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Anxiolytic activity of Moralbosteroid, a steroidal glycoside isolated from *Morus alba*

Gaurav Gupta[1,2], Imran Kazmi[2], Firoz Anwar[2]

[1]Pacific University, Udaipur, Rajasthan, India.
[2]Department of Pharmacology, Siddhartha Institute of Pharmacy, Dehradun, India.

*Corresponding author: gauravpharma25@gmail.com; Tel: +91-9917079006

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Abstract

Mulberry, *Morus alba* L., as a non-toxic natural therapeutic agent, belongs with the family of Moraceae and has been cultivated in many Asian countries such as China, India, Korea, Japan and Thailand where the leaves were used as food for silkworms, is a natural food additive having vitamins, carbohydrates, mineral, lipids, sugars, proteins, fibers, etc. in appropriate proportion. The study aimed to evaluate the anxiolytic activity of Moralbosteroid isolated from *Morus alba*. The anxiolytic study was carried out on elevated plus-maze, light and dark model and open field test. It significantly increased the percentage of time spent (control = 33.27±0.59 sec) and number of entries in open arm (control = 6.39±0.72) in elevated plus-maze apparatus. In light and dark model, moralbosteroid produced significant increase in time spent (control = 54.82 ± 1.04 sec), number of crossing (control = 21.53 ± 0.85) and decrease in the duration of immobility (control = 37.22 ± 1.37 sec) in light box. In open field test, moralbosteroid show significant increase in number of rearing (control = 10.42 ± 0.13), assisted rearing (control = 6.28 ± 0.18) and number of square crossed (control = 57.51 ± 2.19). It is concluded that Moralbosteroid have therapeutic potential for managing anxiety.

Keywords: *Morus alba*; Moralbosteroid; Elevated plus-maze; Light and dark model; Open field test; Anxiolytic

Introduction

Anxiety-related disorders such as generalized anxiety, panic, obsessive-compulsive disorder, phobias or post-traumatic stress are the most common mental illness and a major cause of disability in the world. Mental disorders have been found to be common, with over a third of people in most countries reporting them with sufficient criteria to be diagnosed at some point in their life (WHO, 2000). Although benzodiazepines are among the first line of anxiolytic drugs with well-known benefits, their side effects are prominent, including sedation, muscle relaxation, anterograde amnesia and physical dependence (Kaplan and Sadock,

2005). So development of new anxiolytics has been an area of interest. Many secondary plant metabolites has been reported in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system such as noradrenalin, serotonin, gamma-amino butyric acid (GABA), benzodiazepine (BDZ) neurotransmitters activities.

Morus alba L. is a well-known Chinese herb and is traditionally used for the prevention and treatment of several diseases (Dugo et al., 2009; Piao et al., 2011). It is well documented that this plant possesses antidiabetic (Singab et al., 2005), hypolipidemic, antihypertensive (Lee et al., 2011), antimicrobial (Kim et al., 1993), antioxidant (Arabshahi-Delouee et al., 2007; Wattanapitayakul et al., 2005), antiatherosclerotic (Enkhmaa et al., 2005), anticancer (Choi et al., 2005) and neuroprotective (Niidome et al., 2007) activities. The present study was designed to evaluate the anxiolytic nature of an albosteroid isolated from *Morus alba* stem bark in experimental animals.

Materials and methods

Animals

Swiss albino mice (20–25 g) of either sex were used for the study. The animals were obtained from the animal house Siddhartha Institute of Pharmacy, Dehradun, India, kept at 25±1 °C, 55±5% humidity along with 12 h light/dark cycle. The animals were given standard pellet diet (Lipton rat feed, Ltd., Pune) and water ad libitum throughout the experimental period. The experiments were conducted according to the Institutional Animal Ethics Committee regulations approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (Reg. No. 1435/PO/a/11/CPCSEA).

The animals were divided into four groups, each containing six mice. Group I was served as solvent control and received 0.9% (w/v) of saline (1 ml/100 g). Group II treated as positive control was received diazepam (1 mg/kg). Group III and IV were received DTCP 5.0 and 10.0 mg/kg suspended in 1% Tween 80 (v/v). All the treatments were administered intraperitoneally 30 min prior to start the experiment.

Plant material

Morus alba L. stem bark was collected from a local market in Dehradun and identified by Mr. Imran Kazmi, Assistant Professor, Siddhartha Institute of Pharmacy, Dehra Dun, India (Voucher No. SIP 211).

Extraction and isolation of DTCP

Dried powder of *Morus alba* stem bark (4.5 kg) was extracted with methanol (12 L) at 50°C for 2 days. Extract was concentrated to dryness under reduced pressure to obtain slurry (736 g). The slurry was dissolved in a minimal amount of methanol and was adsorbed onto silica gel (60-120 mesh). The slurry was subjected to a silica gel column using $CHCl_3$/MeOH gradient systems (9:1;2.0L for gradient system); leads to elution of colorless crystals of moralbosteroid (yield 19.3g, 0.43%). Structure of compound was identified by comparison of

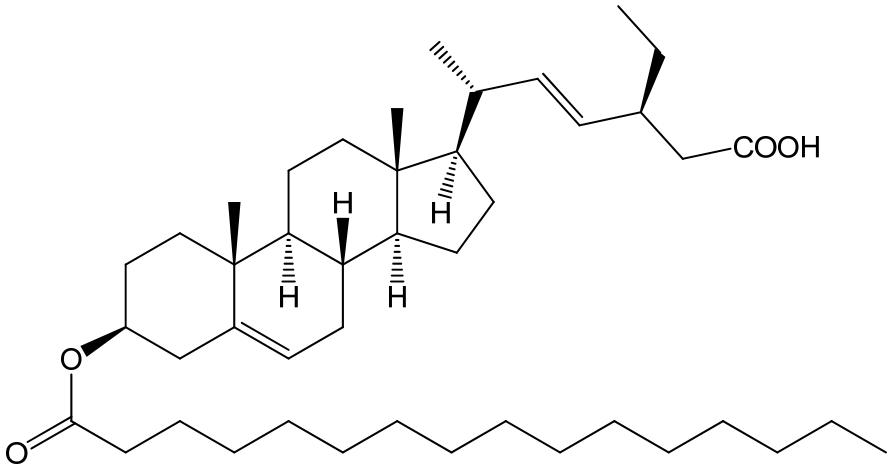

Figure 1. Chemical structure of Moralbosteroid.

their spectroscopic data from the reported literature (Aftab et al., 2012). The structure of moralbosteroid is depicted in Fig.1.

Behavioral parameters used to test anxiolytic activity

Elevated plus-maze test (EPM)

The EPM consisted of four arms elevated 25 cm above the floor, with each arm positioned at 90° relative to the adjacent arms. Two of the arms were enclosed with high walls (35 × 5 × 20 cm), and the other arms were connected via a central area (5 × 5 cm) to form a plus sign. The maze floor and the walls of enclosed arms were painted black. The room was illuminated with a 40-W lamp at the central platform. The animals were treated with vehicle, diazepam (1 mg/kg) and moralbosteroid (5.0 and 10.0 mg/kg) intraperitoneally, 30 min prior to the test. The experiment was performed between 09:00 and 14:00 hours, and the mice became accustomed to the dimly lit experimental laboratory for 30 min prior to behavioural testing. Each mouse was individually placed on the central platform facing toward an open arm. The frequency and duration of entries into the open and closed arms were observed for 5 min. An entry was counted when all four paws of the mouse entered an open or closed arm. Subsequently, the percentage of time spent (duration) in the open arms (100 × open/[open + enclosed]) and percentage of the number of open arm entries (frequency, 100 × open/total entries) were calculated for each animal. The apparatus was thoroughly cleaned after each trial (Adeyemi et al., 2006).

Open field test

The apparatus consisted of a wooden box (60 × 60 × 60 cm). The arena of the open field was divided into 16 squares (15 × 15 cm): the four inner squares in the center and 12 squares in the periphery along the walls. The experimental room was a sound attenuated, dark room. The open field arena was illuminated with a 40-W lamp, focusing on the field from a height of about 75–100 cm. After 30 min of oral treatment with vehicle, diazepam (1 mg/kg) and moralbosteroid (5.0 and 10.0 mg/kg), animals were placed individually in one of

the corner squares and number of rearing, assisted rearing (forepaws touching the walls of the apparatus) and number of squares crossed were observed for 5 min (Yadav et al., 2008).

Light and dark test

The L and DT apparatus consisted of open top wooden box. Two distinct chambers, a black chamber (25 cm long × 35 cm wide × 35 cm deep), painted white and brightly illuminated with 40-W white light source, were placed 25 cm above the open box. The two chambers were connected through a small open doorway, (7.5 cm long × 5 cm wide) situated on the floor level at the centre of the partition. The mice were placed individually in centre of the light box after 30 min of oral treatments and observed for 5 min (Ambavade et al., 2006).

Statistical analysis

Results are expressed as mean ± S.E.M. The statistical analysis of data was done using the one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability level less than 0.05 was considered statistically significant.

Result and discussion

In Elevated plus-maze test, vehicle-treated mice spent 33.27±0.59 s in open arm and 145.3±1.64 s in the closed arm, with 6.39±0.72 entries into the open arm and 25.28±0.14 entries into closed arm. Diazepam (1 mg/kg) and moralbosteroid (5.0 and 10.0 mg/kg), significantly ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) increase in the occupancy in the open arm, whereas significant ($P < 0.01$ and $P < 0.001$) decrease in number of entries and time spent in closed arm. Percentage of time spent in the open arms and number of open arm entries was significantly ($P < 0.01$ and $P < 0.001$) increased by moralbosteroid (5.0 and 10.0 mg/kg) and diazepam (Table 1).

In open field test, animals treated with diazepam (1 mg/kg) and moralbosteroid (5.0 and 10.0 mg/kg) showed significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) increase in the time spent in the lighted box and decrease in the time spent in the dark box and also both diazepam (1 mg/kg) and moralbosteroid (5.0 and 10.0 mg/kg) showed significant ($P < 0.05$, $P < 0.01$ and $P < 0.001$) increase in the number of crossing and decrease in the duration of immobility (Table 2).

In Light and dark test, vehicle-treated mice crossed 57.51 ± 2.19 squares and showed 10.42 ± 0.13 self-rearing and 6.28 ± 0.18 assisted rearing during the test interval of 5 min.

Table 1. Effect of DTCP on behavior of mice in elevated plus maze test.

Treatment (mg/kg, p.o.)	No. of entries		Time spent (sec)		% OAE	TSOA (sec)
	Open arm	Closed arm	Open arm	Closed arm		
Vehicle	6.39±0.72	25.28±0.14	33.27±0.59	145.3±1.64	20.17±0.83	18.63±0.26
Diazepam (1)	10.67±0.93[c]	13.25±0.09[c]	74.18±1.20[c]	127.61±1.03[c]	44.60±0.91[c]	36.76±0.53[c]
DTCP (5.0)	9.90±0.51b	15.93±0.34[b]	69.12±1.06[a]	176.27±1.73[b]	38.32±0.60[b]	28.16±0.37[b]
DTCP (10.0)	11.60±0.58[c]	15.05±0.82[c]	71.60±1.24[c]	125.94±1.07[c]	43.52±0.41[c]	36.24±0.53

Values are the mean SEM of six mice/treatment.
Whereas a= $P < 0.05$, b= $P < 0.01$ and c= $P < 0.001$ compared with Control.

Table 2. Effect of DTCP on behavior of mice in light and dark model.

Treatment (mg/kg, p.o.)	Time spent in lighted Box (sec)	Time spent in dark Box (sec)	No. of crossing	Duration of immobility (sec)
Vehicle	54.82 ± 1.04	207.9 ± 4.72	21.53 ± 0.85	37.22 ± 1.37
Diazepam (1)	149.0 ± 1.29[c]	97.20 ± 4.21[c]	39.07 ± 0.30[c]	21.72 ± 0.93[c]
DTCP (5.0)	82.39 ± 1.61[b]	174.0 ± 4.03[a]	25.59 ± 0.27[a]	31.50 ± 1.03[b]
DTCP (10.0)	126.3 ± 1.15[c]	138.8 ± 4.32[b]	32.61 ± 0.69[c]	20.47 ± 1.20[c]

Values are the mean SEM of six mice/treatment.
Whereas a= $P < 0.05$, b= $P < 0.01$ and c= $P < 0.001$ compared with Control.

Table 3. Effect of DTCP on behavior of mice in open field test.

Treatment (mg/kg, p.o.)	No. of rearing	No. of assisted rearing	No. of squares crossed
Vehicle	10.42 ± 0.13	6.28 ± 0.18	57.51 ± 2.19
Diazepam (1)	23.94 ± 0.06[c]	20.16 ± 0.40[c]	213.28 ± 4.72[c]
DTCP (5.0)	14.29 ± 0.17a	9.05 ± 0.61[a]	89.32 ± 2.17[b]
DTCP (10.0)	22.23 ± 0.16[c]	23.34 ± 0.20[c]	193.24 ± 4.37[c]

Values are the mean SEM of six mice/treatment.
Whereas a= $P < 0.05$, b= $P < 0.01$ and c= $P < 0.001$ compared with Control.

Diazepam and moralbosteroid (5.0 and 10.0 mg/kg), significantly ($P < 0.01$ and $P < 0.001$) increased in the number of squares crossed. The self-rearing and assisted rearing was significantly ($P < 0.05$, and $P < 0.001$) increased by moralbosteroid (5.0 and 10.0 mg/kg) and diazepam (Table 2).

In elevated plus maze, native mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by fear of open spaces. In our study, moralbosteroid, dose-dependently induced significant increases in both the number of entries and time spent in the open arms, whereas decreases in the number of entries and time spent in the closed arm, thus indicates the anxiolytic activity (Hellion-Ibarrola et al., 2006).

The open field model examines anxiety related behaviour characterized by the normal aversion of the animal to an open, brightly lit area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters (Mechan et al., 1995). Moralbosteroid, dose dependently, significant increase in the number of self-rearing, number of assisted rearing and number of squares crossed, which indicates its anxiolytic effect by reducing such fearful behaviour of animals in open field.

The light and dark box test is based on the natural aversion of mice to brightly lit places (Bourin and Hascoet, 2003). Moralbosteroid reduce the natural aversion to light and increase the time spent in the lit compartment, dose dependently. The observed activity may be due to the agonistic effect on GABA/benzodiazepine receptor complex.

Steroids are apparently involved in the regulation of large number of biological activities including electrolytic and hormonal balance as well as reaction to allergy. Steroids

actained from different plant sources have already reported for various neuroprotective activity (Kumar and Khanum, 2012).

The results obtained in this study suggest that the moralbosteroid isolated from *Morus alba* stem bark possesses anxiolytic activity which is possibly mediated through the $GABA_A$-BZD mechanism. Thus, moralbosteroid has potential clinical application in the management of anxiety.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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