Evaluation of the Anxiolytic and Antidepressant Effects of Alcoholic Extract of *Kaempferia parviflora* in Aged Rats

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**Abstract:** To date, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has significantly progressed. The present study was performed to evaluate the anxiolytic and antidepressant like activities of the *K.parviflora* rhizome extract. Aged male Wistar rats were orally administered the alcoholic extract of this plant at various doses ranging from 100, 200 and 300 mg kg⁻¹ BW once daily for 7 days. The anxiolytic and antidepressant activities were performed after both single and repetitive treatment for 7 days using elevated plus maze and forced swimming tests respectively. The results showed that the extract decreased immobility time with the increase swimming time. However, no changes in number of open arm entries and time spent in open arm were observed. These results suggested the anti-depression activity of the plant extract. Therefore, *K.parviflora* may be served as a potential resource for natural psychotherapeutic agent against depression. However, further studies were still required.

**Key words:** *Kaempferia parviflora*, anxiolytic, antidepressant, elevated plus maze, forced swimming test

**INTRODUCTION**

At present, anxiety and depression are the most frequent psychiatric conditions commonly found. A number of the population suffer from these conditions at some time during their life. 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 should be considered as alternative/complementary medicines. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly[1]. This has been reflected in the large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models.  

*Kaempferia parviflora* Wall. or Krachai Dam, a plant in a family of Zingiberaceae, is very popular for health promotion in Thailand. Rhizomes of *K. parviflora* have been used as traditional medicine for various medicinal purposes including a tonic for rectifying male impotence, body pains and gastrointestinal disorders among local people in the Northeast of Thailand[2]. At present, a tonic drink made from the rhizomes of *K. parviflora* is commercially available and is believed to relieve impotent symptoms and promote longevity. Despite widely used as health promotion drink, the scientific evidence to support its beneficial effect is very limited. Recently, Yenji and coworkers have reported that this plant contained high amount of flavonoids[2]. These substances have been reported to a neuroprotector against various brain pathological conditions and served as a valuable resource for treating neuropsychological diseases[3]. Therefore, this raises the possibility that *K.parviflora* should possess some neuropsychological diseases. However, there is no scientific evidence about potential effects of this plant against neuropsychological diseases. The present study is carried out to determine the effect of the plant extract against these disorders particularly against anxiety and depression, the most psychological diseases commonly found.

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MATERIAL AND METHODS

Plant material and the preparation of the extract: *Kaempferia parviflora* rhizomes were collected from Amphoe Na Haeo, Loei, Thailand, authenticated and prepared as alcoholic extract by Associate Professor Bungorn Sripanidkulchai, Director of Center for Research and Development of Herbal Health Product, Khon Kaen University, Thailand. The voucher specimen was deposited at Center for Research and Development of Herbal Health Product. The dried plant rhizome powder was macerated in 95% ethanol for 4 days (1 kg/ 2 liters) with occasionally stirring. After filtration, the residual was further repeat macerated with 95% ethanol, then the filtrate were combined and dried by freeze dryer. The percent yield of the final product was 4.187. Suspensions of alcoholic extract of *K. parviflora* were prepared in 2% carboxymethylcellulose as the suspension agent in order to deliver through oral route (gavage).

Animals: Aged male Wistar rats (350-450 gm, 16 weeks old)) were obtained from National Animal Center, Salaya, Nakorn 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.

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 saline and administered via oral route.

Experimental protocol: All rats were randomly divided into 5 groups. Each group contained 8 rats. The first group was control naïve intact rats, the second group was treated with 2% carboxymethyl cellulose which used as vehicle. The third to fifth groups were treated with the extract of *K. parviflora* at doses of 100, 200 and 300 mg kg⁻¹ BW, respectively via oral route for 7 days once daily. The doses used in this study are based on the preliminary study of our group. In the determination of anxiolytic and anti-depression activities, the animals were divided into 6 groups. The first to fifth groups were treated as mentioned above and the sixth group was treated with diazepam in the determination of anxiolytic activity whereas fluoxetine was treated in the determination of anti-depression.

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 (7 days). All animals were submitted to the following behavior tasks a) elevated plus maze b) spontaneous motor behavior c) forced swimming test. Diazepam (2 mg kg⁻¹ 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.

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 treatment.

Forced swimming test: In order to assess the antidepressant activity of plant extract, the modified Porsolt test[4] was conducted. In the first trial, the rats which has 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. In the next exposure, antidepressant activity of repetitive doses of extract was assessed after 7 days of treatment within 75 min after the last dose of administration. 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-min test. Upon removal from the water, rats were towel-dried and finally returned to their home cage.
Spontaneous motor behavior: The test was performed in group of 8 rats each. The first group was control or naïve intact rat, the second group was received 2% carboxymethylcellulose as vehicle while the animals in the third to fifth groups received the extract of *K. parviflora* at doses of 100, 200 and 30 mg kg\(^{-1}\) BW for various periods ranging from 1 and 7 days. Thirty min after the treatment, all animals were observed stereotyped behaviors including grooming and rearing for 5 min.

Statistical analysis: Data are presented as mean ± standard error of mean (S.E.M). One-way analysis of variance (ANOVA), followed by Newman-Keuls post hoc test. A probability level less than 0.05 was accepted as significance.

RESULTS

Anxiolytic activity: The present results demonstrated that diazepam at dose of 2 mg kg\(^{-1}\) BW significantly increased both number of the opened arm entries and time spent in the opened arm after the single and repetitive (7 days) administration of drugs as shown in Fig. 1 and 2. No significant changes both in number of opened arm entries and time spent in opened arm were observed after the single and repetitive administration of *K. parviflora* extract at dosage ranged used in this study.

Antidepresant-like activity: Both single and repetitive administrations of vehicle did not produce significant changes in the immobility time in forced swimming test. Single administration of fluoxetine significantly decreased the immobility time (p-value <.05) as shown in Fig. 3 whereas the repetitive treatment produced no significant changes as shown in Fig. 4. The results also showed that in accompanied with the decrease in immobility time, fluoxetine also increased swimming time without significant change in climbing time. Single administration of *K. parviflora* at all dosage rage used in this study failed to show significant changes on percentage of changes of immobility, swimming and climbing time as shown in Fig. 3. The plant extract at dose of 100 mg kg\(^{-1}\) BW markedly decreased the immobility time and increased swimming time but produced no change in climbing time after daily treatment for 7 days. However, the low dose of the plant extract significantly decreased immobility time whereas increased swimming time after 7 days of treatment. No significant changes in all parameters
DISCUSSION

The present study investigates the putative psychotherapeutic effects of *Kaempferia parviflora* as anxiolytic and anti-depressant. Our results show that *K. parviflora* extract can decrease immobility time in forced swimming test in aged rats with no sedative effect. It is found that *K. parviflora* can produce antidepressant-like activity at dose of 100 mg kg\(^{-1}\) BW after 7 days of treatment whereas no significant changes are observed at all other doses used in this study. The decrease in the immobility time is accompanied with the increase in swimming time. Previous studies demonstrated that

were observed after repetitive treatment at doses of 200 and 300 mg kg\(^{-1}\) as shown in Fig. 4

**Spontaneous motor activity:** Figure 6, 7 and 8 showed that the plant extract at dosage range used in our study did not produce the significant changes in the grooming, licking and rearing behaviors both after the single and repetitive administration.
many neurotransmitters were involved in the pathophysiology of depression. Numerous studies have demonstrated that antidepressant drugs, such as fluoxetine, facilitated the action of serotonin. This drug is widely used as antidepressant drugs and agreed with studies in animal models[5], such as forced swimming test. It was also demonstrated that swimming behavior was sensitive to serotonergic compounds, such as the selective serotonin reuptake inhibitor fluoxetine, whereas, climbing behavior was sensitive to drugs with selective effects on noradrenergic transmission[6,7,8]. Based on these findings, it can be supposed that the extract which is able to decrease the immobility time and increases swimming behavior in the animals exposed to these paradigms can exert its effect through a mechanism similar to that of the fluoxetines via the serotonin system. However, the precise mechanism underlying this change has still required further investigations.

Since, the decrease in immobility was also under the influence of motor behavior and sedative effects, we also determined the effect of the plant extract on the behaviors just mentioned. The present results showed that the extract did not produce significant changes of these parameters. Thus, the decrease immobility time observed in this study seemed to be the real antidepressant like activity of the extract.

The effect of extract was observed only at low dose (100 mg kg⁻¹ BW). The increasing doses did not produce significant changes. The possible explanation for this change might partly due to the difference in concentration of various constituents in the crude extract. It is possible that each chemical constituents of medicinal plants exhibits the biological activity influencing on the neurobehaviors involving depression activity in different aspects and the increasing some constituents can masking the effect of active constituent which shows anti-depressant effect.

In conclusion, our study is the first study to demonstrate the anti-depressant like activity of K. parviflora rhizome in rats. However, further studies are necessary to confirm and extend these results before the application in human.

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