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Aphrodisiac Activity of *Kaempferia parviflora*

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Abstract: Problem statement: The increasing prevalence of sexual dysfunction and the limitation of therapeutic efficacy nowadays give rise to the requirement of novel therapeutic strategy. *Kaempferia parviflora* or Krachai-Dun has been long term used in Thai traditional folklore to treat this condition. Unfortunately, no scientific document is available until now. Therefore, this study was undertaken to determine the effect of this medicinal plant on male sexual behavior of aging rats subjected to stress. **Approach:** Aging rats were divided into 2 groups; the vehicle+stress and the *K.parviflora*+stress. All animals were administered the assigned substance 45 m before they were subjected to the 12-hr stress exposure for 3 weeks. They were determined male sexual behaviors including both latency and frequency of mounting, intromission and ejaculation behaviors after single dose and every week until the end of experimental period. In order to investigate the possible underlying mechanism, we also determined the alteration of DA1-immunopositive stained neurons density in hypothalamus. **Results:** Our results showed that after the single administration of the plant extract, the intromission frequency increased, it was also found the rats subjected *K.parviflora* treatment significantly increased the frequency of mounting, intromission and ejaculation while decreased the latencies of all sexual behaviors mentioned earlier. Moreover, *K.parviflora* also increased D1-immunopositive stained neurons density in hypothalamus. Therefore, the aphrodisiac activity of *K.parviflora* might be attributed in part to the enhanced dopaminergic function in hypothalamus. **Conclusion:** Present findings provide experimental evidence that the crude extract of *K.parviflora* can enhance male sexual behaviors. Therefore, it will be further developed as the functional food or health product for men especially for men who are risk for sexual dysfunction.

Key words: *Kaempferia parviflora*, aphrodisiac activity, sexual dysfunction, traditional medicine, vehicle plus stress, European Community, Sodium Carboxy MethylCellulose (SCMC), parameters, room temperature

INTRODUCTION

Aging and stress exposure are the inevitable phenomena. A large body of evidence has demonstrated that male sexual behaviors gradually decline with age (Smith *et al*., 1992, Phanijo, 2000, Bretschneider and McCoy, 2011). Numerous factors including both physical and mental factors are regarded as the important etiology for sexual dysfunction in elderly. Stress is regarded as one important factor to induce sexual dysfunction. It was reported that chronic exposure to a variety of mild stressors significantly decreased male sexual behavior (Brotto *et al*., 1998; D'Aquila *et al*., 1994; Retana-Marquez *et al*., 1996; Sato *et al*., 1992; Miwa *et al*., 2011; Hawley *et al*., 2011). It had been estimated that more than 152 million men worldwide subjected to sexual dysfunction and this number might increase to approximately 322 million by the year 2025 (Aytac *et al*., 1999; Hawton, 1985; NIH, 1993). This condition produces great impacts not only on the patient but also on their marital life. Despite its increasing prevalence and high impact on quality of life, the therapeutic efficacy is still limited. Therefore, traditional medicine still plays a significant role in the lives of many people.

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Aphrodisiac substances or foods have been long term used for treating sexual dysfunction and enhancing the sex lives in traditional folklore. In Thai traditional folklore, numerous plants have been claimed for their aphrodisiac activities including Krachai-Dum or *Kaempferia parviflora.*

Kaempferia parviflora Wall.Ex Baker, a plant in a family of Zingiberaceae, is very popular for health promoting, stimulating and vitalizing 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 (Yenjai *et al*., 2004). Recently, we have found that *K. parviflora* also processes the anti-depressant activity (Hawiset *et al*., 2011). However the advocated sexual stimulant activities of the *K.parviflora* are not scientifically tested and validated, this study was undertaken to investigate the effects of *K.parviflora* on the male sexual behavior of aging rats exposed to stress.

MATERIALS AND METHODS

Plant material and extraction: *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 percent yield of 95% alcohol was 4.187% of dried rhizome. 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: Healthy aged male Wistar rats (350-600 gm, 18 weeks old) were obtained from National Animal Center, Salaya, Nakorn Pathom and were housed in group of 6 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 labium. 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.

Stress procedure: The restraint stress was performed during the night cycle from 6.00 p.m. to 6.00 a.m. The restrainer was made of transparent perforated plastic tube, 20 cm long and 7 cm in diameter. The rats were put into the restrainer, head first and once in, the tubes were closed with plexiglass lids. The animals fit tightly into the restrainers and it was not possible for them to turn around. None stressed control rats were at the same time briefly handled and returned to their home cages.

Evaluation of sexual behaviors: Male aged Wistar rats of proven fertility were randomly divided into 2 groups of 6 animals each as following; (1) Vehicle plus stress (2) *K.parviflora* plus stress. Rats in group 1 were administered with 2% Sodium Carboxy MethylCellulose (SCMC) which used as vehicle for the plant extract then expose to the 12 h- restraint stress whereas rats in group 2 were administered with the plant extract at dose of 200 mg kg^{-1} BW (the optimum dose which produced significant changes in the central nervous system) plus stress exposure as mentioned earlier.

Since the selected dose produced the optimum effect on the brain function, therefore, we hypothesized that this dose might produce the positive modulation effect on the hypothalamus, the area contributing important role on the regulation of sexual behaviors. All substances treatments were administered 45 m prior to the 12 h-restraint stress exposure. The treatment and stress exposure were performed once daily and the sexual behaviors assessments were performed blindly. The animals were allowed to rest in order to refresh the animals 3 h after the removal from restraint cage and then they were assessed the sexual behaviors between 7.00-9.00 p.m. at room temperature 26-28°C after single dose,1 and 2 weeks of treatment.

In order to assess the sexual behaviors, estrous female rats were paired with male treated with single or repeated doses of extract. Female rats were induced to estrous by sequential administration of estradiol benzoate (Sigma, St. Louis, MO) at dose of 2 µg kg^{-1} BW and progesterone (Sigma, St.Louis, MO) at dose of 500 µg kg^{-1} BW were injected before the determination of copulatory behaviors via subcutaneous route 48 h and 6 h respectively.

Sexual behaviors were monitored in a separate room for 2h in a clear plastic box via blind observer 30 min at the start of first hour whereas the whole duration of observation (2 h) was recorded by digital video recording. The assessed sexual parameters were including the following parameters:

- Mounting number: The number of mounts without intromission from the time of introduction of the female until ejaculation

- Intromission number: The number of intromissions from the time of introduction of the female until ejaculation
- Mount latency: The time interval between the introductions of the female to the first mount by the male
- Intromission latency: The interval from the time of introduction of the female to the first intromission by the male
- Ejaculation number: The number of ejaculation which characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity
- Ejaculation latency: The time interval between the first intromission and ejaculation

Tissue reparation: Following anesthesia with sodium pentobarbital (60 mg kg^{-1} BW), fixation of the brain was carried out by transcardial perfusion with fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3. The brains were removed after perfusion and stored over a night in a fixative solution that used for perfusion. Then, they were infiltrated with 30% sucrose solution for approximately 4°C. The specimens were frozen rapidly and 30 µM thick sections were cut on cryostat. They were rinsed in the phosphate buffer and picked up on slides coated with 0.01 % of aqueous solution of a high molecular weight poly L-lysine.

Immunohistochemical Study of Dopamine Receptor (Type I): A series of brain slices containing hypothalamus from various groups (Control, Vehicle plus stress and *K.parviflora* plus stress) were reacted in parallel experiments using a mouse monoclonal antibody directed against dopamine receptor (type I) (Sigma, USA) and a modification of a previously described protocol employing the DAKO Strept ABC Complex/HRP duet kit. In brief, the brain slices were eliminated endogenous peroxidase activity by 0.5% H_2O_2 in methanol. brain slices were washed in running tap water and distilled water for 1 min each, then rinsed in KPBS and KPBS-BT for 5 min per each process. Excess buffer was removed and then incubated for 30 min in a block solution composed of 5% normal goat serum in KPBS-BT. The brain slices were then incubated in primary antibody against D1 (dopamine receptor type I) diluted 1:100 in KPBS BT (two washes ×7 min), incubated for 1 h in biotinylated goat anti-mouse IgG antibody, rinsed in KPBS-BT (two washes ×7 min) and then incubated in Strept ABC Complex/HRP for 4 h. In preparation for visualization step, brain slices were rinsed in KPBS-BT (1 min) and KPBS (twowashes ×10 min). D1 immunoreactivity was visualized using 0.025% 3, 3' diaminobenzidine (DAB, Sigma) and 0.01%H_2O_2 for 48 h. Finally, brain slices were rinsed in running tap water, air dried and cover slipped using permount.

Morphological analysis: Five coronal sections of each rat in each group were studied quantitatively. D1 immunoreactived neurons in hypothalamus were counted by eye using a×40 objective with final field 255 μm^2 according to the following stereotaxic coordinates: AP -0.4 mm, lateral±0-2 mm, depth 8-9 mm. The observer was blind to the treatment at the time of analysis. Counts were made in five adjacent fields and the mean number extrapolated to give total number of neurons per 255 μm^2. All data are represented as number of neurons per 255 μm^2.

Statistic analysis: All data were expressed as mean ± SEM value. The significant differences among various groups were compared by ANOVA and followed by Duncan's test. The statistical difference was regarded a p-value < 0.05.

RESULTS

Determination of the sexual enhancing effect of *K. parviflora* in aging rats with stress exposure: The results obtained from this study showed that the alcoholic extract of *K. parviflora* could decrease mount, intromission and ejaculation latencies when compared to vehicle plus stress group after 2 weeks of treatment (p-value <0.05) as shown in Fig. 1, 3, 5 respectively.

In addition, the plant extract also increased mounting, intromission and ejaculation numbers after single administration and after 2 weeks of treatment (p-value <0.05) as shown in Fig. 2, 4 and 6. It was also demonstrated that the significant change of ejaculation number was observed only after 2weeks of treatment, the increasing duration of treatment further to 3 weeks did not produce significant changes.

Determination of D1 immunoreactived cell density in hypothalamus: Since D1-receptor had been proposed to play a crucial role in regulating sexual behaviors, the present study was carried out to determine the effect of *K. parviflora* on the density of D1-immunoreactived neurons in hypothalamus. The results were shown in Fig. 7. It was found that the plant extract treated rats significantly increased the density of D1-immunoreactived neurons in hypothalamus.

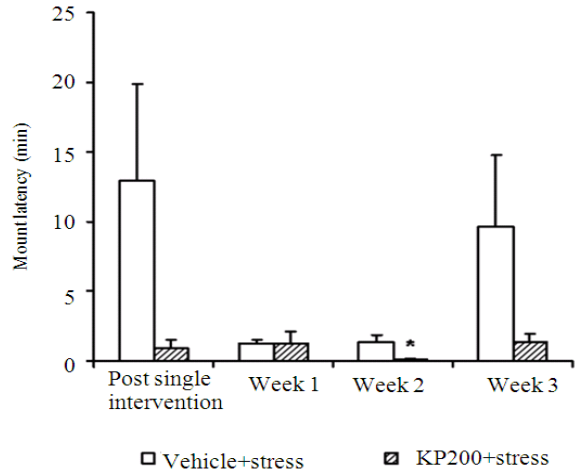

Fig. 1: The effect of *K. parviflora* (KP) extract on mount latency. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization stress exposure for 3 weeks (n = 6). Mount latencies were recorded in different group of rats. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

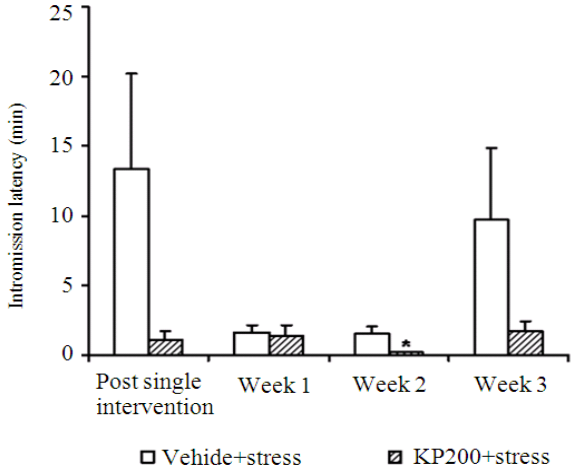

Fig. 3: The effect of oral administration of *K. parviflora* (KP) extract on intromission latency. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization stress exposure for 3 weeks (n = 6). Intromission latencies were recorded in different group of rats. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

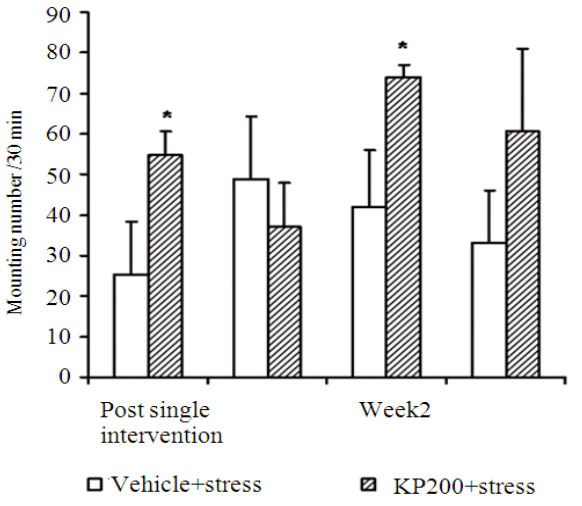

Fig. 2: The effect of *K. parviflora* (KP) extract on mounting number. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization stress exposure for 3 weeks (n = 6). Mounting numbers were recorded in different group of rats. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

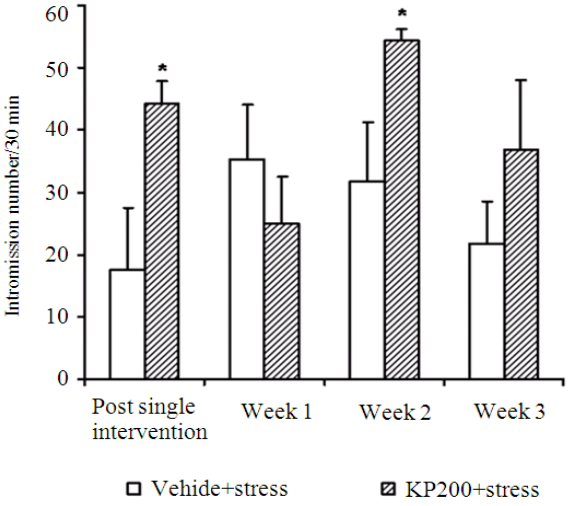

Fig. 4: The effect of oral administration of *K. parviflora* (KP) extract on intromission number. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization stress exposure for 3 weeks (n = 6). Intromission numbers were recorded in different group of rats. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

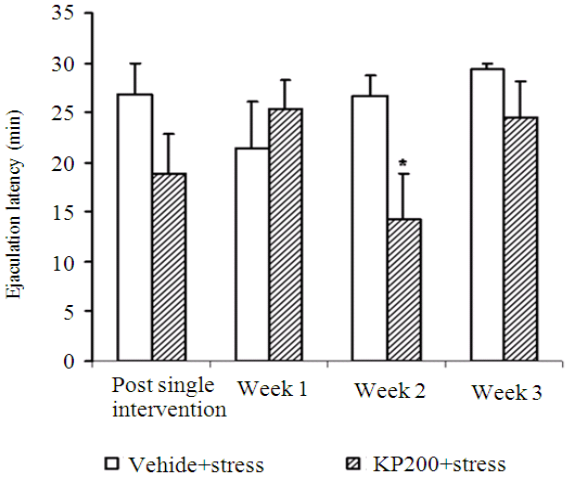

Fig. 5: The effect of oral administration of *K. parviflora* (KP) extract on ejaculation latency. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization tress exposure for 3 weeks (n = 6). Ejaculation latencies were recorded in different group of rats. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

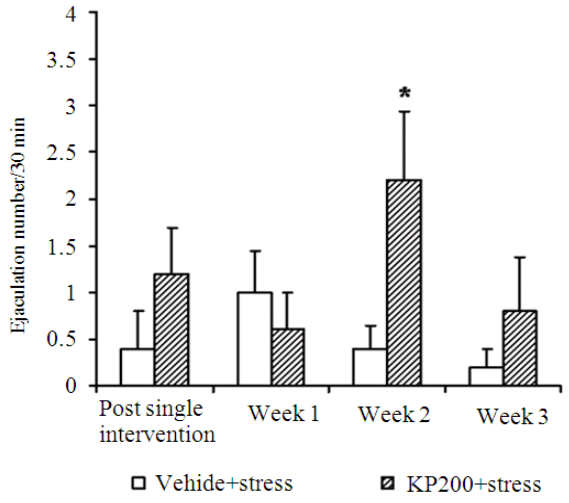

Fig. 6: The effect of oral administration of *K. parviflora* (KP) extract on ejaculation number. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization stress exposure for 3 weeks (n = 6). Ejaculation numbers were recorded in different group of rats. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

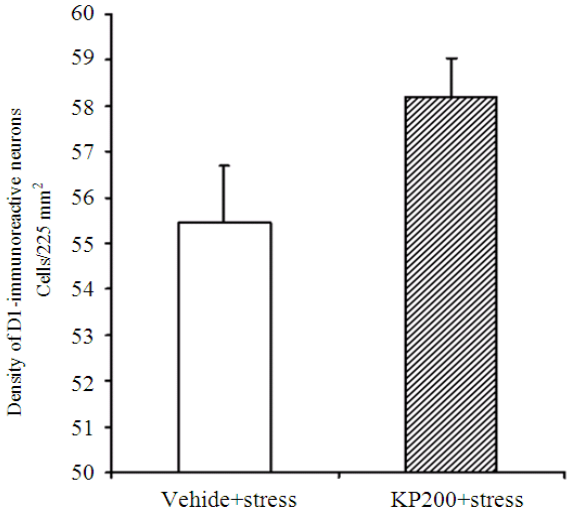

Fig. 7: The effect of *K. parviflora* extract on the alteration of D1immunoreactived neurons in hypothalamus. Rats were received the alcoholic extract of *K. parviflora* at dose of 200 mg kg^{-1} BW via the intragastric route 45 min prior to the 12 h of immobilization stress exposure for 3 weeks. After the last dose of administration, they were sacrificed and the brains were cut as coronal sections at 30 µm thick. The sections were stained with monoclonal antibody against D1receptor. The D1-immunoreactived neurons were determined under light microscope at 40X magnification. Data were expressed as mean ± S.E.M. *p-value<0.05; compared to vehicle+stress

DISCUSSION

The current study provides evidence that the crude extract of *K.parviflora*, a Thai medicinal plant reputed for aphrodisiac activity, enhances the expression of male sexual behaviors in sexually active rats. It was found that the oral administration of *K.parviflora* extract effectively facilitated several aspects of copulatory behavior.

In this study, the aphrodisiac activity or prosexual effect of the extract was observed after *K. parviflora* treatment at dose of 200 mg kg^{-1} BW for 2 weeks. In addition to arousal effect, the extract also increased sexual performance.

It has been reported that besides hormonal control system, a neural control system is also recognized as one key factor to regulate sexual behavior. Stimulation and inhibition of dopamine receptors were reported to enhance and impair sexual behavior respectively (Dominguez and Hull, 2005). Previous study

demonstrated that the dopamine activity increased in several sex-relevant brain regions before and/or during copulation (Hull *et al*., 2004). It was proposed that dopamine acted by removing the tonic inhibition in brain regions that were important for male sexual behaviors, thereby, enhancing sensorimotor integration, in the presence of a sexually eliciting stimulus or during copulation. A common feature of dopaminergic action in nigrostriatal, mesolimbic and hypothalamic system including the action via Medial Preoptic Area (MPOA) was the enhancement of sensorimotor integration by removing GABAergic tonic inhibition resulting in the facilitation of male sexual behaviors (Britan and Hull, 1987; Chevalier and Deniau, 1990; Hull *et al*., 2004; Melis and Argiolas, 1995; O'Donnel *et al*., 1999; Furth *et al*., 1995).

Dopamine release was elicited in each of the three integrative systems as a result of sensory cues from a sexually exciting stimulus and/or the act of copulation. Briefly, increased dopaminergic activity in the nigrostriatal system enhanced the readiness of motor system to respond to sexual stimuli whereas the increase dopaminergic activity in the mesolimbic system was important for the motivation and reinforcement and the increase dopaminergic activity in the medial preoptic area was important for genital reflex, motor pattern of copulation and possibly sexual motivation (Dominguez and Hull, 2005).

It was found that dopamine should interact with dopaminergic receptors various types resulting in the opposite postsynaptic effects. Previous studies demonstrated that D1 and D2 receptors in MPOA had different activation thresholds and exerted different effects on the autonomic control of genital reflexes. The low threshold D2 in this area was demonstrated to play important role in the disinhibition of genital reflexes without directly stimulating them whereas the high threshold D2 was shown to involve the sympathetically mediated ejaculations. The D1 receptor in the MPOA was reported to involve in the parasympathetically mediated erections (Dominguez and Hull, 2005).

Based on the previous reported about the role of D1 receptor in the control of parasympathetically mediated erection, the effect of *K. parviflora* plus chronic stress on the expression of D1 receptor in the hypothalamus was determined. The current results showed that the extract treatment showed increase in the density of D1positive immunoreactived neurons. Therefore, the increase in sexual behavior induced by *K. parviflora* plus chronic stress compared to vehicle plus chronic stress observed in this study might in part due to the induction of D1 receptor expression.

CONCLUSION

In conclusion, this study is the first study which provides the scientific data to confirm the traditional believe that *K.parviflora*, a Thai medicinal plant, possesses the aphrodisiac and sexual enhancing effects in aged rats. The increase in copulatory behaviors induced by the plant extract may partly occur via the induction of D1 receptors expression in hypothalamus. Therefore, it will be further developed as the functional food or health product for men especially for men who are risk for sexual dysfunction. However, further investigation about the active ingredients exhibiting these effects and the precise underlying mechanism of the extract are still required.

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