The Antidepressive Effect of Barakol in the Forced-Swimming Test

Wanida Pumpaisalchai[1]*, Sayam Kaewichit[1], Tawat Taesothikul[2], Wirat Niwatananun[3] and Kittipong Sanichwankul[4].

[1]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand*

[2]*Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

[3]*Department of Pharmaceutical Care, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand*

[4]*Suanprung Hospital, Department of Mental Health, Ministry of Public Health , Chiang Mai 50100, Thailand*

**Corresponding author. E-mail: pumpaisalchai@yahoo.com*

ABSTRACT

The forced-swimming test is a behavioral test developed to predict the efficacy of antidepressant treatments. The immobility time indicates behavioral despair. Many antidepressants have been found to reduce immobility time in FST. The aim of this study was to investigate if barakol had an antidepressive effect in FST in rat. The rats were divided into 6 groups. Each group received either water, imipramine 25 mg/kg or barakol 5, 10, 15 and 30 mg/kg orally for 7 days. Rats were forced to swim 5 minutes in the last three days after one hour of drug administration. The duration of immobility, struggling and swimming were recorded during the last 5-minute test. This experiment showed that all groups of barakol- and imipramine-treated significantly reduced immobility time in comparison to the water controlled group, ($p<0.05$). Only the barakol 5-mg/kg-treated group increased struggling behavior where as the barakol 30-mg/kg-exposed animals increased swimming time ($p<0.05$). The result indicated that barakol had potential antidepressant effect. Further study should be conducted in other models to confirm whether barakol produces the same antidepressive effect as demonstrated in this study.

Key words: Barakol, Cassia siamea Lamk, Antidepressive effect, Forced-swimming test, Immobility

INTRODUCTION

Considerable effort has been exerted to establish animal models for the screening of depressant drugs. Of these models, the forced-swimming test developed by Porsolt et al., (1977a, 1978) has gained considerable acceptance. It is a behavioral test which predicts the efficacy of antidepressant treatments (Porsolt et al., 1977b; Porsolt et al., 1978). The test consists of placing a rodent in a cylinder tank of water for a 15-min "pretest" and then returning the animal to the water 24 hours later for a 5-min "test". Rats respond vigorously during the early part of the test but then display little motor activity during later parts of the test period, which Porsolt termed "immobility". The characteristic behavior of the test, "immobility", develops when a rodent has been placed in a tank of water for an extended period of time and makes only those movements necessary to keep its head above water (Porsolt et al., 1977b). If antidepressant drugs are administered between the pretest and test periods, the rats

are more active or less immobile in the test (Porsolt et al., 1977b; Porsolt et al., 1978; Porsolt, 1981).

The results from the forced-swimming test could be related to the mood state of the animal and brain levels of noradrenaline, dopamine, serotonin and cholinergic neurotransmitters (Willner, 1984). These neurotransmitters are involved in the pathophysiology of some types of depression and play a permissive role in the antidepressant activities (Willner, 1995; Ferigolo et al., 1998). The enhancement of serotonin, norepinephrine and dopamine content in the brain can reduce the immobility time of rats in the forced-swimming test (Borsini et al., 1984; Cervo and Samanin, 1988; Cervo et al., 1990; Detke et al., 1995b; Calapai et al., 2001). In addition, antidepressant drugs decrease immobility but affected active behaviors of climbing and swimming that changed specifically in different antidepressant groups (Detke et al., 1995a). Selective serotonin reuptake inhibitors (SSRIs) decreased immobility but increased swimming behavior (Detke et al., 1995a; Detke and Lucki, 1996). Enhancement of dopamine (Cervo et al., 1992; Cabib et al., 1995; Cryan et al., 2003) and norepinephrine neurotransmission (Detke et al., 1995a) may mediate climbing in the forced-swimming test.

In an effort to enhance the sensitivity of the traditional forced-swimming test in the rat, several simple procedural modifications have been made (Lucki, 1997). These developments include increasing the water depth to 30 cm from traditional depth of 15–18 cm, and using a time sampling technique to rate the predominant behavior over a 5-s interval, e.g., 10 to 30 min long test (Abel et al., 1992a; Miura et al., 1993). The repetition of forced swimming for several days tends to increase immobility (Abel and Hannigan, 1992b; Abel, 1993) and to produce anhedonia, similar to procedures which produce chronic stress (Katz, 1982; Willner et al., 1987). Prolonged antidepressant administration has been found to reverse the immobility behavior. SSRIs were seen to decrease immobility if given for prolonged periods (Okada et al., 1997). It was shown that chronic but not acute treatment of rats with the antidepressant fluoxetine displayed significantly longer times of immobility (Uz and Manev, 2001). Potentiation by chronic administration of an antidepressant effect detectable in treatment appears also to describe the clinical situation (Frazer et al., 1985). The major advance of the modified forced-swimming test over its traditional counterpart is that it reveals that catecholaminergic agents, e.g., dopamine and norepinephrine, decrease immobility with corresponding increase in climbing behavior, whereas 5-HT-related compounds such as SSRIs also decrease immobility but increase swimming behavior (Lucki, 1997; Cryan and Lucki, 2000).

Barakol, an active ingredient in Cassia siamea Lamk., has been shown to inhibit *in vitro* dopamine release in a similar manner to dopamine D2 -like receptor agonist, quinelorane (Thongsaard, 1998). In *in vivo* study, it increased rotation behavior induced by apomorphine in rats with a unilateral lesion in the substantia nigra with 6-hydroxydopamine lesions of the nigrostriatal pathway. This drug promoted behaviors, e.g., locomotion, typically attributed to heightened dopaminergic activity in rats (Jantarayota, 1988). Moreover, barakol significantly increased 20 mM[K+]-stimulated 5-HT release from rat hippocampal slices (Thongsaard, 1997). At 25 mg/kg dose, barakol heightened serotonergic activity by increasing head shake behavior as produced by injection of 5-hydroxytryptophan in rat at 90–120 min (Jantarayota, 1988). Thus, barakol can enhance the effect of dopamine and serotonin (Jantarayota, 1988; Thongsaard, 1998). The present study was aimed at testing the antidepressant-like effect of chronic administration of barakol, using the repetition of forced-swimming test in rat.

MATERIALS AND METHODS

Animals:

Thirty-six male Sprague-Dawley rats (purchased from National Laboratory Animal Center, Salaya, Mahidol University, Thailand), weighing 150–180 g were used. The rats were housed in colony, three per cage, with free access to food and water. The colony was maintained on a 12:12 h light:dark cycle (on 7:00 off 19:00) under constant temperature (22±1°C). Experiments were carried out between 9:00 AM and 11:00 AM, in a soundproof and air-regulated experimental room. Rats were handled for 3–5 days prior to behavioral testing.

Drugs:

Barakol was extracted from fresh young leaves of Cassia siamea Lamk. adapted from Kaokeaw (1992). The leaves were cut into small pieces and boiled with 0.5% sulfuric acid. The water extract was alkalinized with concentrated sodium hydrogen carbonate solution, further extracted with chloroform, shaken with 5% aqueous acetic acid until becoming colorless, neutralized with concentrated ammonia solution, and cooled. Crude crystallized barakol was added with concentrated hydrochloric acid and dried immediately with filtrated vacuum. The obtained crystallized-yellow needles was anhydrobarakol hydrochloride. The compound was dried by vacuum filtration.

The identification of the compound was confirmed, using nuclear magnetic resonance (NMR), infrared spectroscopy (IR) and ultraviolet spectroscopy (UV). The physical and spectroscopic characteristics of the compound were evaluated and compared with those in previous reports (Bycroft, 1970; Kaokeaw, 1992).

Imipramine was obtained from Pharminar Ltd, Co. (Harika Drugs Private Ltd., India. IH017012001). Each drug was solubilized in distilled water.

Procedure:

The study protocol was approved by the Animal Ethics Committee of the Faculty of Pharmacy, Chiang Mai University. Six groups of rat received 7 days of daily oral administration of either water (the control group), imipramine 25 mg/kg or barakol 5, 10, 15 and 30 mg/kg. All drugs were dissolved in distilled water and were administered orally in an equal volume for a period of 7 days. At day 5–6 before the test session, the animal was forced to swim for 5 min. Each rat was placed individually in a glass cylinder (diameter 20 cm, height 40 cm) filled with tap water at height of 24 cm. Water temperature was maintained at 23–25°C. Following both swimming sessions, the rats were removed from the cylinders, dried with towels and placed in heated cages for 15 min, and then returned to their home cages. On the seventh day, after 1 h drug administration, each rat was placed again into the water and forced to swim for 5 min. The last session was videotaped for scoring later. The swimming sessions were always conducted between 9:00 and 11:00 AM. (Kitada et al., 1981; Araki et al., 1985; Armario et al., 1988).

Behavioral scoring:

All of the behavioral scoring was done by a single trained rater, blind to experimental conditions. Several test sessions (n=10 subjects), chosen at random, were scored a second time by this rater to determine test-retest reliability. A stopwatch was used to time the duration of each behavior.

The scorer would rate the rat's behavior as one of the following three behaviors: 1) immobility - a rat was judged to be immobile when it remained in the water without struggling and was making only those movements necessary to keep its head above water; 2)

swimming - a rat was judged to be swimming if it was making active swimming motions, more than necessary to merely maintain its head above water, e.g., moving around in the cylinder; and 3) struggling or climbing - a rat was judged to be struggling when it was making active movements with its forepaws in and out of the water, usually directed against the walls, or sweeping its head around or raising its head above the water while its forelimbs are against the wall. Diving-when rat's entire body was submerged was judged to be struggling.

Statistical analysis:

Data obtained from activity monitors were evaluated, using one way ANOVA followed by LSD post-hoc tests for differences between groups. Significant level was set at $p<0.05$.

RESULTS

The determination of the test-retest reliability by the rater was analyzed. T-test analysis did not show significant difference between the first and the second scoring.

In the FST, Figure 1 shows the influence of barakol on the duration of immobility behaviors. The ANOVA revealed that the treatment with imipramine or barakol was significantly effective in reducing immobility time in comparison to the control group. Despite struggling time in imipramine or barakol treatment which seemed to be higher than in control group, only 5 mg/kg dose of barakol increased this behavior significantly (Figure 2). The barakol 30 mg/kg treated-group showed a significant increase in swimming time (Figure 3).

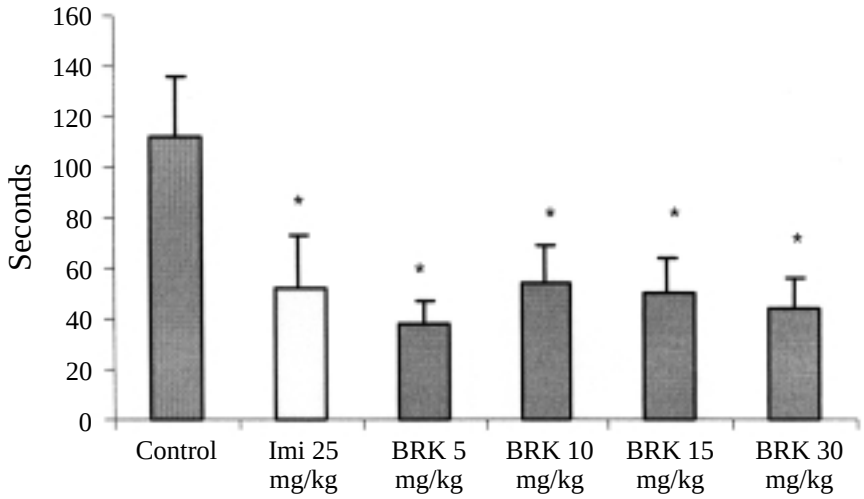

Figure 1. The duration (sec) of immobility time in rat after receiving barakol (BRK) 5–30 mg/kg, imipramine (Imi) 25 mg/kg, p.o., once a day for 7 days comparing with the controlled group. Chronic barakol- and imipramine-treated rats significantly reduced immobile behavior. Each bar represents the mean time (sec). Vertical bars represent ±SEM. * represents significant difference at $p<0.05$ in comparison to control.

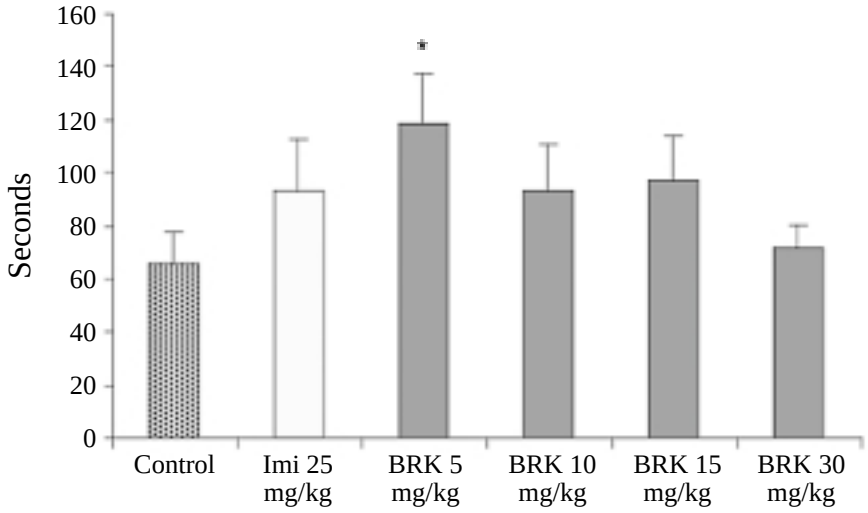

Figure 2. The struggling time in rats after receiving barakol (BRK) 5–30 mg/kg, imipramine (Imi) 25 mg/kg, p.o., once a day for 7 days comparing with the controlled group. Only barakol 5 mg/kg-treated rats significantly increased struggling behavior. Means and +S.E.M. are presented. * statistically-significant difference at $p<0.05$ in comparison to control.

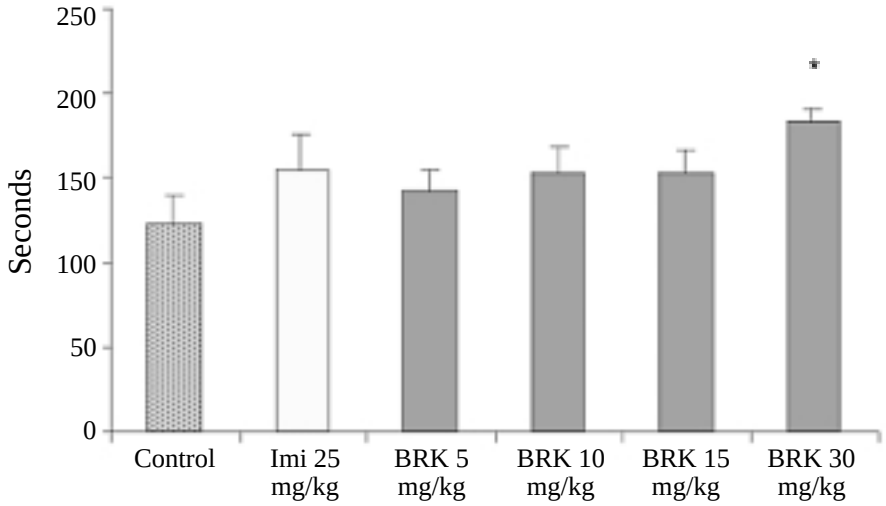

Figure 3. The swimming time in rat after receiving barakol (BRK) 5–30 mg/kg, imipramine (Imi) 25 mg/kg, p.o., once a day for 7 days comparing with the controlled group. Only barakol 30 mg/kg-treated rats significantly increased swimming behavior. Means and +S.E.M. are presented. * statistically-significant difference at $p<0.05$ in comparison to control.

DISCUSSION

The forced-swimming test, the most-widely used tool for assessing antidepressant activity preclinically, is sensitive to the effects of all of the major classes of antidepressant drugs (Detke, et al., 1995a). Immobility time is reduced by clinically-relevant doses of tricyclic and atypical antidepressants, 5-HT uptake inhibitors and monoamine oxidase inhibitors in mice and rats (Porsolt et al., 1977b; Lucki et al., 1994). The present study revealed that chronic barakol treatment, given orally, was effective in forced-swimming test.

Both barakol and imipramine significantly reduced immobility behavior which indicates depression (Figure 1). In general, all antidepressants take 2–4 weeks to reach maximum therapeutic effect. However, some of the depressive symptoms are relieved after the first week (Travis, 1998). The reduction of immobility behavior after multiple, 7 days, administration of barakol was in accordance with the clinical onset of antidepressive effect.

The behaviors of animal exhibited in the forced-swimming test could be related to the mood state of the animal (Willner, 1984) and brain levels of noradrenaline, dopamine, serotonin and cholinergic neurotransmitters. These neurotransmitters decrease immobility but affected active behaviors of climbing and swimming which changed specifically in different groups (Detke et al., 1995a). Serotoninergic drugs decreased immobility but increased swimming behavior (Detke et al., 1995a; Detke and Lucki, 1996). Enhancement of dopamine (Cervo et al., 1992; Cabib et al., 1995; Cryan et al., 2003) and norepinephrine neurotransmission may mediate climbing in the forced-swimming test (Detke et al., 1995a). In agreement with prior study (Ferigolo et al., 1998), imipramine produced a mixed effect, increasing both mobility-type behaviors, climbing and swimming. In contrast, barakol, differing from imipramine, enhanced climbing at low dose (5 mg/kg) (Figure 2). This effect could probably be explained by its predominantly-dopaminergic property at low dose. Serotonin neurotransmitters decreased immobility but increased swimming behavior (Detke et al., 1995a; Detke and Lucki, 1996). Barakol, increasing swimming at high dose (30 mg/kg) (Figure 3), may play a role in serotonergic system.

Although the mechanism of action of the drug is not clear, these results indicate that the effect of barakol may depend on dose of administration. These results are also in line with previous findings in vitro and in vivo which demonstrated that barakol may act at both pre-synaptic dopamine D2 and/or D3 receptors. At pre-synaptic nerve terminal, barakol may act at both dopamine D2 and D3 receptor to inhibit dopamine release. At post-synaptic nerve terminal, low dose of barakol (10 mg/kg) may act at the D2 receptor to exhibit anxiolytic, exploratory and hyperlocomotive behaviors, while high dose of barakol may act at the D3 receptor to produce hypolocomotion and sedation (Thongsaard, 1998). In *in vivo* study, barakol (25 mg/kg, i.p.) may enhance serotonergic activity by increasing head shake behavior, as produced by injection of 5-hydroxytryptophan in rat at 90-120 minute (Jantarayota, 1988).

The present study indicated that barakol had potential antidepressive effect. However, the underlying mechanism for this behavioral observation deserves further attention. Further study should be conducted in other models to confirm whether barakol produces the antidepressive effect as in the forced-swimming test.

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