Pharmacological Evaluation of Anxiolytic Activity of Ursolic Acid Stearoyl Glucoside Isolated from *Lantana camara*

Imran Kazmi, Gaurav Gupta, Muhammad Afzal, Mahfoozur Rahman & Firoz Anwar

Siddhartha Institute of Pharmacy, Dehradun, India

Correspondence

Firoz Anwar, Siddhartha Institute of Pharmacy,
Dehradun-248001, Uttarakhand, India.

Tel.: +91-941-293-7329;

Fax: +91-135-260-7784;

E-mail: firoz_anwar2000@yahoo.com

Received 27 March 2012; revision 6 April

2012; accepted 20 April 2012.

doi: 10.1111/j.1755-5949.2012.00351.x

Anxiety disorders are the most common psychiatric disorders [1]. Present epidemiological data suggest half of the population suffers from this disorder [2]. Many secondary plant metabolites are used in traditional medicine practice, either directly or indirectly affecting the central nervous system and altering gamma-amino butyric acid (GABA), serotonin noradrenalin, benzodiazepine (BDZ) neurotransmitter activities [3]. Dried powder of *Lantana camara* leaves (4 kg) was extracted with methanol (12 L) at 50°C for 1 day. The slurry was dissolved in methanol and was adsorbed on silica gel column (60–120 mesh). Using CHCl₃/MeOH (49:1; 2.0 L) gradient system in the column leads to the elution of colorless crystals of UASG (yield, 11.2 g; 0.28%) [4]. Isolated UASG crystals were examined for anxiolytic potential.

The animals were divided into four groups with six mice each: Group I, solvent control (received 0.9% [w/v] of saline); Group II, a positive control (diazepam, 1 mg/kg); and Groups III and IV received 25 and 50 mg/kg of UASG suspended in 1% Tween 80 (v/v). All the animals were administered the aforementioned drugs/solvent intraperitoneally 30 min prior to start of the experiment.

Elevated plus-maze test (EPM): Vehicle, diazepam (1 mg/kg), and UASG (25 and 50 mg/kg) were intraperitoneally administered 30 min prior to the test. Each animal was placed on the central platform. The movement and duration of entries into the closed and open arms were observed for 5 min. The percentage of time spent in the open arms and percentage of the number of open arm entries were calculated for each mouse [5].

Open-field test: Treatment was initiated with vehicle, diazepam (1 mg/kg), and UASG (25 and 50 mg/kg), by placing the animals in corner squares, and the number of rearing, assisted rearing, and number of squares crossed were observed for 5 min [6].

Light and dark test: Each animal was placed in center of the light box and was observed for 5 min [7].

The animals were more stable in the open arm when treated with diazepam (1 mg/kg) and UASG (25 and 50 mg/kg), with significant *P*-values ($P < 0.05$ and $P < 0.001$), whereas significant

($P < 0.01$ and $P < 0.001$) reduction was observed in the number of entries and the time spent in the closed arm. UASG (25 and 50 mg/kg) and diazepam significantly ($P < 0.001$) increased the percentage of the time spent and entries in open arm (Table 1).

Diazepam and UASG (25 and 50 mg/kg) displayed significant effect ($P < 0.05$ and $P < 0.001$) on the number of squares crossed. The self-rearing and assisted rearing were increased by UASG (25 and 50 mg/kg) ($P < 0.05$ and $P < 0.001$) (Table 2). Diazepam (1 mg/kg) and UASG (25 and 50 mg/kg) showed marked change ($P < 0.05$, $P < 0.001$, and $P < 0.001$) in time spent, which was increased in lighted box and decreased in the dark box. UASG (25 mg/kg) failed to have any effect on the number of crossing and duration of immobility, whereas diazepam (1 mg/kg) and UASG (50 mg/kg) showed significant ($P < 0.05$ and $P < 0.01$, respectively) increase in the number of crossing and decrease in the duration of immobility (Table 2).

The time spent on the central platform appears to be related to decision making and/or risk assessment, and total arm entries is a contaminated measure reflecting changes in anxiety or general activity [8]. UASG, in a dose-dependent manner, induces significant increases both in the number of entries and in time spent in the open arms, and vice versa in the closed arm, proving its anxiolytic nature. The open-field model evaluates anxiety-related activities such as location, fear characterized to an open and bright area [9]. UASG significantly changes anxiety parameters, which results in the increase in the number of self-rearing, number of assisted rearing, and number of squares crossed, exhibiting its anxiolytic effect. The light and dark box method is a natural test used for rodents to avoid bright places. UASG reduces the fear of animal, which may be due to the agonistic effect on GABA/BDZ receptor complex. UASG, a derivative of ursolic acid, may be responsible for anxiolytic activity. In a nutshell, UASG from the above experiment and results demonstrates marked anxiolytic activity. Exact mechanism of action needs to be under study.

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

Treatment (mg/kg, i.p.)	No. of entries		Time spent (second)		% OAE	TSOA (second)
	Open arm	Closed arm	Open arm	Closed arm		
Vehicle	4.86 ± 0.28	33.8 ± 0.74	53.3 ± 1.52	135.4 ± 1.62	23.8 ± 0.74	35.9 ± 0.78
Diazepam (1)	9.88 ± 0.63***	23.9 ± 0.47***	74.6 ± 1.41***	85.5 ± 1.73***	49.6 ± 1.79***	56.2 ± 1.00***
UASG (25)	6.10 ± 0.45*	17.6 ± 0.24**	57.9 ± 1.20	110.6 ± 1.93**	52.4 ± 2.05***	42.3 ± 0.58**
UASG (50)	8.36 ± 0.52***	20.0 ± 0.38***	74.5 ± 1.19***	86.8 ± 1.71***	52.2 ± 2.20***	54.9 ± 1.16***

Values are the mean SEM of six mice/treatment. Significant * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with control.

Table 2 Effect of UASG on behavior of mice in open field test and light and dark model

Treatment (mg/kg, i.p.)	Open field test			Light and dark model			Duration of immobility (second)
	No. of rearing	No. of assisted rearing	No. of squares crossed	Time spent in lighted box (second)	Time spent in dark box (second)	No. of crossing	
Vehicle	17.4 ± 0.44	18.9 ± 0.88	84.8 ± 1.58	86.4 ± 1.51	201.1 ± 2.01	24.1 ± 1.98	37.8 ± 2.92
Diazepam (1)	31.7 ± 1.46***	33.3 ± 0.95***	172.8 ± 1.36***	175.0 ± 1.82***	121.4 ± 2.62***	32.5 ± 1.63*	28.2 ± 2.31*
UASG (25)	28.0 ± 0.40***	26.0 ± 0.97**	105.4 ± 1.10*	106.2 ± 1.50*	177.2 ± 2.56**	26.0 ± 1.35	34.2 ± 2.23
UASG (50)	28.9 ± 1.64***	29.8 ± 0.74***	147.9 ± 1.34***	156.6 ± 1.69***	125.3 ± 2.39***	31.9 ± 1.93*	25.6 ± 1.65**

Values are the mean SEM of six mice/treatment. Significant * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with control.

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