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Barakol: A Potential Anxiolytic Extracted From *Cassia siamea*

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THONGSAARD, W., C. DEACHAPUNYA, S. PONGSAKORN, E. A. BOYD, G. W. BENNETT AND C. A. MARSDEN. *Barakol: A potential anxiolytic extracted from* Cassia siamea. PHARMACOL BIOCHEM BEHAV 53(3) 753–758, 1996.—The behavioural effects of an extract of *Cassia siamea*, a plant used in Thai traditional medicine, and barakol, its active chemical, were studied on an elevated plus-maze compared with diazepam. An aqueous extract of *C. siamea* (1, 6, and 12 g/kg body wt., orally) produced a small increase in the percentage of the open : total number of arm entries and time, time spent on the end of the open arms, total number of arm entries, and number of rears/min. Barakol [10 mg/kg, intraperitoneally (IP)] significantly increased all of these behavioural parameters in a manner similar to diazepam (1 mg/kg, IP, 30 or 60 min before testing), except that barakol and not diazepam increased both the number of rears and total arm entries. Barakol at 25 and 50 mg/kg increased the percentage of the open : total number of arm entries and time and number of rears. The results indicate that barakol has anxiolytic properties similar to diazepam but differs from diazepam in that it also increases exploratory and locomotor behaviour, as shown by the number of rears and total arm entries.

Cassia siamea Barakol Diazepam Elevated plus-maze Anxiolytic

CASSIA SIAMEA is widely cultivated in Southeast Asia, including Thailand. Different parts of this plant can be used for various medical purposes. For example, the root is used as an antipyretic in fever; the bark is used to treat skin disease and haemorrhoids; the leaves for constipation, diabetes, hypertension, and insomnia; and the flowers for insomnia and asthma (13,14,18). The preparation recommended in primary health care to treat insomnia is an aqueous extract of fresh or dried leaves. In 1949, Arunlakshana (1) reported that an alcoholic extract of the leaves of *C. siamea* can inhibit central nervous activity and increase tension in smooth muscle, and has a diuretic effect. Barakol is a biologically active constituent of the extract of *C. siamea*. It was first extracted and partially characterised by Hassanali-Walji et al. in 1969 (11). Barakol contains the unusual tricyclic 3a,4-dihydro-3a,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene ring system and has been shown to exist as a hydrate (3,10) (Fig. 1A). Previous studies have shown it to have dopamine agonist and possible serotonergic antagonist properties, as it suppresses 5-hydroxytryptophan (5-HTP) head shakes in mice (20).

The present study compared the effects of *C. siamea* extracts and barakol on behaviour in the elevated plus-maze, a behavioural test for anxiolytic drugs (6,9). Furthermore, the effects of barakol and diazepam on the elevated plus-maze were compared to determine whether barakol has a similar behavioural profile to an established anxiolytic drug. Previous studies (16,21) showed that saline-treated control animals spent the greater amount of time on the closed arms of the plus-maze. This preference appears to reflect an aversion towards the open arms, caused by fear and anxiety (2,15), whereas an increased preference towards the open arms after diazepam treatment indicates an anxiolytic effect (16,17). Some of the results in this current study have been presented in preliminary form to the British Pharmacological Society (19).

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Fig. 1A

The structure of Barakol

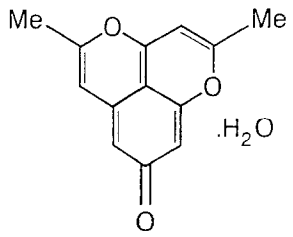

Fig. 1B

Barakol: NMR spectrum (250 MHz)

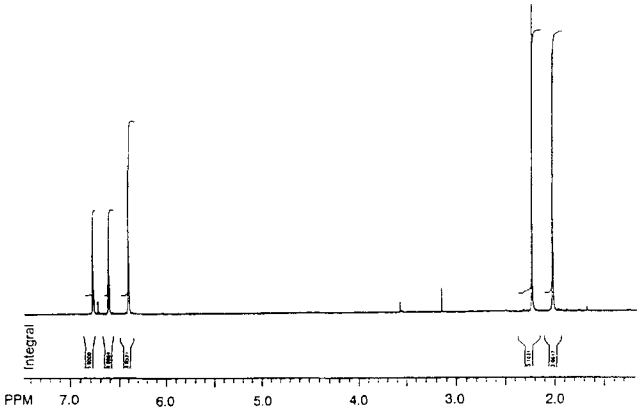

FIG. 1. (A) The structure of barakol. (B) Barakol : NMR spectrum (250 MHz).

METHOD

Animals

All experiments were performed using male Wistar rats, weighing 150–170 g, obtained from the National Laboratory Animal Centre, Mahidol University, Thailand. They were housed in groups of four to six in a room with a 14 L : 10 D cycle and maintained on laboratory pellets (Pokphand Animal Feed Co., Bangkok, Thailand) and tapwater ad lib. Animals were naive to an elevated plus-maze.

Apparatus

The plus-maze, made of wood covered with dark Formica, was elevated to a height of 70 cm and consisted of two open arms, 45 × 15 cm, and two closed arms, 45 × 15 × 10 cm, with an open roof, arranged so that the two pairs of identical arms were opposite each other. There were lines across the arms of the plus-maze at the entrance and halfway along each of the arms. Different aspects of the animals' behaviour were recorded directly by two observers sitting 2 m away from the plus-maze in the same room. The plus-maze was carefully wiped with a wet towel after each animal. All experiments were carried out between 1000 and 1400 h under low-intensity natural light.

Plant Material and Drug

Cassia siamea was obtained from Bangkok, Thailand, and its leaves and flowers were dried by sunlight and pulverised into a powder. The water extract of *C. siamea* was prepared immediately before use by boiling weighed aliquots of the powder in distilled water for 15 min. The boiled material was filtered, cooled, and given orally.

Barakol was extracted and purified by the following method (4). Fresh young leaves of *C. siamea* were cut into small pieces and boiled twice with 2% aqueous acetic acid for 1 h. All fractions of water extract were filtered, combined, and alkalinised with concentrated ammonia solution. The mixture was further extracted with chloroform, which was washed with water. The solution was concentrated and shaken with 5% aqueous acetic acid until the extract became colourless. The acidified chloroform extract was neutralised carefully with concentrated ammonia solution and cooled. The crude barakol was crystallised as greenish yellow needles (4). The purified barakol was shown to be pure by nuclear magnetic resonance (Fig. 1B) and chromatographic techniques. Barakol was dissolved in 0.9% saline immediately before injection.

Diazepam was obtained from Roche (Welwyn Garden City, UK) in a solvent form (10 mg/2 ml ampoule). It was diluted with 0.9% saline to 1 mg/kg before use.

Procedure

The study consisted of three sets of experiments: First, the effects of three doses of *C. siamea* extract were tested on the behavioural changes on the plus-maze; second, the effects of various doses of barakol were similarly examined; and third, a comparative study of the effect of barakol and diazepam was performed. Animals were randomly divided into groups of 10 for each treatment in all experiments. In the first experiment, each group received an oral administration of 1, 6, or 12 g of the original extract of *C. siamea* at 1.5 ml/kg body wt. with controls receiving distilled water. Sixty minutes later, each rat was tested individually for 5 min on the plus-maze apparatus. The rat was placed on the centre of the plus-maze facing an open arm. An arm entry was defined as placing all four legs over the line marking that area (an end of arm entry was when the rat crossed the line halfway along the arm).

The cumulative time spent on, and the number of entries made into, the open end of open, closed, and ends of closed arms, and the total number of supported and unsupported rears on the arms of the maze were recorded. A rear was defined as the elevation of both forepaws off the ground. The open-arm data are expressed as a percentage of the total time spent on, and the total number of entries made into, both the open and closed arms and the rearing data as the rears per minute in all arms. The percentage of the open:total number of arm entries and the time, and the time on the end of the open arms were used as indices of anxiety, whereas the total number of arm entries and the number of rears per minute were used as indices of locomotor activity.

The second experiment was similar to the first, except that the animals received an IP injection of barakol (10, 25, 50, or 75 mg/kg) with control receiving saline, and each rat was tested individually on the plus-maze 30 min after injection.

For the third experiment, the effects of barakol (10 mg/kg, IP) were compared with diazepam (1 mg/kg, IP). In addition to barakol alone and a control (saline) group, further groups of rats received diazepam 30 or 60 min before being placed on the plus-maze; another group received diazepam 30 min be-

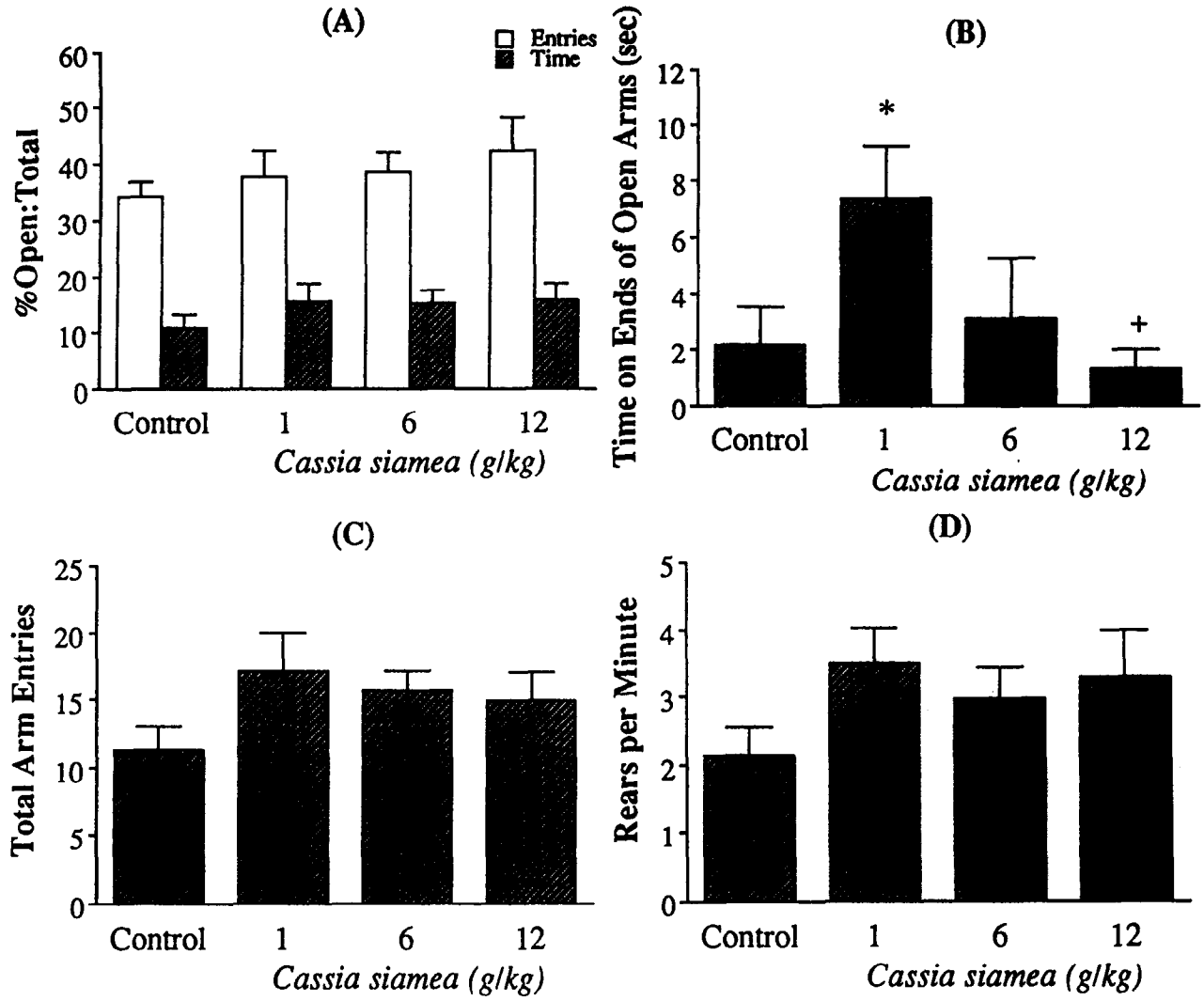

FIG. 2. (A–D) Effects of an aqueous extract of *Cassia siamea* (1, 6, and 12 g/kg, orally; $n = 10$) on (A) the percentage of the open : total number of arm entries and time, (B) the time spent on the end of the open arms, (C) the total number of arm entries, and (D) the number of rears per minute. Behaviour was monitored in the elevated plus-maze for a 5-min period 60 min after drug administration. Data are the means ± SEM. $^{*}p < 0.05$ compared with control, and $^{+}p < 0.05$ compared with *C. siamea* extract (1 g/kg) using a one-way ANOVA with post hoc Duncan's new multiple range test.

fore barakol followed by testing on the plus-maze 30 min later.

Statistics

All data are expressed as the mean ± SEM. The ratios of open : total number of arm entries and time were calculated for each rat individually and the mean ± SEM for each treatment group are presented. All data were analysed using a one-way analysis of variance (ANOVA) with post hoc Duncan's new multiple range test. $p < 0.05$ was considered significant.

RESULTS

Cassia siamea at doses of 1, 6, and 12 g/kg, orally, did not significantly increase the percentage of the open : total arm entries and time, and total arm entries and rears (Fig. 2A, C, and D). The time spent on the ends of the open arms, however, was significantly increased with the 1 g/kg dose compared with the control, whereas the response with 12 g/kg was significantly reduced compared with 1 g/kg (Fig. 2B).

Barakol (10 mg/kg, IP) significantly increased all parameters measured on the plus-maze (percentage of the open : total number of arm entries and time, time spent on the end of the open arms, and total arm entries and rears) (Fig. 3A–D). Barakol (25 and 50 mg/kg, IP) also increased the percentage of the open : total number of arm entries and time, but had no significant effect on the other parameters compared with control. These were significantly reduced when compared with barakol (10 mg/kg). With the highest dose of barakol (75 mg/kg, IP), all parameters measured were significantly reduced compared with the response using the lowest dose (10 mg/kg) (Fig. 3A–D).

In the third study that compared the effects of barakol (10

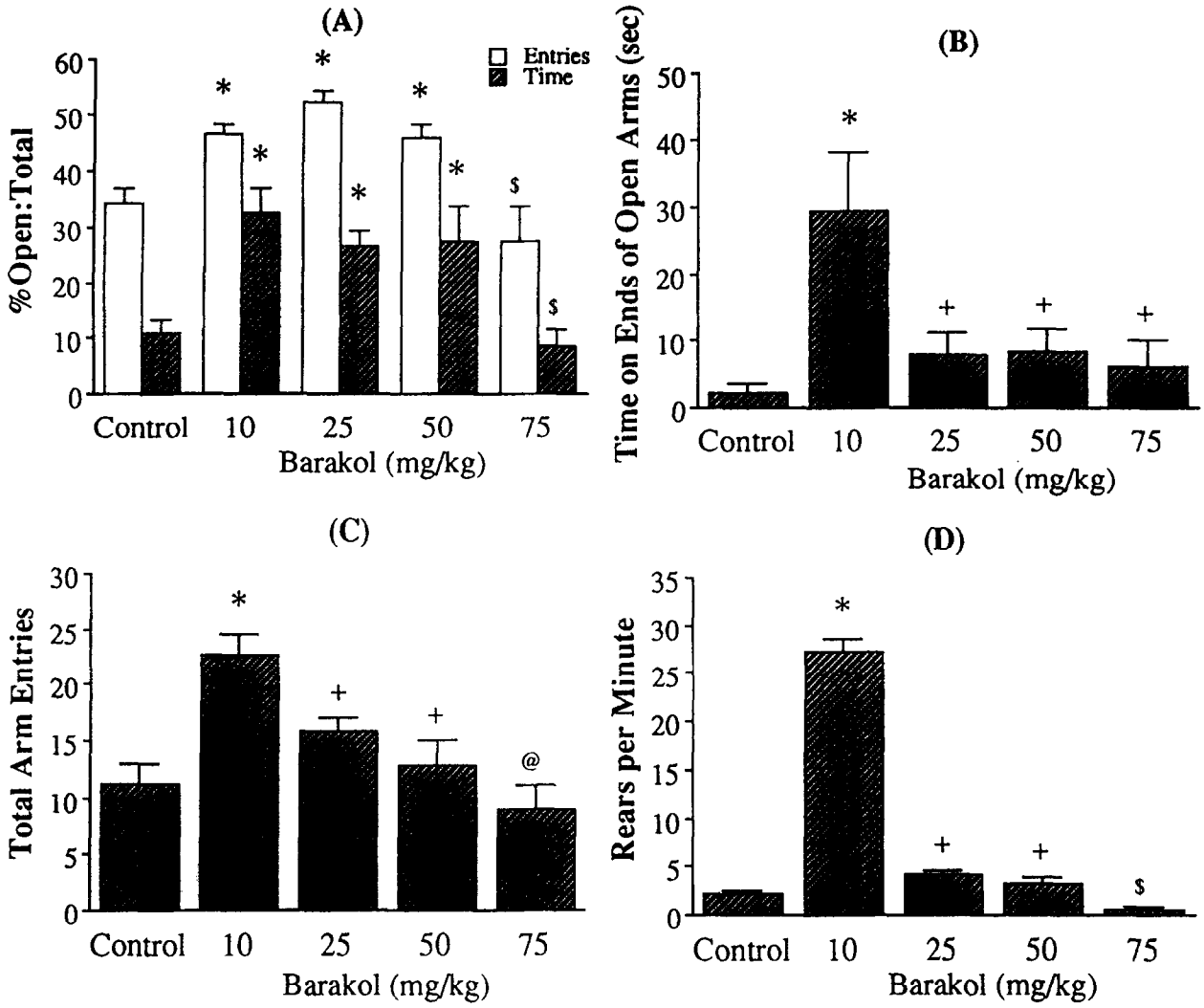

FIG. 3. (A-D) Effects of barakol (10, 25, 50, and 75 mg/kg, IP; $n = 10$) on (A) the percentage of the open : total number of arm entries and time, (B) the time spent on the end of the open arms, (C) the total number of arm entries, and (D) the number of rears per minute. Behaviour was monitored on the elevated plus-maze for 5 min, 30 min after drug administration. Data are the means ± SEM. $^{*}p < 0.05$ compared with control, $^{+}p < 0.05$ compared with barakol (10 mg/kg), $p < 0.05$ compared with barakol (10 and 25 mg/kg), and $^{\$}p < 0.05$ compared with all other doses of barakol using a one-way ANOVA with post hoc Duncan's new multiple range test.

mg/kg) with those of diazepam (1 mg/kg, IP), barakol again increased all parameters measured (Fig. 4A-D). Diazepam given either 30 or 60 min before testing produced a similar behavioural profile on the plus-maze, but in contrast to barakol did not significantly increase the total arm entries and had no effect on rearing (Fig. 4A-D). When diazepam was given 30 min before barakol (60 min before testing), the behavioural profile was similar to that of barakol alone (Fig. 4A-C), except for a marked reduction in the number of rears (Fig. 4D); compared with diazepam (60 min) alone, the only difference was a significant increase in the total number of arm entries (Fig. 4C).

DISCUSSION

The behaviour observed using the plus-maze in the present study confirmed the anxiolytic activity of diazepam as reported previously (16,21). Using this test, extracts of *C. siamea* had no marked effects on behaviour in the plus-maze except for the effect of the low dose on the time spent on the ends of the open arms. However, the active constituent of *C. siamea*, barakol, particularly at the lowest dose, showed significant anxiolytic properties when tested on the elevated plus-maze, similar to that of diazepam. Barakol is a dioxaphenalene derivative which in previous studies (20) had been shown to have dopamine agonist and possible serotonergic antagonist properties: It suppresses 5-hydroxytryptophan (5-HTP) head shakes, indicating an antagonist effect at postsynaptic 5-HT_2 receptors (7).

In contrast to diazepam, barakol also markedly increased the number of rears on the plus-maze. When barakol was given together with diazepam, this increase in rearing was very significantly reduced compared with that of barakol alone. This result suggests that barakol has a potent effect on exploratory behaviour, which is inhibited by diazepam. Barakol also resulted in increased total arm entries, indicating an effect on locomotor behaviour, which was not observed with diazepam.

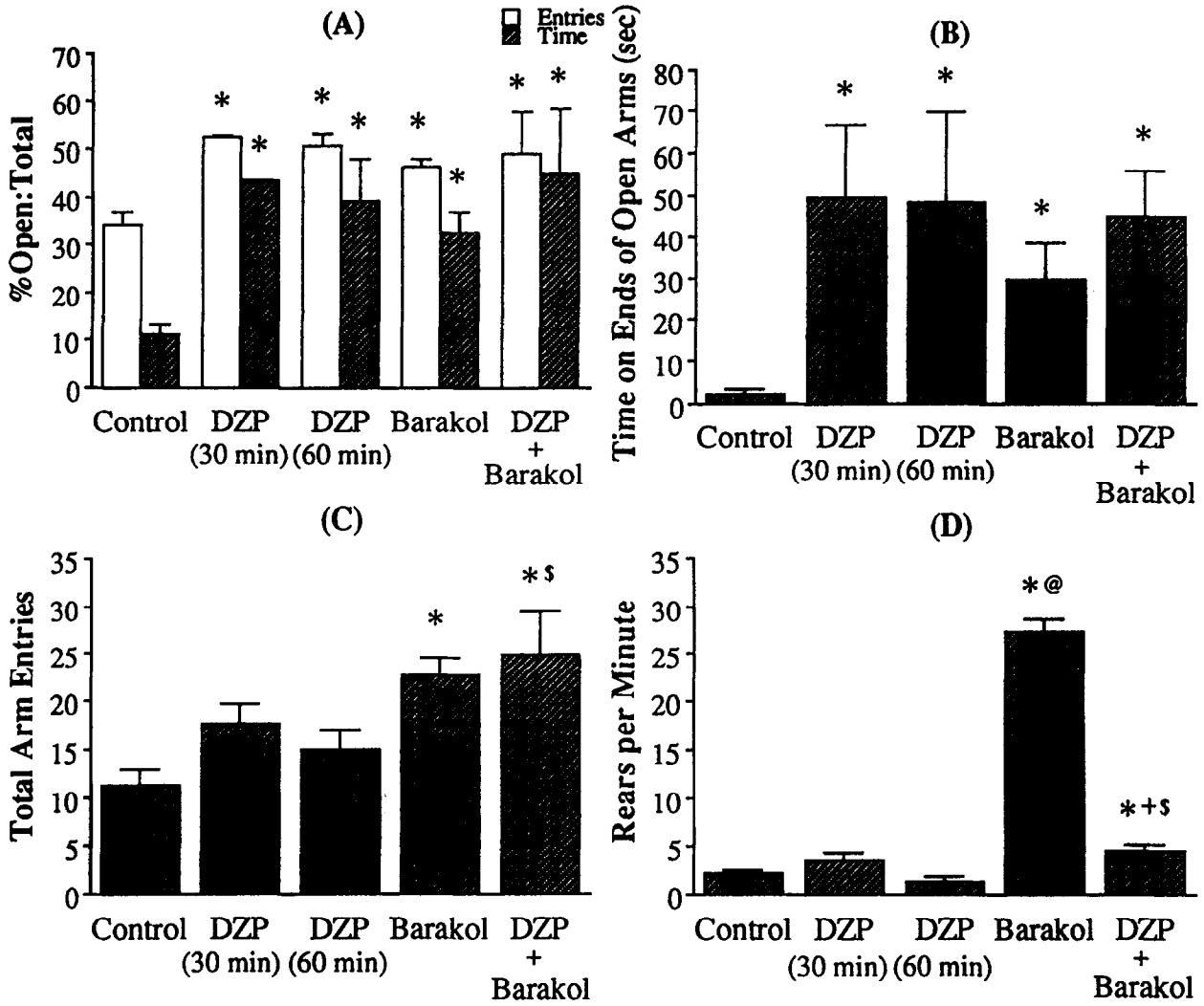

FIG. 4. (A–D) Effects of diazepam (DZP, 1 mg/kg, IP; $n = 10$), barakol (10 mg/kg, IP; $n = 10$), and DZP (1 mg/kg, IP) plus barakol (10 mg/kg, IP; $n = 10$) on (A) the percentage of the open : total number of arm entries and time, (B) the time on ends of open arms, (C) the total number of arm entries, and (D) the number of rears per minute. Behaviour was monitored in the elevated plus-maze for 5 min, 30 or 60 min after DZP and 30 min after barakol administrations. In the combination study, DZP was administered 30 min before barakol. Data are the means ± SEM. $^{*}p < 0.05$ compared with control, $^{+}p < 0.05$ compared with barakol, $p < 0.05$ compared with DZP (30 min), and $^{\$}p < 0.05$ compared with DZP (60 min) using a one-way ANOVA with post hoc Duncan's new multiple range test.

The combination of barakol and diazepam also showed enhanced locomotor behaviour similar to that of barakol alone. This result suggested that whereas diazepam inhibited the effect of barakol on exploratory behaviour, it did not alter the effect of barakol on locomotion.

Thus, barakol (10 mg/kg, IP) has the profile of an anxiolytic drug, but unlike diazepam, this appears to be associated with increased exploratory and locomotor behaviour. It remains to be determined whether barakol affects GABAergic mechanisms similar to diazepam (8), because barakol has been shown not to reverse the convulsant effects of bicuculline (12), which indicates that the compound has no direct action on $GABA_A$ receptors. Barakol may differ from diazepam in its effects on exploratory and locomotor behaviour as a result of an agonist effect at dopamine receptors. Alternatively, because barakol antagonises 5-HT_2 receptor-mediated behaviour in mice (20), and the 5-HT_2-receptor antagonist ritanserin has reported anxiolytic effects (5), the behavioural effects of barakol observed in the present study could also involve 5-HT mechanisms. The anxiolytic properties and increased exploratory and locomotor behaviour of barakol were reduced at higher doses (25 and 50 mg/kg, IP) and not observed at the highest dose studied (75 mg/kg, IP), indicating that other pharmacologic actions come into play at higher doses. The reasons for this need to be clarified in future experiments.

In summary, barakol, an extract of the plant *C. siamea*, exhibits not only anxiolytic activity at low doses similar to diazepam but also exploratory and locomotor behaviour not shown by diazepam. The precise mechanisms involved in these actions need to be identified, but they indicate the value of further investigation of alkaloids with known, or anecdotal, behavioural effects and possible future clinical application.

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