

Involvement of GABA in Nootropic and Anxiolytic Activity of Saponins of *Albizzia lebeck* Leaves

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Abstract

The effect of saponin containing n-butanolic fraction (BF) extracted from dried leaves of *Albizzia lebeck* was studied on cognitive behavior and anxiety in albino mice. An elevated plus maze was used for assessment of both nootropic and anxiolytic activity. The nootropic activity was assessed by recording the effect of BF (10-50 mg/kg) on the transfer latency, anxiolytic activity was evaluated by measuring the effect on duration of occupancy in the open arm. The studies showed that BF possesses anxiolytic activity and nootropic activity. BF inhibited baclofen-induced hypothermia and passivity. Thus the study suggests that saponins act by modifying GABA ergic mechanism.

INTRODUCTION

In recent years, there has been a phenomenal rise in the interest of scientific community in the pharmacological action of herbs, verifying the claims made about them in the official books of Ayurveda. Several plants have been reported to possess nootropic activity (Nadkarni, 1996). Drugs having different chemical structures exhibit nootropic activity. The alkaloids from *Vinca minor* and *Secale cornutum* (Giurgea, 1973), saponins, bacoside A and B from *Bacopa monnieri* (Singh and Dhavan, 1997) and ginsenoside Rb1 and Rb1-influene, the saponins from *Panax ginseng* (Ying et al., 1994), are the active principles responsible for enhancing cognitive behavior. Since the leaves of *Albizzia lebeck* Benth (Fam. Mimosaceae) are rich in saponins (Itoh et al., 1990), we investigated the nootropic activity of this plant.

For many years it was thought that anxiolytic agents impair learning and memory, both in animals and humans. The search for alternative to the BZD lead to the finding that antagonists at the serotonin 5-HT₃ receptor cause anxiolysis and also improve memory simultaneously (Barness et al., 1990; Blackburn et al., 1993). Many researchers have shown that some nootropic agents improve retention but not acquisition (Hasenohrl et al., 1998; Jaiswal and Bhattacharya, 1992). The previous studies on the saponin containing fraction of *A. lebeck* showed no significant effect on active avoidance learning in mice but improved object recognition. The brain concentration of gamma-aminobutyric acid and dopamine was decreased whereas the serotonin level was increased (Chintawar et al., 2002). In the present study, we examined the effect of n-butanolic fraction (BF) on retention of learned task and anxiolytic activity of saponin containing (BF) of *A. lebeck*. We also studied the effect of BF on the behavior mediated *via* GABA because GABA receptors are implicated in long term potentiation and memory (Olpe et al., 1993; Staubli and Xu, 1995).

MATERIALS AND METHODS

Isolation of Saponins

Saponins were isolated from dried leaves of *A. lebeck* as described by Pal et al. (1995). In brief, shade dried leaves of *A. lebeck* were defatted with petroleum ether (60-

80°C) in Soxhlet's extractor. The marc was dried and extracted with methanol. The methanolic extract was evaporated to dryness in a vacuum. The residue was suspended in water, extracted with ethyl acetate and n-butanol (3 x 300 ml each) and the solution was evaporated to dryness in a vacuum to provide ethyl acetate (3.5 g), n-butanol (11.0 g) and water soluble (3.5 g) portions.

The n-butanol soluble fraction (BF) was tested for presence of saponins using haemolysis test and foam test as described by Evans (1996).

Animals

Albino mice (Swiss, 20-22 g) and rats (Wistar, 125-150 g) of either sex were used in this study. The animals were allowed to acclimatize to the laboratory conditions for 10 days after their arrival. The animals were housed in groups of six under standard housing conditions. Food was withheld from animals overnight prior to drug administration and during the experiment. All experiments were carried out during the light period (08:00-16:00 h).

Drugs

Piracetam (Uni-UCB, India), scopolamine (German Remedies, India) (\pm) baclofen (Hindustan Ciba-Geigy Ltd., India) and ANXOL inj. (diazepam, Sigma Labs, India) were used in this study. All drugs were dissolved in distilled water and administered intraperitoneally except when stated otherwise.

1. Assessment of Nootropic Activity. An elevated plus maze consisting of two open arms (35 x 6 cm) and two enclosed arms (35 x 6 x 15 cm) was used. The maze was elevated to the height of 25 cm. Mice were placed individually at the end of an open arm facing away from the central platform and the time it took from the end of open arm to either of the closed arms (transfer latency, TL) was recorded (Pellow and File, 1986). On the first day, mice were allowed to explore the plus maze after the measurement of TL. On the following day, mice received vehicle, piracetam (100 mg/kg) or BF (10-50 mg/kg) 30 min before their placement on the elevated plus maze as before and TL was noted again. The TL was expressed as retention after 24 hr by calculating the "inflection ratio" using the formula described by Jaiswal and Bhattacharya (1992): inflexion ratio = $(L_1 - L_0)/L_0$, where L_0 = transfer latency after 24 hr and L_1 = initial transfer latency in seconds.

Anxiolytic Activity

Mice were individually placed in the center of the plus maze (same dimension as used for measurement for TL) facing an enclosed arm. The time spent by the mouse during the next 5 min on the open and closed arms was recorded along with the number of entries into the closed arm. Animals were treated with vehicle, BF (10-50 mg/kg) or diazepam (1 mg/kg) 30 min before their placement on the maze. Each group contained 6 animals.

Behavioral Studies

1. Baclofen-induced Hypothermia. Baclofen-induced hypothermia was used to assess the effect of drugs influencing GABA mediated behavior (Jackson and Nutt, 1991). Albino rats were taken into groups of 5 each. BF (25 mg/kg) was administered 30 min before baclofen and rectal temperature was recorded, using telethermometer (Electrolab, India), every 30 min after baclofen (10 mg/kg) until 180 min.

2. Baclofen-induced Passivity. Rats were divided into groups of six each and administered baclofen (10 mg/kg) 30 min after BF, 25 mg/kg and passivity was scored every 30 min until 180 min as described by Turner (1972). In brief, rat was grasped with the thumb and index finger, which held the dorsal skin of the neck while the rat was walking.

3. Scoring System. An unaffected rat escaped (score 0). The rat still grasped in the same manner held in vertical position, struggled (score 2), the unaffected rat when placed in supine position on the back of observers hand so that the thumb can support head of the

rat, escaped (score 4), the unaffected rat tried to escape when held vertically by one forepaw (score 6) or by one hindpaw (score 8). Intermediate scores were used when struggle was diminished but not abolished.

4. Statistics. The observations are given as mean \pm SEM. The data was analyzed by one way ANOVA followed by Student's t test. Baclofen-induced passivity was analyzed by Mann-Whitney U test. $P < 0.05$ was considered significant.

RESULTS

Nootropic Activity

The retention of learned task was studied after 24hr as transfer latency on the elevated plus maze. The effect on transfer latency was expressed by inflexion ratio (IR). Increase in IR after 24 hr indicated improved retention of learned task. Piracetam showed significant increase in IR compared to vehicle treated group and antagonized the effect of scopolamine also. The BF in all the three doses increased the IR and antagonized the effect of scopolamine $F(7, 40) = 8.3, P < 0.05$ (Fig. 1).

Anxiolytic Activity

The vehicle treated mice spent 220.16 ± 13.0 sec with 4.16 ± 0.7 number of entries into enclosed arm. Diazepam (1 mg/kg) and BF (25 and 50 mg/kg) showed significant ($P < 0.01$) decrease in time spent in enclosed arm whereas BF, 10 mg/kg showed insignificant decrease in the time spent in enclosed arm. Both diazepam and BF, 25 mg/kg exhibited increased entries in the open arm. The observations are shown in Fig. 2.

Baclofen-induced Hypothermia

Baclofen produced decrease in rectal temperature from $35.6 \pm 0.04^\circ\text{C}$ to $32.9 \pm 0.3^\circ\text{C}$ at 90 min. The peak hypothermic effect was observed 90 min after baclofen in the vehicle treated group. Prior treatment with BF (25 mg/kg) significantly ($P < 0.01$) inhibited the hypothermic activity of baclofen. The observations are shown in Fig. 3.

Baclofen-induced Passivity

Baclofen gradually induced passivity in all the animals and peak effect was observed 120 min after baclofen, when the passivity score was 7.5 ± 0.4 . The prior administration of BF (25 mg/kg) significantly reduced the passivity and the maximum score of 4.0 ± 0.4 was observed after 90 min. The observations are shown in Fig. 4.

DISCUSSION

The study observed that the saponin-containing fraction (BF) of leaves of *Albizzia lebeck* possessed nootropic and anxiolytic activity. This indicated dissociation between hypomnesic and anxiolytic activity as observed previously by Hasenohrl and his associates (Hasenohrl et al., 1998). The BF inhibited the GABA_B mediated behavior as indicated by diminished hypothermic effect of baclofen in presence of BF. The higher dose of the BF (50 mg/kg) preceded neurological deficit and approximate oral median lethal dose was 100 mg/kg. This suggests that the BF has a narrow margin of safety.

The nootropic drugs belong to the category of psychotropic agents with selective facilitatory effect on intellectual performance, learning and memory (Giurgea, 1973). The elevated plus maze is a widely accepted model to study nootropic activity (Itoh et al., 1990). The increase in the inflexion ratio by BF proved that *A. lebeck* possessed nootropic activity. Thus, the BF meets a major criterion for nootropic activity, improvement of memory in absence of cognitive deficit (Poschel, 1988). The BF in higher dose i.e. 50 mg/kg showed diminished nootropic activity which may be because of sluggishness induced by the depressant activity associated with this dose. The antagonistic action of BF against scopolamine-induced amnesia substantiates its nootropic activity.

The BF also exhibited dose related anxiolytic activity in mice. This finding is

interesting with regards to the previous contention that anxiogenic agents can improve cognitive behavior and anxiolytics can impair learning and memory (Arolfo and Brioni, 1991; Cole and Jones, 1995; Janak and Martinez, 1992; Venault et al., 1986). Despite extensive experimental and clinical studies the neurochemical basis of learning and memory remains poorly understood. Although the role of central cholinergic system is fairly well established, the involvement of other neurotransmitter systems cannot be ignored (Hollander et al., 1986). It is well known that diazepam impairs memory and the inhibition of GABA_B receptor facilitates learning and memory (Olpe et al., 1993; Staubli and Xu, 1995). The antagonistic action of BF on the behavior mediated by baclofen suggests that the observed effects on cognitive behavior and anxiolysis may be mediated via GABA. Sarter et al. (1992) have postulated that GABA antagonists may enhance cholinergic activity by blocking neurons that reach cholinergic nerve cells of the basal forebrain.

Thus it is concluded that the *n*-butanolic fraction of petroleum ether extract of *A. lebbeck* leaves, which contained saponins, possessed nootropic and anxiolytic activity. The inhibition of GABAergic transmission may be responsible for the nootropic and anxiolytic activity of the saponins of *A. lebbeck*.

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Figures

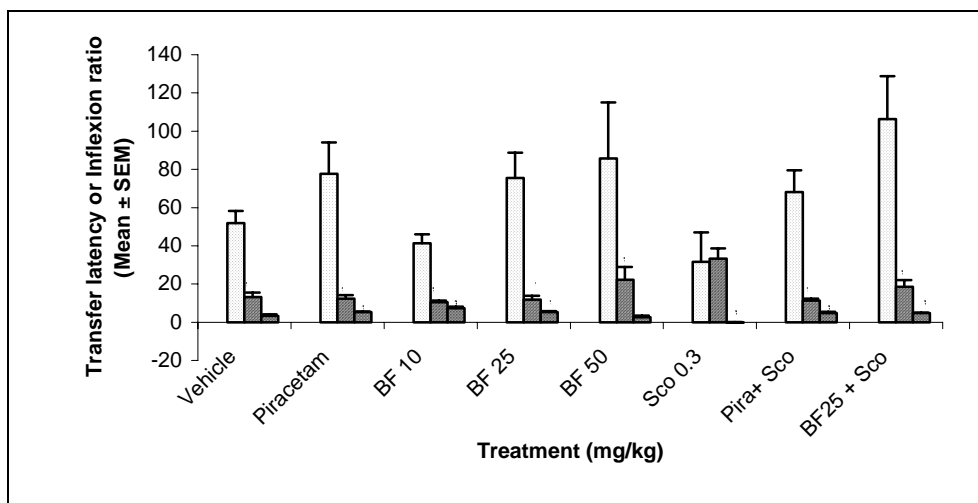


Fig. 1. Effect of n-butanolic fraction (BF) of *A. lebeck* on transfer latency and inflexion ratio in elevated plus maze. * $P < 0.01$ vs. respective control (Student's t test).

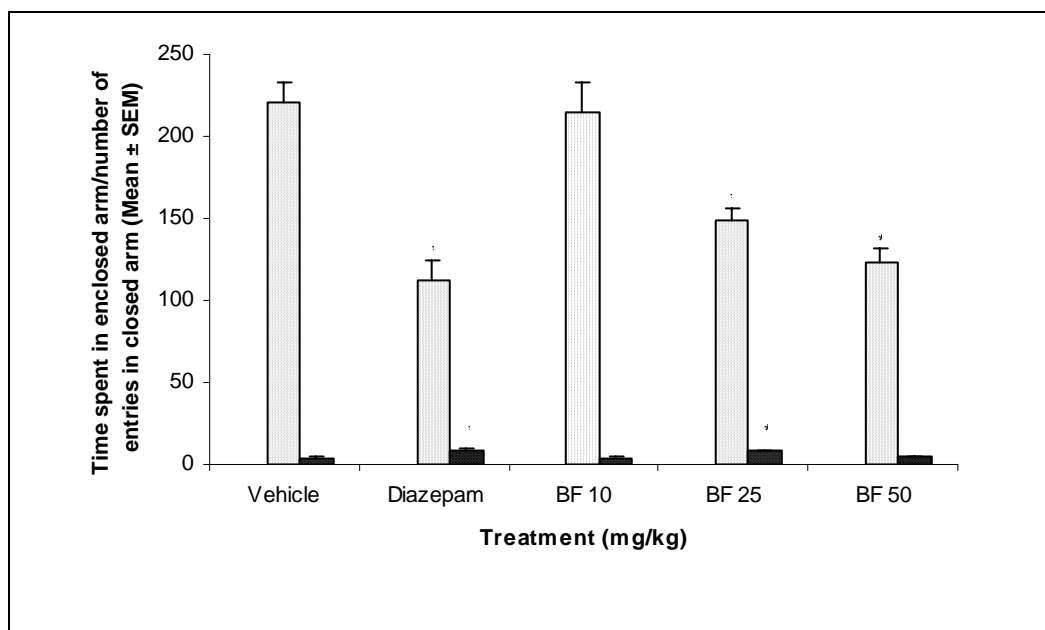


Fig. 2. Effect of n-butanolic fraction (BF) of *A. lebeck* on time spent in enclosed arm and number of entries into enclosed arm. * $P < 0.01$ (Student's t test).

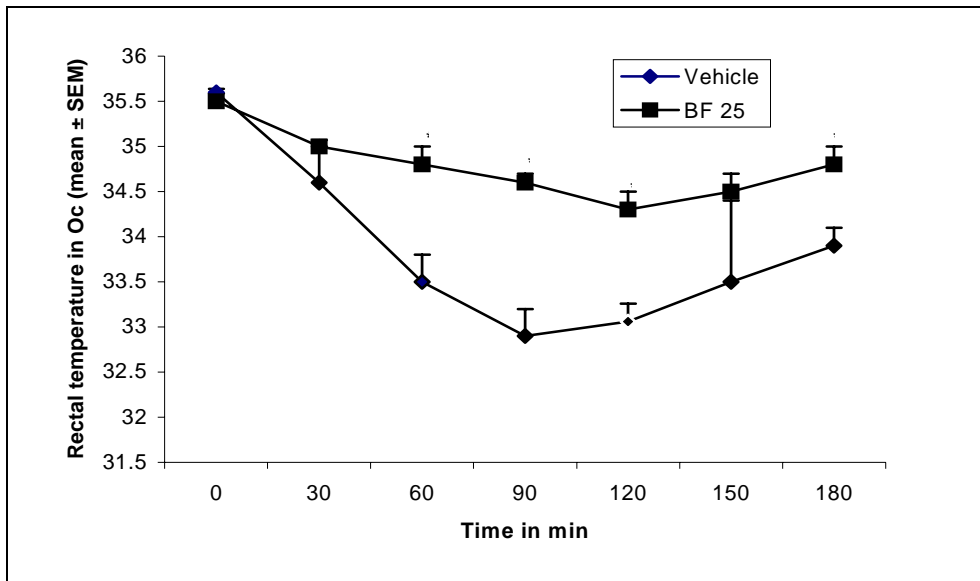


Fig. 3. Effect of n-butanolic fraction (BF) of *A. lebeck* on baclofen induced hypothermia in rats. $P < 0.05$ (Mann-Whitney U test).

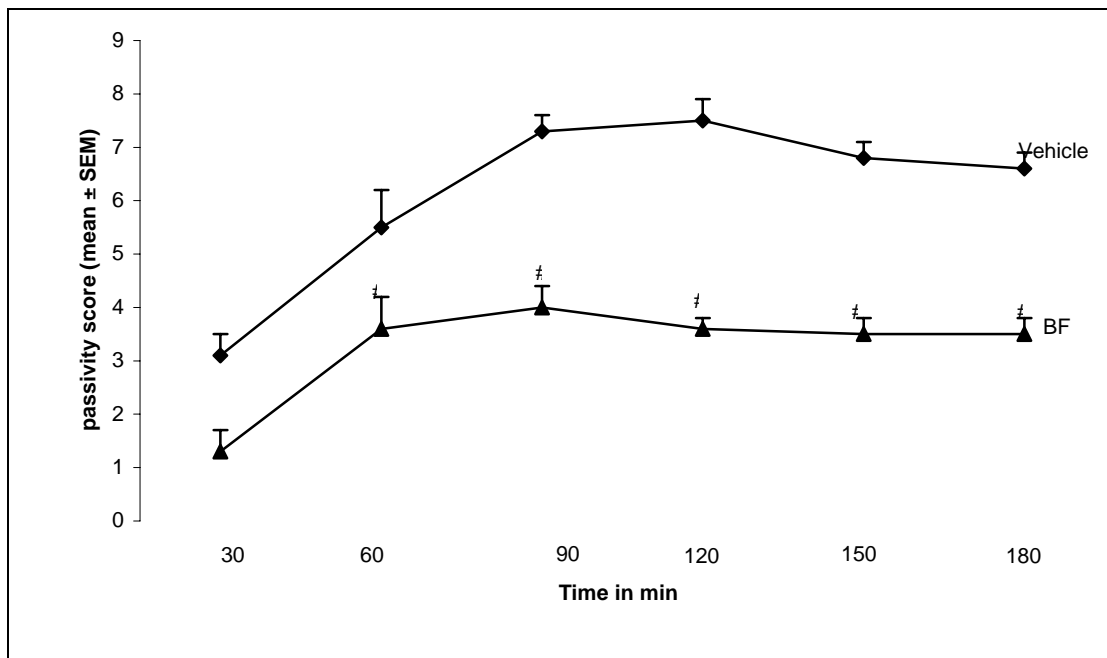


Fig. 4. Effect of n-butanolic fraction (BF 25) of *A. lebeck* on baclofen induced passivity in rats. # $p < 0.01$ vs. Baclofen (Mann-Whitney U test).