not yet treated were forced to swim in a glass aquarium (22 cm in diameter, 40 cm in height) containing 20 cm high fresh water at 25 °C for 15 min. Then, the assessments were performed after single, 1, 2, 3 and 4 weeks of treatment. During the test session, the immobility time, swimming and climbing times were recorded by blind observer who has been trained for the observation. The rats were considered immobile when neither hind leg was moving, the rats were slightly hunched forward. The total duration of immobility was measured during the 5-minutes test. Upon removal from the water, rats were towel-dried and finally returned to their home cage.

2.4.3. Spontaneous motor behavior

In order to assure that the anxiolytic activity, anti-depression like activity and cognitive enhancing effect which determined by various tests just mentioned earlier were not false positive due to the effect of piperine on motor behavior, we also determined the effect of piperine on the spontaneous locomotor activity. All animals in group 1–2 and group 4–6 were assessed spontaneous motor behaviors including grooming and rearing behaviors for 5 min. The performance was determined 60 min after the substances administration.

2.4.4. Morris water maze test

The water maze consisted of a metal pool (170 cm in diameter × 58 cm tall) filled with tap water (25° C, 40 cm deep) divided into four quadrants. In the center of one quadrant was a removable escape platform below the water level and covered with a nontoxic milk powder. The pool was divided into four quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the center of the pool. For each animal, the location of invisible platform was placed at the center of one quadrant and remained there throughout training. The rats must memorize the platform location in relation to various environmental cues because there was nothing directly shows the location of the escape platform in and outside the pool. Therefore, the placement of the water tank and platform were the same in all acquisition trials. Each rat was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim until it found and climbed onto the platform. During training session, the subject was gently placed on the platform by the experimenter when it could not reach the platform in 60 s. In either case, the subject was left on the platform for 15 s and removed from the pool. The time for animals to climb on the hidden platform was recorded as escape latency. In addition to the acquisition test, the determination of retention memory was carried out on the next day. According to this test, the platform was removed and the animals were placed into the water maze for 60 s. The retention of memory or the time that the animal spent to swim around the previous location of platform before removing the platform on the test occurring in the next day was also recorded. It has been postulated that if the spatial memory of the rat for the trained platform location is accurate, the rat will swim to the platform location and search around the exact location. Therefore, the more accurate the spatial memory is, the greater the number of times rat swim across the trained platform. In each trial, the animal was quickly dried with towel before being returned to the cage. All test in Morris water maze tests were carried out within 30 min after the oral administration of the substances.

2.5. Statistical analysis

Data are presented as mean ± standard error of mean (± SEM). One-way analysis of variance (ANOVA), followed by Tukey post hoc test. A probability level less than 0.05 was accepted as significance.

3. Results

3.1. Anti-depression activity

The results obtained from this study (Figs. 1–3) showed that vehicle treatment alone did not produce significant changes in immobility, climbing and swimming times at all treatment durations used in this study. Fluoxetine, a serotonin uptake inhibitor, significantly decreased immobility time ($p < .001$ all) but increased swimming time at all duration of treatment ($p < .001$ all). The significant changes in climbing time induced by fluoxetine were observed after single administration and 4 weeks of treatment ($p < .001$ all). Piperine at all dosage range used in this study produced significant changes in both immobility and swimming times at all treatment duration in the same pattern as those occurred after fluoxetine treatment ($p < .001$ all). No significant changes in climbing time were observed at all dosage range and treatment duration used in this study. Our data failed to show both a dose-dependent effect and a treatment duration-dependent of piperine.

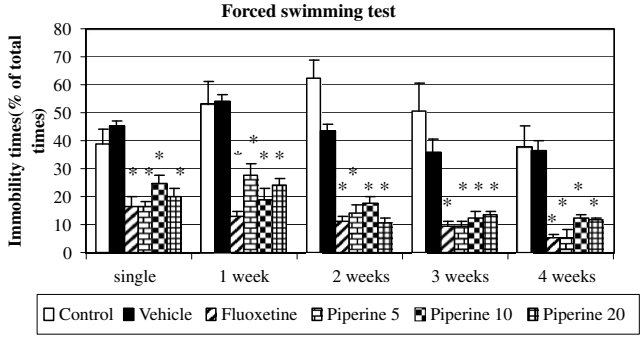

Fig. 1. Immobility time of rats orally given vehicle or fluoxetine or piperine at various doses ranging from 5, 10 and 20 mg/kg BW for 4 weeks in forced swimming test. ($n = 8$ animals per group). Data are represent as mean ± SEM. Comparisons were made by using a one-way ANOVA $^{*}p < .001$ compared with vehicle group.