

Effects of Aqueous Extract of *Prismatomeris glabra* Root on Non-Spatial Memory in Rats Using Object Discrimination Test

(Kesan Ekstrak Akues Akar *Prismatomeris glabra* ke atas Memori Bukan Ruang Tikus Menggunakan Ujian Diskriminasi Objek)

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ABSTRACT

The aqueous extract of *Prismatomeris glabra* root has been used traditionally in Malaysia by the aborigines and certain rural Malays for its ergogenic effects, to maintain wellness and to enhance physical stamina. It has also been used as an aphrodisiac for generations in the east coast of Peninsular Malaysia. Previous studies have shown that plants with ergogenic effects may also act as a stimulant and impair cognitive function. Therefore, we seek to investigate the effects of *P. glabra* on non-spatial memory in male Sprague Dawley rats using object recognition test. Trial rats were injected intraperitoneally with an aqueous extract of *P. glabra* roots at doses of 50 and 100 mg/kg for the acute (30 min) and subacute (7 days) studies. Scopolamine (0.3 mg/kg) was used as a positive control only in the acute study meanwhile control rats were injected with saline. The locomotor activity of rats was also determined in the same test. We demonstrated that groups treated with 50 and 100 mg/kg of the extract lost their ability to discriminate the novel from familiar object in choice phase and did not alter the locomotor activity in both studies. Our results also indicated that the deficits in non-spatial working memory occurred at these doses were not due to impaired locomotor activity.

Keywords: Aqueous extract; locomotor activity; non-spatial memory; object discrimination test; prismatomeris glabra

ABSTRAK

Ekstrak akues akar *Prismatomeris glabra* telah digunakan secara tradisi di Malaysia oleh Orang Asli dan penduduk Melayu luar bandar untuk kesan ergogenik, memelihara kesihatan dan meningkatkan stamina fizikal. Rebusan airnya juga digunakan sebagai afrodisiak sejak turun-temurun di kawasan pantai timur Semenanjung Malaysia. Kajian lepas menunjukkan bahawa tumbuhan yang memberi kesan ergogenik boleh bertindak sebagai perangsang dan mengganggu fungsi kognitif. Oleh itu, kajian ini dilakukan untuk mengkaji kesan ekstrak ke atas memori bukan ruang tikus jantan Sprague Dawley menggunakan ujian diskriminasi objek. Tikus disuntik dengan ekstrak akues akar *P. glabra* secara intraperitoneum pada dos 50 dan 100 mg/kg untuk kajian akut (30 minit) dan subakut (7 hari). Skopolamina (0.3 mg/kg) digunakan sebagai kawalan positif untuk kajian akut sahaja manakala tikus kawalan disuntik dengan salin. Aktiviti lokomotor tikus juga ditentukan dalam ujian yang sama. Hasil kajian menunjukkan bahawa kumpulan rawatan yang disuntik dengan 50 dan 100 mg/kg ekstrak *P. glabra* masing-masing tidak berupaya membezakan objek baru dengan objek biasa dalam fasa pemilihan dan tiada kesan ke atas aktiviti lokomotor dalam kedua-dua kajian. Didapati gangguan memori bekerja bukan ruang yang berlaku pada kedua-dua dos ekstrak ini bukan disebabkan oleh gangguan aktiviti lokomotor.

Kata kunci: Aktiviti lokomotif; ekstrak akues; memori bukan ruang; prismatomeris globra; ujian membezakan objek

INTRODUCTION

Prismatomeris glabra (Rubiaceae) or locally known as 'Haji Samat', is usually found in the mixed dipterocarp and keranga forests of up to 700 m altitude in Peninsular Malaysia, Sumatra and Borneo (Slik 2006). This species has simple and oppositely arranged leaves with white-purplish flowers and green-whitish fruits in the form of berries. In Malaysia, the aqueous extracts of the roots of *P. glabra* have been used traditionally by the aborigines and certain rural Malays for wellness, enhancing stamina and for its ergogenic effects. In addition, as stated by a local taxonomist, this

plant has been used as an aphrodisiac for generations in Kelantan and Terengganu (Kamarudin 2008). To the best of our knowledge, scientific studies on the biological activities of this plant have not been published. Currently, there is no evidence which support the purported beneficial effects of *P. glabra*. In addition, its chemical compounds have not been fully isolated and identified. However, a preliminary study carried out recently by a group in Universiti Teknologi Mara (UiTM) has demonstrated that the aqueous extract of *P. glabra* administered orally did not produce overt signs of subacute toxicity in mice (unpublished data).

Other plant species in the Rubiaceae family are also widely used as ergogenic agents. For example, caffeine which is found in *Coffea arabica* is the commonly ingested psychoactive drug and ergogenic aid in the world (Rudge & Schifano 2001). Another species, *Mitragyna speciosa* was reported to exert stimulant effects on the central nervous system and improved working capacity (Chittrakarn et al. 2008). The bark of *Pausinystalia yohimbe* is used as an alternative to anabolic steroid to enhance athletes' performance in the United States (Betz et al. 1995). The plant species described above have all been shown to act as stimulants (Gyllenhaal et al. 2000). According to Lieberman et al. (2002), the consumption of herbal products for stimulant effects could give rise to negative effects on cognitive function. The consumption of caffeine at high doses (450 mg per day) could lead to impairment of working memory in humans (Childs & Wit 2006). In addition, *yohimbe* extracts taken at high doses for a prolonged period of time might contribute to memory dysfunction (Schwartz 2005).

Since previous studies have shown that plants with ergogenic effects also act as stimulants (Chittrakarn et al. 2008; Rudge & Schifano 2001) and might impair human memory (Childs & Wit 2006; Lieberman et al. 2002), we hypothesized that *P. glabra* may also affect cognitive function. In the present study, we used the object discrimination test developed by Ennaceur and Delacour (1988) to investigate the effects of aqueous extract of *P. glabra* root in Sprague Dawley rats. This behavioral test works based on the natural propensity of rats to explore novel objects spontaneously (Ennaceur & Delacour 1988). It has been widely used to study and screen compounds which may cause impairment of non-spatial memory in rats (Bertaina-Anglade et al. 2006). Scopolamine was utilized as a positive control due to its ability to impair non-spatial memory (Azmi et al. 2006; Giovanni et al. 1999).

MATERIALS AND METHODS

PLANT MATERIAL

P. glabra roots were purchased from a herbal supplier company, Famildizu Sdn. Bhd. (Pahang, Malaysia). The roots were collected from Balok Reserved Forest, Cherating, Pahang in October 2008 and identified by botanists at the Forest Research Institute of Malaysia (FRIM). The roots were chipped into small pieces, dried at 45 to 50°C and grinded into coarse powder form. The powder was then boiled at 100°C for 30 min and filtered to collect its aqueous extract. Subsequently, the liquid extract was freeze-dried and the yellow-brownish powder produced was stored at -20°C in an amber glass bottle until use.

ANIMALS

Adolescent male Sprague Dawley rats weighing between 150-200 g were purchased from Syarikat Usaha Cahaya

(Kuala Lumpur, Malaysia) and housed in groups of four under controlled conditions (28 ± 1°C, 60 ± 5% humidity and 12 h light/dark cycle) with food and water available *ad libitum*. The rats were acclimatized for a week before experimentation. All experiments were performed in accordance with the procedures approved by the Universiti Kebangsaan Malaysia Animal Ethical Committee (UKMAEC), Faculty of Medicine (FSKB/FARC/2008/NORAZRINA/9-APR/224-APR-2008-DEC-2009).

DRUGS AND DRUG ADMINISTRATION

P. glabra was administered in doses of 50 and 100 mg/kg. Since published biological studies on *P. glabra* are not available, the doses chosen were based on the oral dose which did not produce subacute toxicity in mice demonstrated by the UiTM group using similar aqueous extract of the plant (unpublished data). Scopolamine (Sigma-Aldrich, Germany) at a dose of 0.3 mg/kg was used as a positive control (Azmi et al. 2006) and 0.9% saline (A.N.B. Laboratories, Malaysia) was used as a control. Extracts of the roots of *P. glabra* and scopolamine were dissolved in saline. All drugs and vehicle were injected intraperitoneally in a volume of 1 mL/kg of body weight.

ACUTE EFFECTS OF *P. GLABRA* ON OBJECT DISCRIMINATION TEST AND LOCOMOTOR ACTIVITY

Rats were randomly allocated into four different groups (n = 8) and received saline, 0.3 mg/kg scopolamine, 50 mg/kg or 100 mg/kg *P. glabra*, respectively. Object discrimination test commenced 30 minutes after a single intraperitoneal injection was given.

SUBACUTE EFFECTS OF *P. GLABRA* ON OBJECT DISCRIMINATION TEST AND LOCOMOTOR ACTIVITY

The rats were randomly divided into three different groups (n = 8) and treated with saline, 50 mg/kg or 100 mg/kg *P. glabra*, respectively. Scopolamine treatment was not carried out in this subacute study due to anticholinergic adverse effects associated with prolonged exposure. All rats were given an injection once daily at the same time every day for seven days. Object discrimination test commenced 30 min after the final injection was given on treatment day seven.

BEHAVIOURAL TEST

The object discrimination test described in this study is a modification of the protocol used by Azmi et al. (2006). This test was performed in a clear Perspex box (40×40×40 cm³). The floor was divided into nine identical sectors by black lines for scoring of locomotor activity. The objects to be discriminated were 150 mL glass bottles wrapped either with white masking tape (familiar objects) or white masking tapes with three horizontal lines made using black insulating tape (novel object). The objects were filled with salt so that they were heavy enough and could

not be moved by the rats. In order to increase stability, the objects were secured into holes diagonally located in opposite front and back corners of the box, 7.5 cm away from the adjacent walls.

All rats were habituated in the box for 30 min for three consecutive days prior to the behavioral test. During this pre-test habituation period, no object was placed inside the box. On the test day, animals were habituated for another 3 min followed by a 1 min interval (spent in the home cage). Subsequently, the animals were allowed to explore two identical objects (A1, A2) in an acquisition phase and then subjected to a choice phase where they explored a familiar object (A3), identical to the one used in the acquisition phase, and a novel object (B), replacing the previous familiar object. Each phase lasted for 3 min and was intercepted with a 1 min inter-trial interval. The familiar and novel objects used in each test were counterbalanced to the left and right position to avoid bias for a particular object or location.

Exploration in both trials was defined as any sniffing or touching while orientating the nose towards the objects at a distance less than 2 cm. Other behavior such as sitting on, leaning against or chewing the objects was not considered as exploration. The objects were cleaned with 20% ethanol v/v before the start of each phase to eliminate any olfactory cues. All tests were carried out between 9:00 to 13:00 h in the same level of lighting. The behavioral test was recorded by a video camera (Sony Handycamcorder DCR-SR45E, Japan) suspended above the test arena. Scoring was performed on a replay of the video recording.

BEHAVIORAL AND LOCOMOTOR ACTIVITY MEASUREMENTS

The basic measurements for exploratory activity were the total time spent by the rats exploring each object in both the acquisition and choice phase (namely a1, a2, a3 and b). The following variables were then calculated according to the methods of Ennaceur and Delacour (1988): $e1 = a1 + a2$; $e2 = a3 + b$. $e1$ and $e2$ represent the total exploration time of both objects in the acquisition phase and choice phase, respectively. The basic measurement for locomotor activity was counted based on the total number of black lines crossed by the rats during the acquisition and choice phases.

DATA ANALYSIS

All data are expressed as mean \pm SEM (standard error mean) and analyzed statistically. Student's paired t-test (two-tailed distribution) was performed within the same treatment group to compare the time spent in exploring the two identical objects (A1 and A2) during the acquisition phase, the time spent in exploring the familiar (A3) versus novel object (B) during the choice phase, or the total exploratory activity in the acquisition ($e1$) versus choice phase ($e2$). One-way analysis of variance (ANOVA) followed by *post hoc* Dunnett's Multiple Comparison was performed to test any significant difference in locomotor activity between

different treatment groups. Statistical difference was set at a probability level of $p < 0.05$ for all data analyzed.

RESULTS

ACUTE EFFECTS OF *P. GLABRA* ON OBJECT DISCRIMINATION TEST AND LOCOMOTOR ACTIVITY

In this experiment, three rats were excluded from the study due to low exploratory activity. Figure 1(a) shows that rats in all treatment groups spent similar time exploring both identical objects (A1 and A2) in the acquisition phase. In the choice phase, saline-treated rats spent significantly longer time exploring the novel (B) compared to familiar object (A3) as revealed by the student's paired t-test ($p < 0.05$). In contrast, the rats' ability to discriminate the novel from familiar object was abolished after 30 min of 0.3 mg/kg scopolamine, 50 mg/kg and 100 mg/kg *P. glabra* treatment, whereby there was no significant difference in exploration of the novel and familiar objects as depicted in Figure 1(b). The total exploratory activity in the acquisition phase (Table 1(a)) was significantly higher compared to the choice phase for all treatment groups ($p < 0.05$, student's paired t-test) indicating that the rats were habituated to the experimental conditions. One-way ANOVA revealed a significant effect of treatment on the locomotor activity [$F_{(3,25)} = 5.805, p < 0.01$]. Further *post hoc* Dunnett's Multiple Comparison showed that the locomotor activity was significantly ($p < 0.01$) increased only in the scopolamine treated group as compared to the saline group (Figure 2).

SUBACUTE EFFECTS OF *P. GLABRA* ON OBJECT DISCRIMINATION TEST AND LOCOMOTOR ACTIVITY

Similar to the acute study, rats in all treatment groups spent similar time exploring the two identical objects in the acquisition phase ($p > 0.05$, student's paired t-test) as illustrated in Figure 3(a). In the choice phase (Figure 3(b)), rats in the saline group showed significant discrimination between the familiar and novel object ($p < 0.05$, student's paired t-test). Conversely, rats treated with both doses of *P. glabra* failed to discriminate the novel from familiar object as revealed by student's paired t-test ($p > 0.05$). Analysis using student's paired t-test also revealed that the total exploratory activity in *P. glabra* 50 mg/kg and 100 mg/kg treated groups, but not the saline treated group, was significantly higher during the acquisition phase than the choice phase ($p < 0.05$) (Table 1(b)). One-way ANOVA revealed no significant effect of any treatment on the locomotor activity [$F_{(2,21)} = 0.540, p = 0.590$] (Figure 4).

DISCUSSION

To the best of our knowledge, there has been no scientific evidence of biological studies having been conducted on *P. glabra*. However some phytochemical studies have been carried out on related species, for example, *P. fragrans*

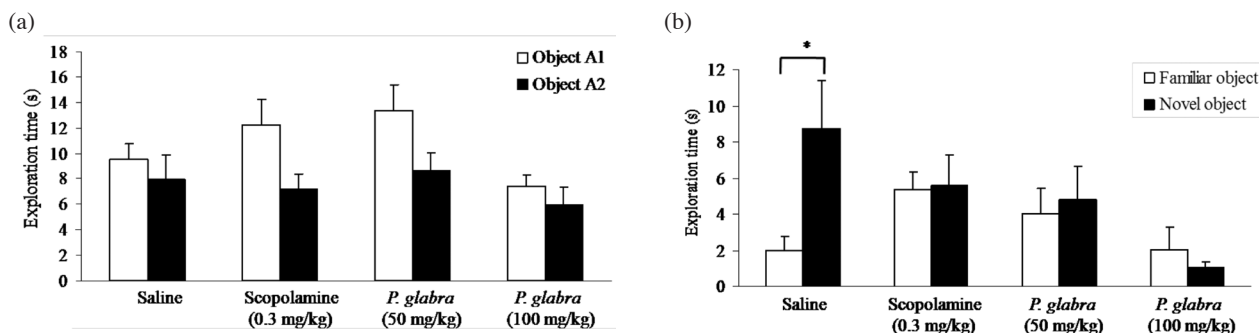


FIGURE 1. Acute effects of *P. glabra* on object exploration in the (a) acquisition and (b) choice phase. Rats in all treatment groups did not show any significant difference in the exploration time of identical objects A1 and A2. Rats treated with 0.3 mg/kg scopolamine, 50 mg/kg *P. glabra* and 100 mg/kg *P. glabra* failed to discriminate the novel from familiar object.

Data expressed as mean time in seconds (+ SEM) with $n=6-8$ per treatment group. * $p < 0.05$ (student's paired t-test, two-tailed distribution)

TABLE 1. The (a) acute and (b) subacute effects of *P. glabra* on total exploratory activity

Treatment Groups	Total Exploratory Activity (s)	
	Acquisition Phase, e1	Choice Phase, e2
(a) Saline	17.54 ± 2.48*	10.69 ± 3.26
Scopolamine 0.3 mg/kg	19.35 ± 2.59*	10.97 ± 2.16
<i>P. glabra</i> 50 mg/kg	22.02 ± 2.03**	8.79 ± 2.71
<i>P. glabra</i> 100 mg/kg	13.38 ± 2.23*	3.03 ± 1.51
(b) Saline	11.62 ± 1.81	6.59 ± 1.42
<i>P. glabra</i> 50 mg/kg	16.75 ± 3.87*	10.01 ± 2.98
<i>P. glabra</i> 100 mg/kg	14.84 ± 2.30*	7.86 ± 2.51

* $p < 0.05$ and ** $p < 0.01$ as compared with total exploratory activity in the choice phase within-group comparison (student's paired t-test, two-tailed distribution). Data expressed as mean ± SEM

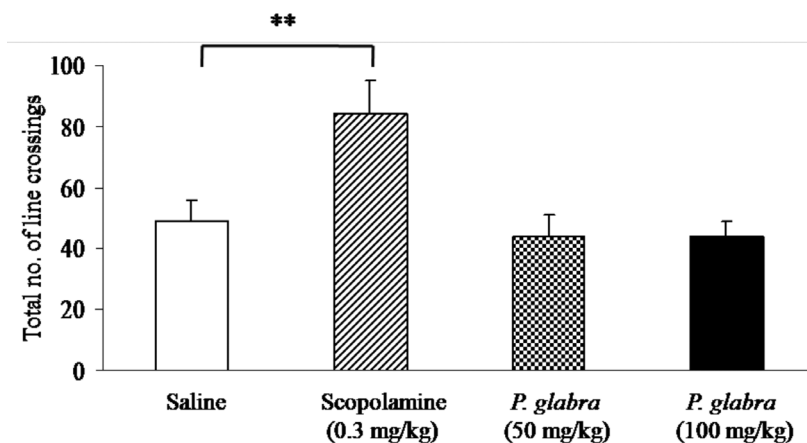


FIGURE 2. Acute effects of *P. glabra* on locomotor activity in rats. 0.3 mg/kg scopolamine treatment significantly increased locomotor activity compared to the saline-treated rats. No significant effects on locomotor activity were observed in rats treated with *P. glabra* at doses of 50 and 100 mg/kg. Data expressed as mean (+ SEM) with $n=6-8$ per treatment group).

** $p < 0.01$ (one-way ANOVA followed by *post hoc* Dunnett's Multiple Comparison test)

(Kanokmedhakul et al. 2005), *P. tetrandra* (Feng et al. 2005; Jiang et al. 2005; Tu et al. 1981), *P. malayana* (Lee 1969) and *P. sessiflora* (Likhitwitayawuid et al. 1999). Kanokmedhakul et al. (2005) have also investigated the biological activities of chemical constituents of the genus

Prismatomeris. The anthraquinones and triterpenoids isolated from *P. fragrans* showed antimalarial, antifungal and antituberculosis activity and were found to be cytotoxic to cultured cells (Kanokmedhakul et al. 2005). Although scientific evidence is lacking on the biological activity

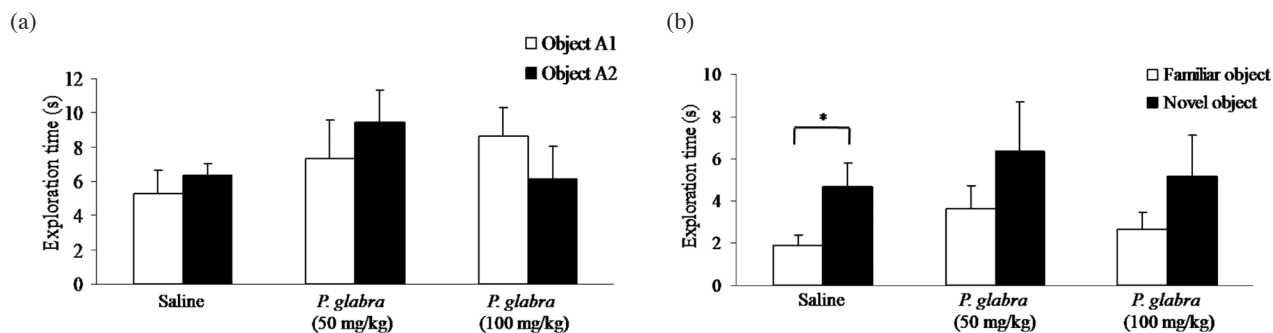


FIGURE 3. Subacute effects of *P. glabra* on object exploration in the (a) acquisition and (b) choice phase. There was no significant difference in the exploration time of object A1 and A2 within all treatment groups. Saline control group explored the novel object significantly longer than the familiar object. The rats' ability to discriminate the novel from familiar object was abolished following subacute treatment with 50 and 100 mg/kg *P. glabra*. Data expressed as mean time in seconds (+ SEM) with $n=8$ per treatment group. * $p < 0.05$, (student's paired t-test, two-tailed distribution)

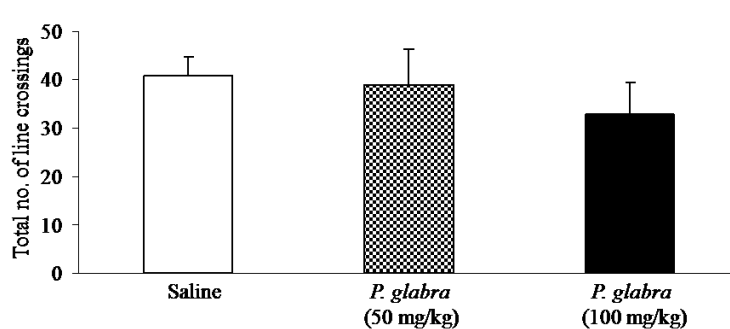


FIGURE 4. Subacute effects of *P. glabra* on locomotor activity in rats. Rats in all treatment groups did not display any significant ($p > 0.05$) difference in the locomotor activity compared to the saline-treated rats. Data expressed as mean (+ SEM) with $n=8$ per treatment group.

of *P. glabra*, a subacute study demonstrated that its aqueous extract was not toxic in mice even at large doses (unpublished data). The lack of scientific studies on this plant has led us to investigating its biological effects in animals.

In the present study, the acute and subacute effects of *P. glabra* aqueous extract (50 and 100 mg/kg) on cognitive function in rats were assessed using object discrimination test. Object discrimination test is widely used to screen the amnesic effect of a novel drug or compound in the setting of safety pharmacology study (Bertaina-Anglade et al. 2006). Since this test does not require any pre-training, it is similar to the recognition test performed on humans and only involves a short period of time (Reed & Squire 1997).

The present study demonstrated that all rats spent similar time exploring both objects A1 and A2 after administration of saline, 0.3 mg/kg scopolamine, 50 and 100 mg/kg *P. glabra* in the acute and subacute studies. This result indicated that the rats did not have preference for any object or location following any of the treatment. However, in agreement with previous studies, only rats treated acutely with saline were able to discriminate the novel from familiar object in the choice phase (Azmi et

al. 2006; Besheer et al. 2001). Treatment with 0.3 mg/kg scopolamine, 50 and 100 mg/kg *P. glabra* impaired the non-spatial memory of rats. Scopolamine has been reported to impair rats' acquisition in the object discrimination test by antagonizing the cholinergic function which is mediated centrally (Azmi et al. 2006; Giovanni et al. 1999; Pitsikas et al. 2001).

The impairment of non-spatial working memory in male Sprague Dawley rats after acute and subacute treatment of 50 and 100 mg/kg *P. glabra* is an important new finding on the biological effect of this plant. The decoction of *P. glabra* roots was long used by the aborigines and certain rural Malays in Malaysia. It was claimed that this decoction is beneficial for health and possesses ergogenic effects. Since this study was believed to be the first study carried out to assess the effects of *P. glabra* extract on cognitive function in rats, the bioactive compound(s) and mechanisms responsible for the *P. glabra*-induced cognitive deficits could not be clearly identified. According to Davis et al. (2006), the synonym for *P. glabra* is *Coffea glabra*. Therefore, *P. glabra* is under the same genus as coffee plants and it is possible that caffeine might be present in different parts of *P. glabra* because secondary metabolites of a plant are genus or species-specific

(Balandrin et al. 1993). Furthermore, caffeine is highly soluble in hot water and can be extracted in boiling water (Horie et al. 2002). This suggests that caffeine might be present in the aqueous extract of the *P. glabra* roots which led to the impairment of the working memory as seen in mice treated with caffeine at doses higher than 10 mg/kg (Angelucci et al. 1999). However, this notion needs to be confirmed with further phytochemical studies.

The locomotor activity of scopolamine treated rats was significantly higher than the saline-treated rats in the acute study. Numerous early studies have reported the ability of scopolamine to induce an increment in the rats' locomotor activity even at low doses (Poorheidari et al. 2002). Scopolamine increases locomotor activity by blocking the muscarinic receptors in the pedunculopontin and laterodorsal tegmental nucleus leading to activation of the dopaminergic neuron (Lavolette et al. 2000; Mathur et al. 1997). In contrast, acute *P. glabra* treatment at 50 and 100 mg/kg did not alter the rats' locomotor activity. This result supports the notion that reduced novelty recognition observed in *P. glabra* treated rats was not due to reduced locomotor activity. There is good evidence that this plant contains phytochemical compounds that may act on the central nervous system. Analysis of brain neurotransmitters in rats treated with *P. glabra* aqueous extract is currently on-going in our group to confirm that deficits in non-spatial working memory observed in the present study is mediated by the brain. The present finding also warrants further studies to be established on the safety profile of *P. glabra* consumption, particularly pertaining to its effects on the central nervous system.

CONCLUSION

The aqueous extract of *P. glabra* impaired non-spatial working memory in rats after a single or repeated administration as demonstrated using object discrimination test. Our results also indicated that failure to discriminate the novel from familiar object following treatment with *P. glabra* at 50 and 100 mg/kg was not due to impairment in locomotor activity or decreased exploratory behavior.

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