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Evaluation of Neuropharmacological Activities of *Stephania venosa* Herb Consumption in Healthy Rats

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ABSTRACT

Public concern on mental health has noticeably increased given the high prevalence of neuropsychiatric disorders especially anxiety and depression. Most of the drugs for these conditions used nowadays have adverse side effects so the need for newer, better-tolerated and more efficacious treatments is remaining high. Growing attention is being paid to traditional herbal medicines. *Stephania venosa* Spreng (*S. venosa*; SV) is a Thai traditional herb which has been used for cancer treatment as well as an aphrodisiac. In addition, accumulating lines of evidence reported that the ethanol extract of SV exhibited an antioxidant and exerted acetylcholinesterase inhibitory. However, the *in vivo* neurophamacological activities of this plant have never been studied. The aim of this study was to examine the neurophamacological effects of a crude extract of SV introduced orally at various doses ranging from 5, 10 and 20 mg kg^{-1} BW once daily for a period of 2 weeks. The anxiolytic and anti-depression like activities were determined after single administration, 1 and 2 weeks of treatment using elevated plus maze and forced swimming test respectively. Only SV 20 mg kg^{-1} treated rats exhibited a significant anxiolytic effect at all treatment duration. Unfortunately, this substance failed to show anti-depression like activity. Our findings provide a potential of the SV consumption might be used as a novel therapeutic strategy for the anti-anxiety disorder. However, further investigations about possible active ingredients and the precise underlying mechanisms are still necessary.

Keywords: *Stephania Venosa*, Anxiolytic Effect, Traditional Herbal Medicine, Anti-Depression, Neuropharmacological Activities

1. INTRODUCTION

Depression and anxiety disorders are the most common mental illness in humans (Wong and Licinio, 2001; Nestler *et al*., 2002; Hyman, 2008). It is not only life threatening but also negatively impacts on functional recovery from other neuropsychiatric disorders (Dere *et al*., 2010). Most of the drugs used nowadays have adverse side effects so the need for newer, better-tolerated and more efficacious treatments is remaining high. Traditional herbal medicines are becoming increasingly popular in worldwide (Watanabe *et al*., 2001). The efficacy of medicinal plants in disease management is established and the World Health Organization has recognized their use in the primary health care delivery system. In view of the complexity of herbal medicines and their inherent biological variations, it is necessary to determine their neuropharmacological activities (Castro *et al*., 2009).

Stephania venosa Spreng (*S. venosa*; SV) is a Thai medicinal plant locally known as "Saboo luad". It is a member of the Menispermaceae and is a rich source of alkaloids. Accumulating lines of evidence reported that the genus *Stephania* could be a potential source of biologically active compounds which might be used as lead molecules for development of novel drugs (Charles *et al*., 1987). Tubers of this plant have been used in Thai traditional medicine for nerve tonic, aphrodisiac (Potduang *et al*., 2003), appetizer and for treatment of asthma, hyperglycemia (Moongkarndi *et al*., 2004) and anti-malarial activities (Likhitwitayawuid *et al*., 1999). In addition, some of the isolated alkaloids were reported to exert acetylcholinesterase inhibitors (Ingkaninan *et al*., 2006), anti-cancer and anti-oxidative activities (Jongsomboonkusol, 2005; Leewanich *et al*., 2011). However, the in vivo neuropharmacological activities of

this plant have never been tested. Moreover, numerous studies demonstrated that substance possessing antioxidant activity could reduce psychiatric symptoms (Jittiwat *et al*., 2009; Phachonpai *et al*., 2010). Thus, it is of interest to study the neuropharmacological effects of this medicinal plant.

Based on all these points, the possible effects of SV herb consumption on anxiolytic and anti-depression like behaviors have been investigated.

2. MATERIALS AND METHODS

2.1. Plant Material

The tubers of SV were collected from Ratchaburi Province, Thailand. The herbarium was authenticated by Professor Dr. Pranom Chandranothai, Department of Biology, Faculty of Science, Khon Kaen University, Thailand. A voucher specimen from this plant was deposited at Center of Research and Development of Herbal Products under the number HHP-2-461.

2.2. Extraction

The fresh tubers of SV were harvested, chopped into small pieces and dried under the sunlight for 2 days and dried at 50°C until the weight was stable, then grounded into powder. The fine powder of 500 gm of SV was reflux with 50% ethanol for 30 min and filtered with gauze. The filtrate was then centrifuged at 800 g for 10 min. The extract procedures were repeated twice. The ethanol of the supernatant fraction was evaporated with lyophilizer and resulted in the final product brown color, kept in the dried and dark bottle until used. The percent yield of the final product was 14.29%.

2.3. Drugs

Diazepam (2 mg tablet^{-1}), fluoxetine (20 mg tablet^{-1}) were purchased from the Government Pharmaceutical Organization. All drugs and SV extract were dissolved in saline solution which used as a vehicle to a desired concentration. Then, they were filtered through gauze and given to the animals via the intragastric feeding tube. All administered substances including the SV suspension were freshly prepared.

2.4. Animals

Male Wistar rats, weighing 180–220 g (8 weeks old), were used for this study. Rats were obtained from the National Laboratory Animal Center, Salaya, Nakorn Pathom, Thailand. They were housed in standard metal cages in air-conditioned room at 22 ± 2°C, on 12:12 h light-dark cycle and allowed free access to pellet diet and water *ad libitum*.

The studies were carried out in accordance with the internationally accepted principles for laboratory use and care of the European Community (EEC directive of 1986; 86/609/EEC) and approved by the Ethical Committee of the Khon Kaen University, Khon Kaen, Thailand.

2.5. Experimental Protocol

The animals were divided into six groups (n = 8 in each group). The first group served as naïve intact control rats. The second group was vehicle treated rats. The third group was the positive control. In each test, the positive control group was treated with the standard drugs used for treating the related disorders. In order to determine the anxiolytic effect, the rats were treated with diazepam (2 mg kg^{-1} BW) while during the determination of anti-depressant like activity, the positive control treated group was treated with fluoxetine (20 mg kg^{-1} BW). The fourth-sixth groups were treated with the alcoholic extract of SV (5, 10 and 20 mg kg^{-1}BW, p.o.) for 2 weeks once daily throughout the experimental period.

The doses of SV were selected on the basis of previous studies conducted in laboratory and those reported in literature.

2.6. Determination

All behavioral tasks adopted in this study were assessed both after the single and repetitive administrations of SV extract (1 and 2 weeks). The behavioral tests in each group were performed and the studies were carried out between 8:00 a.m. to 11:00 a.m. under standard laboratory conditions. Each rat was subjected to the following behavior task forces: (a) Open-field tests (Spontaneous locomotor activities), (b) Elevated plus maze test and (c) Forced swimming test.

All tests were performed and analyzed by subject blind to the experiment. *Open-field tests* (*Spontaneous locomotor activities*): In order to guarantee that anxiolytic and anti-depression like behaviors which determined by various tests just mentioned earlier were not false positive due to the effect of SV on motor behaviors, we also determined the effect of SV on the spontaneous locomotor activities by open field test (Hawiset *et al*., 2011).

The open field area consists of an empty and a bright square arena (diameter 80 cm), surrounded by walls (height 60 cm) to prevent the animal from escaping. The rat is usually place in the center of the arena and its locomotor activities including the number of grooming, licking and rearing recorded within 5 min.

2.7. Elevated Plus-Maze (EPM)

This test has been widely validated to measure anxiety in rodents (Pellow *et al*., 1985). This apparatus was made of wooden and consisted of two open arms (50×10 cm) and two closed arms (50×10 cm) with 40 cm

walls. The arms extended from a central platform (10×10 cm). The maze was elevated 50 cm from the room floor. Each animal was placed at the center of the maze, facing one of the enclosed arms. The number and the time spent in opened arms entries were recorded for 5 min test. Entry into an arm was defined as the animal placing all four paws onto the arm. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution) to remove any residue or odors. Each rat was assessed individually 30 min after the treatment.

2.8. Forced Swimming Test (FST)

The FST is the most widely used pharmacological *in vivo* test for assessing antidepressant activity (Bruchey and Gonzalez-Lima, 2008, Tong-Un *et al*., 2010). The development of immobility when the rat is placed in an inescapable cylinder filled with water, reflects the cessation of persistent escape-directed behavior (Porsolt *et al*., 1977). The apparatus consisted of a clear glass cylinder (40 cm high×22 cm diameter) filled to a 20 cm depth with water (24±1°C). Each rat was placed individually into the cylinder for 5 min-test and observed for immobility time (keeping its head above the water in the way that animal made no further attempts to escape and neither hind leg was moving; the rats were slightly hunched forward) by blind observer who has been trained for the observation.

2.8. Statistical analysis

Data were presented as mean ± Standard Error of Mean (SEM) and based on an Analysis of Variance (ANOVA) followed by the Duncan's test, in which a significant difference was established among groups when the p value was lower than 0.05.

3. RESULTS

3.1. Elevated Plus Maze

Only the high dose of SV extracts administered in rats provoked a significant increase in both the number of open arms entries and time spent in the opened arms after a single dose of administration and these changes still existed when the treatment was prolonged further to 1-2 weeks (p-value < 0.05 all; compared to that of control and vehicle treated groups). It is important to mention that diazepam, a standard drug for the treatment of anxiety used in the positive control group, induced a similar modification to that observed with the plant extract (**Fig. 1-2**).

Unfortunately, the low and medium doses of SV extract did not produce the significant changes on this parameter.

3.2. Forced Swimming Test

Our results demonstrated that all rats subjected to vehicle and SV supplementation did not produce significant changes in the immobility times in forced swimming test throughout the observation time while the rats treated with fluoxetine, a standard drug used for the treatment of depression, which used as the positive control in this study significantly decreased the immobility times at all treatment duration ($p<0.05$ all; compared with control and vehicle treated groups) as shown in **Fig. 3**.

3.3. Open Field Test

The OFT was done in order to determine the effect of the administration of the plant extract upon spontaneous motor activity. The total number of spontaneous locomotor activities including grooming, rearing and licking did not differ significantly between the control, vehicle and SV treated groups throughout the experimental period as shown in **Fig. 4-6**.

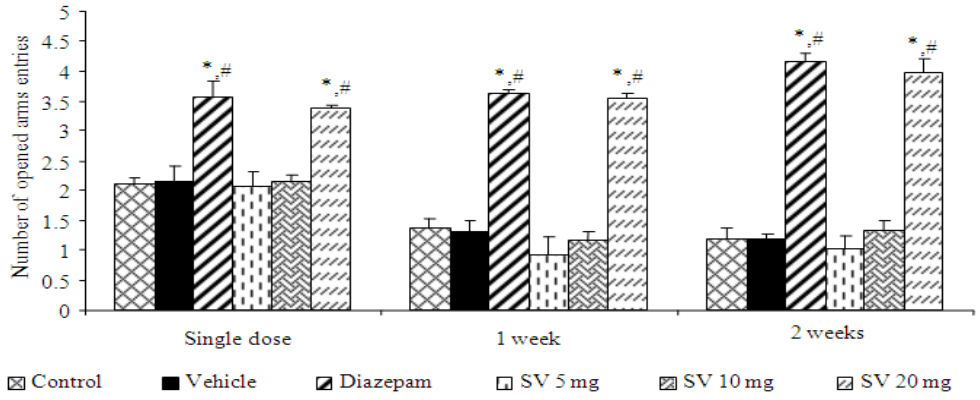

Fig. 1. Effect of diazepam and SV (5, 10 and 20 mg kg^{-1} BW) on the number of open arms entries in elevated plus maze test. The values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to the naïve control group, # p<0.05 as compared to vehicle treated group

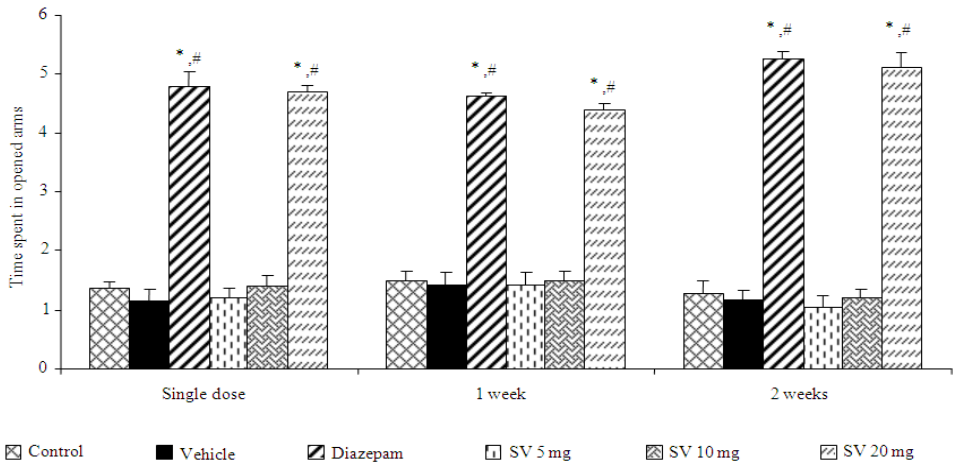

Fig. 2. Effect of diazepam and SV (5, 10 and 20 mg kg^{-1} BW) on the time spent on opened arms in the elevated plus maze test. The values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to the naïve control group, # p<0.05 as compared to vehicle treated group

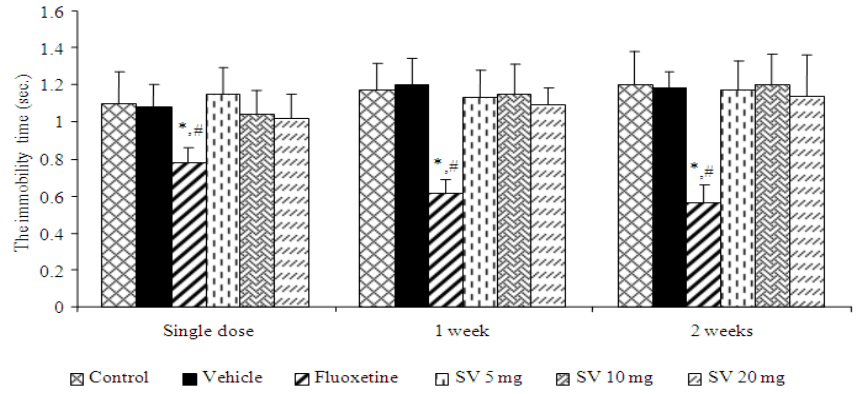

Fig. 3. Effect of fluoxetine and SV (5, 10 and 20 mg kg^{-1} BW) on the immobility time in force swimming test. The values given are the mean ± S.E.M. (n = 8). *p<0.05 as compared to the naïve control group, # p<0.05 as compared to vehicle treated group

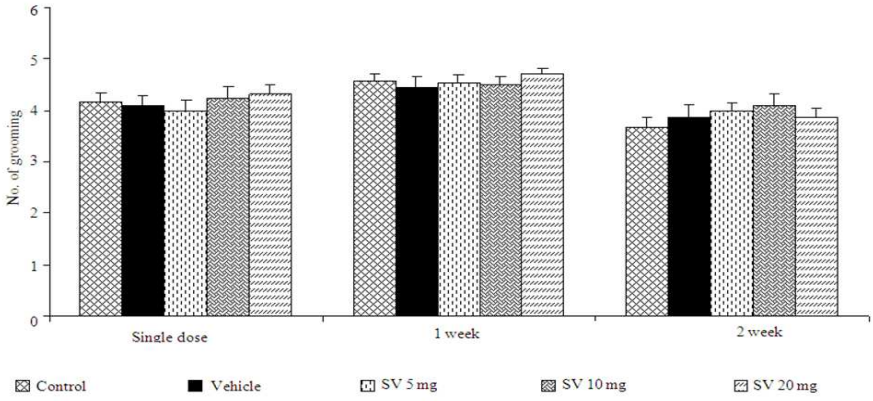

Fig. 4. Effect of SV (5, 10 and 20 mg kg^{-1} BW) on grooming behavior. The values given are the mean ± S.E.M. (n = 8)

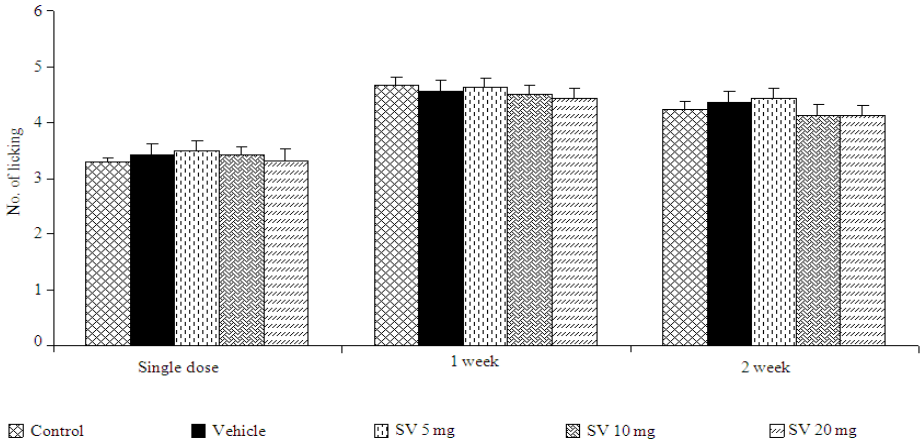

Fig. 5. Effect of SV (5, 10 and 20 mg kg^{-1} BW) on licking behavior. The values given are the mean ± S.E.M. (n = 8)

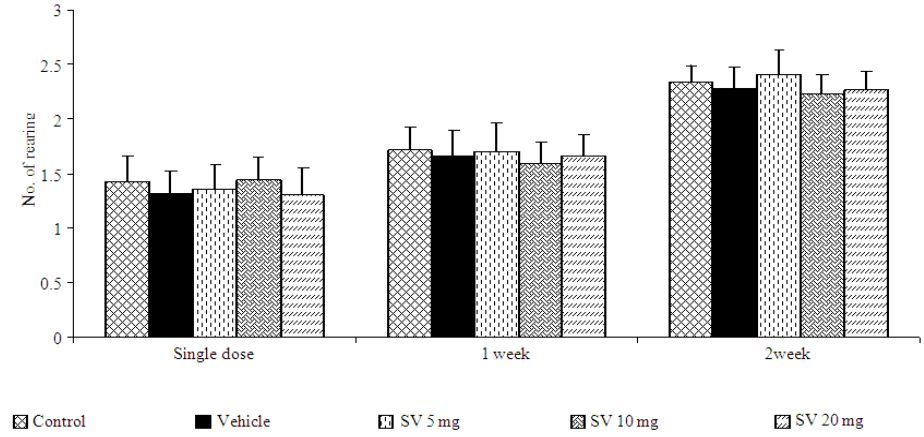

Fig. 6. Effect of SV (5, 10 and 20 mg kg^{-1} BW) on rearing behavior. The values given are the mean ± S.E.M. (n = 8)

4. DISCUSSION

Despite intensive efforts to develop novel psychiatric drugs for anxiety and depression disorders over the past two decades, all drugs have so far failed to show side effects. In this respect, herbal medicines could be an attractive candidate as the therapeutic strategies for these conditions (Fisher and Ward, 1994; Calixto, 2000). Although SV is extensively used in Thai herbal medicine, there is an absence of scientific reports about the determination of its neuropharmacological effects. In this study, it was demonstrated that the administration of the SV extract at a dose of 20 mg kg^{-1} in rats was able to induce anxiolytic effects, without modifying significantly the spontaneous locomotor activities. These encouraging results may have future clinical importance because of the increase using of herbal products by the general population.

EPM test is one of the most frequently used animal models in behavioral Neuro-psycopharmacology for screening drugs with potential anxiolytic effects (Wall and Messier, 2000). In general, the reduction or increase in the number of entries and times spent in the opened arms induced by a given substance had been regarded as good indicators of its anxiogenic or anxiolytic effects respectively (Pellow *et al*., 1985). The results of the present study demonstrated that oral administration of SV at a dose of 20 mg kg1 BW could produce the anxiolytic like effect in this paradigm. However, the low and medium doses of the plant extract did not produce the significant changes on this parameter. One possible explanation for this phenomenon might be due to the low and medium doses of the SV extract fail to raise the concentration of active pharmaceutical ingredient to the therapeutic level.

Previous studies had reported that false positive result could be obtained due to the stimulation effect of the tested substance on locomotor activity and leading to the increase both in the number of open arms entries and time spent in the opened arms in the elevated plus maze

test (Bourin *et al*., 2001). Our results demonstrated that no significant changes in the spontaneous locomotor activities after acute or repetitive administration with SV extract. There is therefore the anxiolytic effect observed in this study should not be the false positive effect.

With the experimental model used in this study (which gives us information about anxiolytic effect). Thus, it is necessary to develop biochemical and pharmacological studies that allow us to establish if the anxiolytic effect here reported is a consequence of the separate activation of nervous structures by one chemical compound by itself, or if the biological activities are produced by different secondary metabolites in the plant.

The neurotransmitter GABA is often reported to be involved in anxiety behavior. Animal studies have shown that increasing GABA can increase anxiety (Ballenger, 1999). Up to date, several lines of evidence demonstrated that numerous neurotransmitters including monoamine such as serotonin and norepinephrine contributed the important role in anxiety. Previous studies reported that increase serotonin synthesis by the increasing the tryptophan supplement could improve social anxiety (Young and Gauthier, 1981). In addition, the level of norepinephrine and the activity of sympathetic nervous system were prolonged in patients with anxiety disorder (Sullivan *et al*., 1999).

It had been reported that the alcoholic extract of SV comprised of various types of alkaloids (Guinaudeau *et al*., 1982; Pharadai *et al*., 1985; Tantisewie *et al*., 1989; Banerji *et al*., 1994) such as tetrahydropalmatine displayed diverse pharmacological activities e.g., analgesic (Chang and Lin, 2001) and hypnotic (Leung *et al*., 2003) actions. In addition, the tetrahydropalmatine alkaloid was also found to be potentially useful for treating cocaine addiction (Mantsch *et al*., 2007) and act as modulator of gammaaminobutyric acid (GABA) receptors (Halbsguth *et al*., 2003).

Take-in all data together, it was possible that the anxiolytic effect observed in our study might be related to the effect of SV extract exerted the regulators to modify the function of monoamine and GABA system or occur via its action of tetrahydropalmatine alkaloid. This observation needs further investigation in the near future.

5. CONCLUSION

The data here obtained allows us to propose this plant species as an excellent candidate for isolating novel substances with potential anxiolytic activity. However, further investigations about possible active ingredients and the precise underlying mechanisms are still required.

6. ACKNOWLEDGMENT

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