

Acute effects of barakol and serotonergic drugs on exploratory behaviour in rats

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ABSTRACT

Barakol (10 mg/kg, i.p.), an active ingredient extracted from *Cassia siamea*, has been shown to have anxiolytic properties on the elevated plus-maze at a low dose similar to diazepam and to increase exploratory and locomotor behaviour. Drugs increasing serotonergic function also alter exploratory activity. The mechanism underlying the effects of barakol on exploratory behaviour has not been studied, therefore, the aim of the present study was to investigate exploratory activity in rats treated with barakol compared with drugs known to have specific action on the 5HT system. Exploratory behaviours were monitored in the rat using the holeboard model. The results show that the 5HT_{1A} receptor agonist 8-OH-DPAT (1 mg/kg, i.p.) significantly ($P<0.05$) decreased exploratory behaviours including rearing, number of head dips and time spent head dipping and the effects were antagonised by the selective 5HT_{1A} receptor antagonist WAY 100635 (1 mg/kg, s.c.). Paroxetine (20 mg/kg, i.p.) also significantly ($P<0.05$) reduced all exploratory activity and grooming. In contrast, barakol (10 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) had no effect on exploration. In conclusion, a low dose of barakol has no effect on exploratory behaviours using the holeboard test indicating that 5HT mechanism and 5HT_{1A} receptor may not be involved in the anxiolytic effects of barakol.

Keywords : barakol; holeboard; exploration; 5HT_{1A} receptors; serotonin

Abbreviations : 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamine) tetralin ; WAY 100635, *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride; 5, 7-DHT , 5, 7-dihydroxytryptamine

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INTRODUCTION

Barakol is an active ingredient extracted from *Cassia siamea* Lam. of the family *Caesalpiniaceae*, a plant used in Thai traditional medicine. Barakol has both the behavioural effects and neurochemical actions¹⁻³. On the elevated plus maze, barakol has been shown to produce both an anxiolytic effect in rats similar to diazepam but also to increase exploratory and locomotor behaviour¹. However, later studies have shown no anxiolytic effect of barakol in rats or mice either on the plus-maze or using the shock-probe burying test^{3,4}. Administration of barakol to mice which has been reported to reduce spontaneous locomotor activity, increase the number of sleeping animals and prolong the sleeping time induced by thiopental, suggesting a sedative effect.^{3, 5, 6} The possible mechanisms underlying the effects of barakol on the central nervous system have focused on the dopaminergic system^{2,3}. Barakol reduced K⁺-stimulated release of endogenous dopamine from rat striatal slices in a manner similar to dopamine receptor agonists and this effect was inhibited by a D₂ receptor antagonist². Furthermore, barakol has been shown to inhibit both the hyperlocomotor activity and the increases in striatal dopamine release and dopamine turnover induced by methamphetamine, indicating an inhibitory effect on the dopaminergic system³.

The effect of barakol on the serotonergic system has not been extensively studied. Barakol has been shown to suppress 5-hydroxytryptophan-induced head shakes in rats, suggesting a 5HT₂ antagonist action of barakol⁷. In a preliminary study by Thongsaard and colleagues² barakol increased K⁺-stimulated endogenous 5HT release from rat hippocampal slices. There is some evidence for the

involvement of the serotonergic system in exploratory behaviour^{8,9}. For example, the 5HT_{1A} receptor agonists, flesinoxan and 8-hydroxy-2-(di-n-propylamine) tetralin (8-OH-DPAT), but not benzodiazepines dose-dependently decreased all exploratory behaviours in mice⁸ while *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl} -*N*-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride (WAY 100635), the selective 5HT_{1A} receptor antagonist significantly increased the frequency of exploration and reduced fear-induced avoidance of the figure-eight maze⁹. In mice naïve to the maze, infusions of WAY-100635 into the median raphe nuclei increased open arm exploration¹⁰. Therefore, the effect of barakol in the rat on exploratory behaviour remains debatable. As does the possibility that the mechanism by which barakol might alter exploration could involve serotonergic function.

The aim of the present study was to compare the effect of barakol administered acutely on exploratory behaviour using a holeboard test, an animal model of exploration with the effects of drugs that alter 5HT_{1A} mediated serotonergic function.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200 - 250 g (The National Laboratory Animal Centre, Mahidol University, Thailand) were used. The rats were housed in groups of four in a room with a light/dark 12:12 cycle (0700 - 1900 light on) and maintained at a temperature of 22 ± 0.5 °C. Laboratory pellets (National Laboratory Animal Center, Thailand) and water were available *ad libitum*. The rats were inexperienced to the holeboard apparatus.

Chemicals

Barakol was extracted and identified by the method described in Thongsaard and colleagues, 2001¹¹. Briefly, a *C. siamea* plant was harvested from the Ratchaburi Province, Thailand between July-August. The fresh young leaves and flowers were cut into small pieces and boiled in 0.5% sulfuric acid for 30 min. The mixture was blended and filtered. Then, the filtrate was alkalinized with concentrated sodium bicarbonate and subsequently extracted with chloroform. The chloroform extract was filtered, mixed with 5% acetic acid and neutralized with 25% ammonium hydroxide to get greenish crystals, crude barakol extract with 0.3% yield. Finally, concentrated hydrochloric acid was added to obtain barakol hydrochloride and the mixture was dried by vacuum filtration to form yellowish crystals, anhydrous barakol hydrochloride. The identity of the compound was checked by thin layer chromatography and nuclear magnetic resonance. Barakol was freshly dissolved in 0.9% saline before injection.

Diazepam (DZP)(10 mg/2 ml ampoule), purchased from Roche (Welwyn Garden City, UK), was diluted with 0.9% saline to 1 mg/kg before use. Paroxetine (Glaxo Smith Kline, Middlesex, UK) was dissolved in distilled water (20 mg/2 ml distilled water). 8-OH-DPAT and WAY 100635, obtained from the Department of Medicinal Chemistry, Wyeth Research, UK) were dissolved in 0.9% saline.

The holeboard apparatus

The holeboard apparatus, raised to a height of 7 cm above floor level, was made of wood covered with dark Formica (62 x 62 x 36 cm) with

sixteen holes, each 4 cm in diameter, equally spaced in the floor. The apparatus was cleaned between rats by wiping the walls and the floor with 20% ethanol. The behaviours were recorded by video camera hanging from the ceiling above the center of the apparatus. The number of rears, head dips, grooming and the time spent head dipping were manually scored by two observers from the video tapes. A dip was considered to take place when the head was introduced into the holes at least to the level of the eyes.

Experiment procedures

All experiments were performed in a quiet room between 8.00 - 12.00 am under low-intensity natural light. The procedures used for the holeboard were modified from that described by File & Wardill (1975)¹². Rats were transferred to the behavioural observation room about 30 min prior to the experiment. Barakol (10 mg/kg, i.p.) was administered 30 min before exposure to the holeboard either in the presence of vehicle (0.9% saline), paroxetine (20 mg/kg, i.p.), 8-OH-DPAT (0.5 mg/kg, s.c.) or diazepam (1 mg/kg, i.p.). In one study the 5HT_{1A} receptor antagonist WAY 100635 (1 mg/kg, s.c.) was given 30 min before 8-OH-DPAT to determine whether the effects of 8-OH-DPAT on exploratory behaviors were mediated via 5HT_{1A} receptor. For the test, rats were placed individually in the centre of the floor of the box and their exploratory behavior examined throughout the 10 min exposure to the holeboard. At the end of the experiment, the animal was returned to its home cage.

The experimental protocol was approved by the Animal Ethics Committee of Srinakharinwirot University for the use of animal subjects and the

procedures are in compliance with the International Guiding Principles for Biomedical Research Involving Animals provided by the National Research Council of Thailand.

Statistical analyses

All data are expressed as mean \pm SEM. The results were analysed using One-way analysis of variance (ANOVA) followed by post hoc Duncan's test. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Rearing behaviour

Both 8-OH-DPAT (0.5 mg/kg, s.c.) and paroxetine (20 mg/kg, i.p.) significantly ($p < 0.05$) lowered the number of rears per minute compared to controls. Prior administration of WAY-100635 (1 mg/kg, s.c.) significantly ($p < 0.05$) reversed the 8-OH-DPAT-induced decrease in rearing. Neither DZP (1 mg/kg, i.p.) nor barakol (10 mg/kg, i.p.) had any significant effect on rearing (Fig. 1).

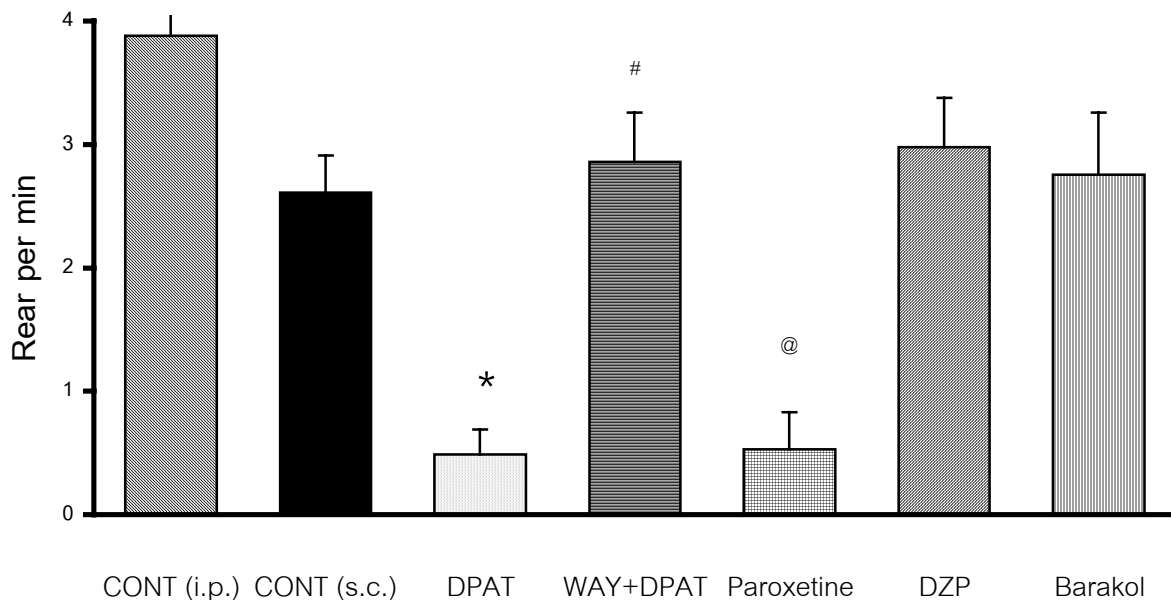


Fig. 1. Effects of barakol (10 mg/kg, i.p.), diazepam (DZP; 1 mg/kg, i.p.), 8-OH-DPAT (DPAT; 0.5 mg/kg, s.c.), 8-OH-DPAT (0.5 mg/kg, s.c.) + WAY-100635 (WAY; 1 mg/kg, s.c.) and paroxetine (20 mg/kg, i.p.) compared to controls (CONT; 0.9% saline either i.p. or s.c.) on number of rear per minute during a 10 min exposure to the holeboard test. Data are expressed as mean \pm SEM of the number of rearing per minute. * $P < 0.05$ compared to subcutaneous injection, # $P < 0.05$ compared to 8-OH-DPAT injection alone, @ $P < 0.05$ compared to controls (i.p.) (n = 10 rats/treatment group) (one way ANOVA with post hoc Duncan's test).

Number of head dips

8-OH-DPAT (0.5 mg/kg, s.c.) alone significantly ($p < 0.05$) decreased the number of head dips while administration of WAY-100635 (1 mg/kg, s.c.) prior to 8-OH-DPAT fully reversed the effects of 8-OH-DPAT. Paroxetine (20 mg/kg, i.p.) also significantly ($p < 0.05$) decreased the number of head dips while DZP (1 mg/kg, i.p.) and barakol (10 mg/kg, i.p.) had no significant effects (Fig 2).

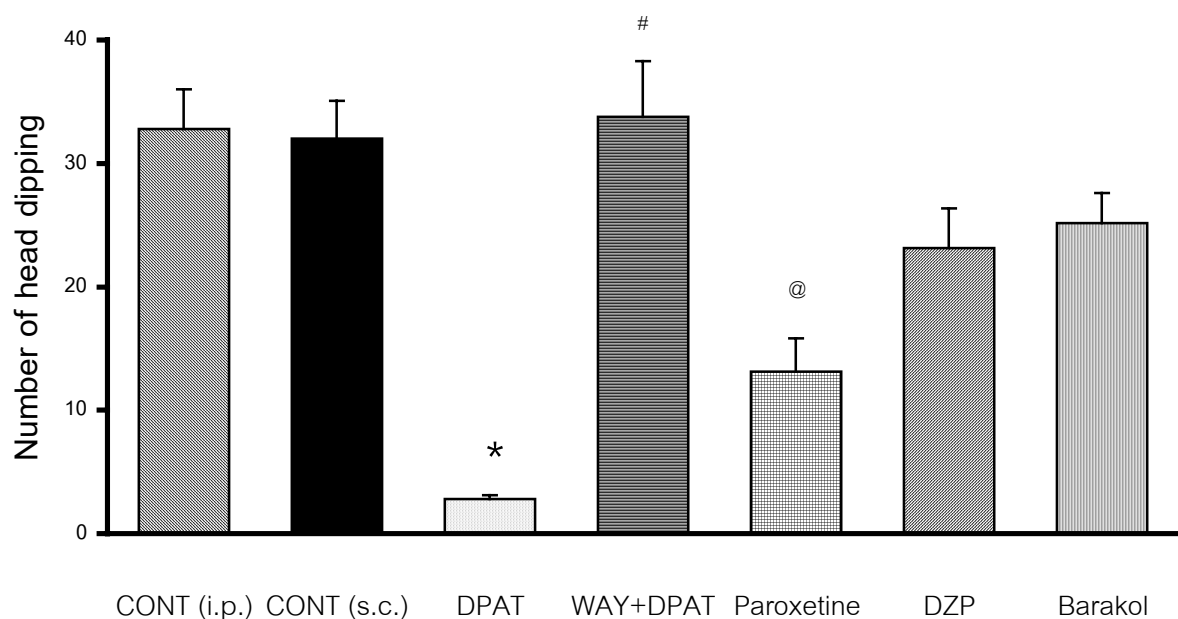


Fig. 2. Effects of barakol (10 mg/kg, i.p.), diazepam (DZP; 1 mg/kg, i.p.), 8-OH-DPAT (DPAT; 0.5 mg/kg, s.c.), 8-OH-DPAT (0.5 mg/kg, s.c.) + WAY-100635 (WAY; 1 mg/kg, s.c.) and paroxetine (20 mg/kg, i.p.) compared to controls (CONT; 0.9% saline either i.p. or s.c.) on number of head dipping during 10 min exposed to holeboard test. Data are expressed as mean \pm SEM of the number of head dipping. * $P < 0.05$ compared to subcutaneous injection, # $P < 0.05$ compared to 8-OH-DPAT injection alone, @ $P < 0.05$ compared to controls (i.p.) (n = 10 rats/treatment group) (one way ANOVA with post hoc Duncan's test).

Time spent head dipping

Both 8-OH-DPAT (0.5 mg/kg, s.c.) and paroxetine (20 mg/kg, i.p.) significantly ($p < 0.05$) reduced the time spent head dipping, and the effects of 8-OH-DPAT were reversed by WAY-100635 (1 mg/kg, s.c.) Again, neither DZP (1 mg/kg, i.p.) nor barakol (10 mg/kg, i.p.) altered time spent head dipping compared to control rats (Fig 3).

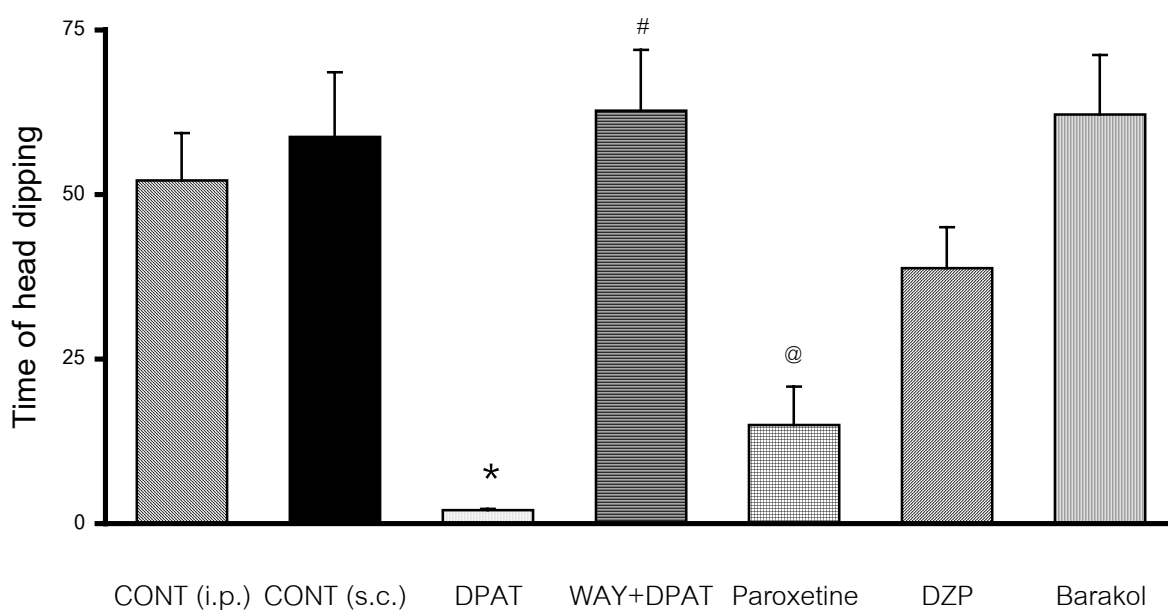


Fig. 3. Effects of barakol (10 mg/kg, i.p.), diazepam (DZP; 1 mg/kg, i.p.), 8-OH-DPAT (DPAT; 0.5 mg/kg, sc.), 8-OH-DPAT (0.5 mg/kg, sc.) + WAY-100635 (WAY; 1 mg/kg, sc.) and paroxetine (20 mg/kg, i.p.) compared to controls (CONT; 0.9% saline either i.p. or s.c.) on time of head dipping during 10 min exposed to holeboard test. Data are expressed as mean \pm SEM of time of head dipping. * $P < 0.05$ compared to subcutaneous injection, # $P < 0.05$ compared to 8-OH-DPAT injection alone, @ $P < 0.05$ compared to controls (i.p.) ($n = 10$ rats/treatment group) (one way ANOVA with post hoc Duncan's test).

Grooming

Paroxetine (20 mg/kg, i.p.) significantly ($P < 0.05$) decreased the number of grooms while 8-OH-DPAT (0.5 mg/kg, s.c.), WAY-100635 (1 mg/kg, s.c.) prior to 8-OH-DPAT, DZP (1 mg/kg, i.p.) or barakol (10 mg/kg, i.p.) had no effect on grooming (Fig. 4).

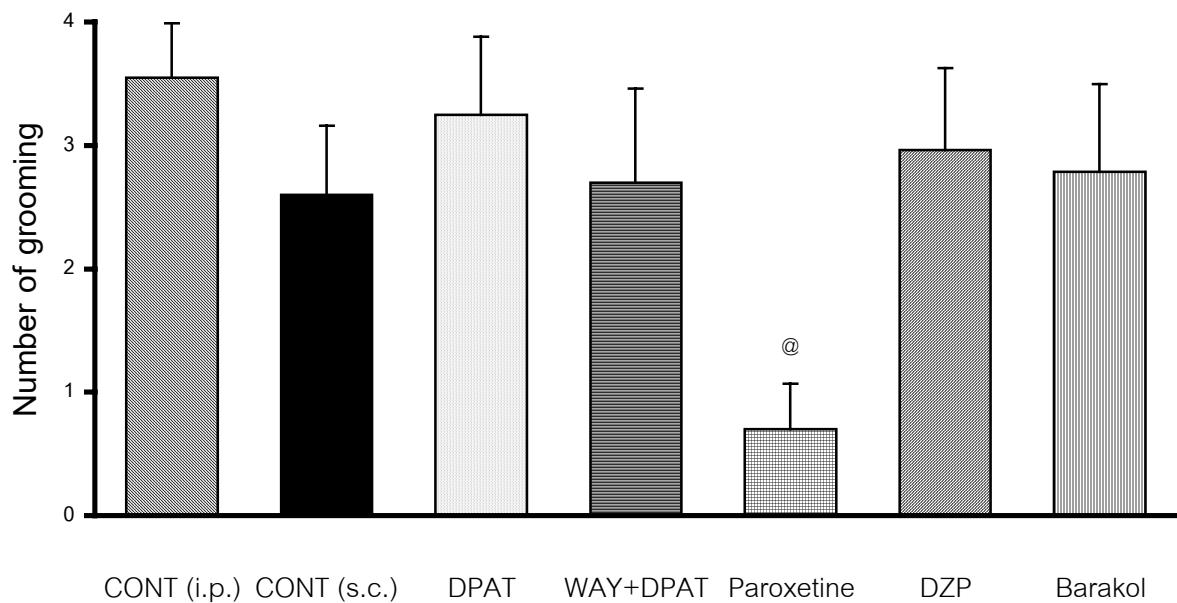


Fig. 4. Effects of barakol (10 mg/kg, i.p.), diazepam (DZP; 1 mg/kg, i.p.), 8-OH-DPAT (DPAT; 0.5 mg/kg, s.c.), 8-OH-DPAT (0.5 mg/kg, s.c.) + WAY-100635 (WAY; 1 mg/kg, s.c.) and paroxetine (20 mg/kg, i.p.) compared to controls (CONT; 0.9% saline either i.p. or s.c.) on number of grooming during 10 min exposed to holeboard test. Data are expressed as mean \pm SEM of the number of grooming. @ $P < 0.05$ compared to controls (i.p.) ($n = 10$ rats/treatment group) (one way ANOVA with post hoc Duncan's test).

DISCUSSION

The present study confirms previous findings that 8-OH-DPAT (0.5 mg/kg, s.c.), but not the benzodiazepine diazepam (1 mg/kg, i.p.) significantly decreased exploratory behaviours including, rearing, number of head dips and time spent head dipping and these effects were antagonised by the 5HT_{1A} receptor antagonist, WAY 100635 (1 mg/kg, s.c.). Acute paroxetine (20 mg/kg, i.p.) also inhibited all exploratory activity and grooming. In contrast, barakol (10 mg/kg, i.p.) like diazepam had no effect on exploratory behaviour using the holeboard.

The present data show that acute administration of 8-OH-DPAT (0.5 mg/kg, s.c.) to rats clearly inhibited exploration including the number of rears per min, the number of head dips and time spent head dipping. This result is consistent with a previous study⁸ showing that 8-OH-DPAT (0.03 - 1 mg/kg, i.p.) but not benzodiazepine dose-dependently decreased all of the exploratory behaviours in mice observed using a holeboard. WAY 100635 (1 mg/kg, s.c.) reversed the inhibition of exploratory behaviour produced by 8-OH-DPAT, suggesting that the effects on exploration of the holeboard are mediated by 5HT_{1A} receptor and not 5HT₇ receptor which are also activated by 8-OH-DPAT¹³.

Acute administration of paroxetine (20 mg/kg, i.p.), a 5HT re-uptake inhibitor, significantly decreased the number of grooms, rears per min, number of head dips and time spent head dipping. These results indicate that the mechanism by which paroxetine inhibit exploratory behaviour may be associated with increased extracellular 5HT resulting from 5HT reuptake inhibition causing 5HT_{1A} receptor activation. Conversely, it has been

shown that 14 days after intracerebroventricular injection of the neurotoxin 5, 7- dihydroxytryptamine (5, 7-DHT), which depletes 5HT there was a significant increase exploration in rat on holeboard compared to controls¹⁴, a finding supported by the present study. However, in a previous study some other selective 5HT uptake inhibitors, fluoxetine, fluvoxamine and citalopram, had no effect on any parameters measured with the holeboard test¹⁵.

A previous study found that chronic treatment with DZP (2 mg/kg, oral) throughout gestation resulted in reduced holeboard exploration in offspring mice (mean age 30.6 days) compared to controls¹⁶. There is however no evidence that acute DZP administration affects exploratory behaviour using the holeboard test,⁸ a finding replicated in the present study.

Surprisingly, barakol produced no change in exploratory activity with the holeboard test in contrast to previous results using the same dose (10 mg/kg) with the elevated plus maze². The results may indicate that barakol does not have effects on serotonergic function that can result in a behavioural change in exploratory behaviour using the holeboard. These results may further indicate that alterations in serotonin function are not important in the potential anxiolytic properties of barakol.

In summary, the present study demonstrated acute treatment of barakol and diazepam had no effect on holeboard exploratory behaviour while 5HT_{1A} receptor activation reduced exploration. Future studies on the effect of barakol on holeboard exploration need to be extended using a wider dose range of barakol and to investigate whether chronic treatment with barakol alters exploratory behaviour.

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REFERENCES

1. Thongsaard W, Deachapunya C, Pongsakorn S, et al. Barakol: a potential anxiolytic extracted from *Cassia siamea*. *Pharmacol Biochem Behav* 1996;53:753-8.
2. Thongsaard W, Pongsakorn S, Sudsuang R, et al. Barakol, a natural anxiolytic, inhibits striatal dopamine release but off uptake in vitro. *Eur J Pharmacol* 1997;319:157-64.
3. Sukma M, Chaichantipyuth C, Murakami Y, et al. CNS inhibitory effects of barakol, a constituent of *Cassia siamea* Lamk. *J Ethnopharmacol* 2002 ; 83:87-94.
4. Fiorino DF, Treit D, Menard J, et al. Is barakol anxiolytic? *Behav Pharmacol* 1998;9:375-8.
5. Arunlakshana O. Pharmacological study of the levels of *Cassia siamea*. *Sririraj Hospital Gazette* 1949;1:434-44.
6. Jantarayaota P. Effects of barakol extracted from leaves of *Cassia siamea* on the central nervous system. Thesis submitted for Master of Science degree in Pharmacy, Chulalongkorn University, Bangkok.
7. Tongroach P, Jantarayota P, Tantisira B, et al. Barakol, a neuroactive compound from *Cassia siamea*. In *The First JSPS-NRCT Joint Seminar in Pharmaceutical Science; Advance in Research on Pharmaceutical Science; Advance in Research on Pharmacologically active Substances from Natural Source*. pp. OP21. Chiangmai, Thailand.
8. Tsuji M, Takeda H, Matsumiya T. Different effects of 5-HT_{1A} receptor agonists and benzodiazepine anxiolytics on the emotional state of naive and stressed mice: a study using the hole-board test. *Psychopharmacology (Berl)* 2000;152:157-66.
9. Barros M, Mello EL, Maior RS, et al. Anxiolytic-like effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 in non-human primates. *Eur J Pharmacol* 2003;482:197-203.
10. Canto-de-Souza A, Luiz Nunes-de-Souza R, Rodgers RJ. Anxiolytic-like effect of WAY-100635 microinfusions into the median (but not dorsal) raphe nucleus in mice exposed to the plus-maze: influence of prior test experience. *Brain Res* 2002;928:50-9.
11. Thongsaard W, Chainakul S, Bennett GW, et al. Determination of barakol extracted from *Cassia siamea* by HPLC with electrochemical detection. *J Pharm Biomed Anal* 2001;25:853-9.
12. File SE, Wardill AG. The reliability of the hole-board apparatus. *Psychopharmacologia*. 1975;44:47-51.
13. Kennaway DJ, Rowe SA, Ferguson SA. Serotonin agonists mimic the phase shifting effects of light on the melatonin rhythm in rats. *Brain Res* 1996;737:301-7.
14. Mogensen J, Wörtwein G, Plenge P, et al. Serotonin, locomotion, exploration, and place recall in the rat. *Pharmacology Biochemistry and Behavior* 2003;75:381-95.
15. Durcan MJ, Lister RG, Eckardt MJ, et al. Interactions of 5HT reuptake inhibitors and ethanol in tests of exploration and anxiety. *Adv Alcohol Subst Abuse* 1988;7:113-7.
16. Barbier P, Breteau J, Autret E, et al. Effects of prenatal exposure to diazepam on exploration behavior and learning retention in mice. *Dev Pharmacol Ther* 1991;17:35-43.