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Neurotoxicity of *Coscinium fenestratum* stem, a medicinal plant used in traditional medicine

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Abstract

Coscinium fenestratum is a common medicinal plant widely used in the Indochina region, but scientific data on its safety is very limited. This study aimed to observe the effect of this plant on neurotoxicity and neurobehavior. Oral administration of plant alcoholic extract at dosages of 5, 10 and 20 mg/kg BW for 14 days increased the rats body weight and decreased the neuron density in the cerebral cortex, hippocampus and striatum. The plant extract significantly increased stereotyped behavior in licking but did not cause anxiolytic activity, anti-depression, sensory motor co-ordination impairment and ataxia. It is concluded that the plant possesses neurotoxicity and is able to induce neurobehavioral changes in rats. Therefore, the application of this plant as either drug or supplementary food should be reconsidered.

Keywords: *Coscinium fenestratum*; Neurotoxicity; Neurobehavior

1. Introduction

Coscinium fenestratum (Gaertn) Colebr, commonly known as "Ham" or "Ka-min-kreu", is a common medicinal plant in the family of Menispermaceae and is widely used in the Indochina region. In Thailand, this plant is distributed mainly in the northeast of Thailand especially in Nong Khai and Nakorn Panom provinces. Decoction of the stem of *C. fenestratum* has been used in Thai traditional medicine for rural people in the northeast of Thailand for a long time. Its stem has been claimed to possess hypoglycaemic hypotensive laxative and anti-diabetic activities. In addition, the product of this plant in Sri Lanka is also claimed to be a therapeutic agent for various conditions including opthalmopathy, inflammation, ulcers, skin disease, abdominal disorders, jaundice, fever and general debility.

Previous studies have reported that the alcoholic extract of this plant possesses anti-oxidant activity, It could protect against hepatotoxicity induced by carbon tetrachloride (Venukumar and Latha, 2002). The extract also exhibited strong anti-feeding (Javasinghe et al., 2003) and hypotensive activities (Singh et al., 1990). In addition, it has been reported that the main components in the stem of *C. fenestratum* are protoberberine alkaloids (Pinho et al., 1992). Berberine and its derivative have been reported to exert profound influences on the nervous system including the

Abbreviations: BW, body weight; *C. fenestratum*, *Coscinium fenestratum*; h, hour; rpm, round per minute; s, second; vol, volume; DZP, diazepam; sec, second.

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anti-amnesic effect against memory defect induced by scopolamine (Peng et al., 1997).

Recently, this plant has been used as one of the active ingredients in functional food and claimed to contain various health promotion effects. Despite long term use, however, we have little scientific data to support the safety of this plant. Therefore, it is important that medicinal plant which have folklore reputation for medicinal effects should be investigated in order to establish their safety and efficacy.

Based on the unpublished data concerning the same comment of consumers about headache and dizziness after consumption in some cases, the present study was carried out to determine the effect of *C. fenestratum* on the neurobehavioral changes and neurotoxicity in various brain areas of rats.

2. Methods

2.1. Plant materials

The stem of *C. fenestratum* was purchased from Nakorn Panom province during the month of August, 2001. The tree specimen was identified and a voucher specimen from this plant was deposited at the Center of Research and Development of Herbal Health Products, Khon Kaen University under the number HHP-2-462.

2.2. Preparation of the extract

Stems of *C. fenestratum* were washed, dried at room temperature and minced into small pieces. Plant materials are then extracted with 50% ethanol by reflux method. Then the extract was centrifuged at 2500 rpm for 10 min to remove residual debris. The clear supernatant was evaporated under reduced pressure and dried by lyophilizer. The percent yield of extract was 8.53%. The extract was stored at −20 °C in a dark bottle until used.

2.3. Animals

Adult male Sprague–Dawley rats (180–200 g, 8 weeks old) were obtained from National Animal Center, Salaya, Nakorn Pathom, and were housed in groups of five 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 after the approval of the protocol by the Ethical Committee of the Institution, and every effort was made to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of the European Community (EEC directive of 1986; 86/609/EEC).

2.4. Drugs

Diazepam (1 mg/tablet), and fluoxetine (20 mg/tablet) (Government Pharmaceutical Organization) were used as standard drugs in this study. They were dissolved in normal and administered via oral route.

2.5. Experimental protocol

All rats were randomly divided into four groups. Each group contained eight rats. The first group was treated with normal saline which used as vehicle. The second to fourth groups were treated with the extract of *C. fenestratum* at doses of 5, 10 and 20 mg/kg BW. respectively via oral route once daily. The doses used in this study are based on the recommendation of traditional herbalists in northeast region of Thailand. In the determination of anxiolytic and anti-depression activities, the animals were divided into five groups. The first to fourth groups were treated as mentioned above and the fifth group was treated with diazepam in the determination of anxiolytic activity whereas fluoxetine was treated in the determination of anti-depression.

2.5.1. Determination of the neurobehavioral effects

The rats were divided into various groups as mentioned earlier. The behavioral profiles were assessed on day 14th after the last dose of treatment. All animals were submitted to the following behavior tasks: (a) elevated plus maze; (b) rotarod test; (c) stereotyped behavior; (d) forced swimming test; (e) walking pattern and ataxia. Diazepam (2 mg/kg BW) and fluoxetine were used as reference drugs for administration to rats belonging to positive control group for the evaluation of anxiolytic and depression activities, respectively.

2.5.1.1. 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. 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 last dose of treatment.

2.5.1.2. Forced swimming test. In order to assess the anti-depressant activity of plant extract, the modified Porsolt test (Porsolt et al., 1977) 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. The second exposure, anti-depressant activity of repetitive doses of extract was assessed after 14 days of treatment within 75 min after the last dose of administration. During the test session, the immobility time was 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-min test. Upon removal from the water, rats were towel-dried and finally returned to their home cage.

2.5.1.3. Rotarod motor co-ordination test. Since the elevated plus maze test was affected by motor defect, the motor co-ordination test was also conducted in order to rule out the motor defect. Motor co-ordination was assessed within 300 s (s) using rotarod test. A rotarod apparatus was used to evaluate motor co-ordination according to the method of Dunham and Miya (1957). Briefly, The animals were placed on a rotating cylinder, 10 cm in diameter covered with texture rubber, suspended 15 cm above an automated stop/start platform. Each rat was subjected to an initial habituation trial at the speed of four rotations per minute (rpm), followed by three test trials at accelerating speed, for three consecutive days. Each trial started at 4 rpm and the speed was accelerated continuously until the animal fell down or up to a maximum of 40 rpm in 300 s. The latency for the animals to fall down onto the platform and the corresponding speed were recorded. When the duration of riding exceeded 300 s, the rat was removed from the rod.

2.5.1.4. Stereotyped behavior. The test was performed in group of eight rat each. The first group received saline as vehicle while the animals in the second to fourth groups received ethanolic extract of *C. fenestratum* at doses of 5, 10 and 20 mg/kg BW for 14 days. Forty five minutes after the last dose of treatment, all animals were observed stereotyped behavior including grooming, rearing and licking for 5 min.

2.5.1.5. Walking pattern and ataxia. After 14 days of treatment, rats were placed in the center of a horizontal 1 m length wooden rod. Ataxia and gait analysis were determined within 90 min after treatment via the ability