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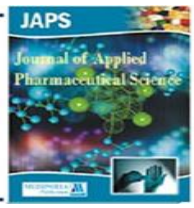

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Anxiolytic effect of chronic administration of ursolic acid in rats

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ABSTRACT

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed it is a universal human emotion, closely allied with appropriate fear presumably serving pyschobiologically adaptive purposes. Anxiety is a normal emotional behaviour. When it is severe and/or chronic, however, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects. Ursolic acid has been identified as the active principle of *Ocimum sanctum*. From our laboratory we have established the antianxiety, anticateleptic and antidepressant activity of ethanolic extract of leaves of *Ocimum sanctum*. In the present study, we have attempted to evaluate the anxiolytic- activity of ursolic acid in rats by employing, elevated plus maze and bright and dark arena. The rats were divided into five groups, each group containing six animals. The effects of the test drug ursolic acid (at 0.05, 0.1 and 0.2 mg/kg doses), the standard anxiolytic, diazepam (1.0 mg/kg) and control group 14% dimethyl sulfoxide (10ml/kg) were assessed after repeated doses administration for ten days. The results suggest that, ursolic acid exhibited anxiolytic like activity comparable to diazepam.

Key words: Anxiolytic, ursolic acid, diazepam, rats.

INTRODUCTION

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed it is a universal human emotion, closely allied with appropriate fear presumably serving pyschobiologically adaptive purposes (Ross et al., 2006). Anxiety is a normal emotional behaviour. When it is severe and/ or chronic, however, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance upon chronic use. In ayurvedic medicine, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs (Pari et al., 1999). Ursolic acid is a triterpenoid compound which exists widely in natural plants in the form of free acid or aglycones for triterpenoid saponins(Mahato et al., 1988). Various plants having ursolic acid as an active ingredient have shown hepatoprotective activity (Lin et al., 1988). Ursolic acid has also been implicated in inhibition of lipoxygenase and HL60 leukemic cells(Simon et al., 1992), inhibition of mutagenesis in bacteria (Young et al., 1994), antitumor promotion (Ohigashi et al., 1986), inhibition of histamine release (Tsuruga et al., 1991), inhibition of lipid peroxidation and protection against adriamycin toxicity (Balanehru et al., 1994), antimicrobial activity (Collins et al., 1987) and anti-inflammatory action (Kosuge et al., 1985). Ursolic acid has been identified as the active principle of *Ocimum sanctum*. From our laboratory we have established the antianxiety (Sudhakar et al., 2010), anticateleptic (Sudhakar et al., 2007)

and antidepressant (Sudhakar P et al., 2010) activity of ethanolic extract of leaves of *Ocimum sanctum*. With this background, in the present study we have investigated the antianxiety effect of ursolic acid in rats using two pharmacologically validated experimental models namely elevated plus maze (Pellow G et al., 1985), and bright and dark arena (Costall B et al., 1988).

MATERIALS AND METHODS

Animals

Adult male wistar albino rats weighing 150 to 180g (90 to 110 days old) bred in the central animal house of Kasturba Medical College, Mangalore, were used for the study. They were housed in clean, clear, polypropylene cages in groups of four and maintained at 24.0±2ºC with 12 hrs light and dark cycle and had free access to food and water *ad libitum*. Animals were kept in experimental lab for seven days prior to experiment to acclimatize laboratory conditions. Each rat was used only once. Experiments were conducted between 9:00 to14:00 hrs. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) and the study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs and dosage

The test drug, ursolic acid (Sigma Aldrich Chemicals Pvt. Ltd, United Kingdom, HS No. 29181985900), standard anxiolytic drug, Diazepam (Ranbaxy Ltd. India) were suspend- ed suspended in 14% dimethyl sulfoxide (DMSO) and was administered orally. Each drug solution was prepared freshly just before the administration. Drugs and vehicles were administered orally. The doses of each drug are selected on the basis of earlier findings with *Ocimum sanctum* (Sudhakar P et al., 2010). Drugs, dosage and number of animals used per treatment are shown in (Table 1). Drugs/vehicle was administered 60 minutes prior to the experiment.

Table1. The animals are divided into five groups with six animals in each group

Groups (n = 6)	Treatment	Dose
I	Control – 14% DMSO	10.0 ml/kg
II	Diazepam	1.0 mg/kg
III	Ursolic acid	0.05 mg/kg
IV	Ursolic acid	0.1 mg/kg
V	Ursolic acid	0.2 mg/kg

(n, number of animals in each group)

Apparatus

Elevated plus maze

The wooden maze consisted of two open arms (length 50 cmX breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type were opposite to each other, with a central square of 10 cm. The maze was elevated to a height of 50 cm above the floor.

Bright and dark arena

The apparatus consisted of an open top wooden box. Two distinct chambers, a black chamber (20 X 30 X 35cm) painted black and illuminated with dimmed red light and a bright chamber (30 X 30 X 35 cm) painted white and brightly illuminated with100W white light source, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 X5 cm) situated on the floor level at the centre of the partition.

Behavioral assessment

Each animal was tested initially in plus maze and, then, in bright and dark arena paradigm in a single setting. In this study 60 minutes after drug or vehicle administration, each animal was placed in the centre square of the plus maze, facing one of the closed arms. The number of entries into and the time spent in open and closed arms and the number of rears in each arm in a five-minute period was noted. Following the elevated plus maze test, the animal was placed at the centre of the brightly lit arena in the bright and dark arena. The number of entries into and the time spent in the bright arena, the number of rears in the bright and dark arenas and the duration of immobility were noted. Following each trial, the apparatus was cleaned with hydrogen peroxide to mask the odour left by the animal in the previous experiment. Hand operated counters and stop watches were used to score the behaviour of animals.

Statistical analysis

The data were analysed by one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed by applying Dunnet's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

a. Elevated plus maze

The results given in table 2 indicate that the diazepam (1.0 mg/kg) treated rats showed a significant increase in number of open arm entries, percentile ratio of open arm to total arm entries, time spent in the open arms, number of rears in open arms and reduction in the time spent in the closed arms. Ursolic acid treated rats exhibited a significant increase in open arm entries (0.1mg/kg), decrease in number of total arm entries (0.2 mg/kg), increase in the percentile ratio of open arm to total arm entries (0.2mg/kg), whereas significant difference in time spent in the open arms, time spent in the closed arms and number of rears in all the doses tested (0.05, 0.1& 0.2 mg/kg).

b. Bright and dark arena

The results given in table 3 indicate that the diazepam (1.0mg/kg) treated rats significantly increased the number of bright chamber entries, time spent and the rears in bright arena, and

Table 2. Effect of chronic administration of diazepam and ursolic acid on behaviour of rats in elevated plus maze

Drug groups (n=6)	Number of open arms entries	Number of total arm entries	Percentage ratio of open/total arm entries	Time spent in open arms(Sec)	Time spent in closed arms(Sec)	Number of rears in open arms
14%DMSO (10.0 ml/kg)	4.00±0.36	10.50±0.56	38.49±3.57	67.00±4.21	218.00±4.50	1.83±0.30
Diazepam (1.0 mg/kg)	5.50±0.34*	9.16±0.65	60.23±1.16*	229.66±14.35**	59.00±11.33**	6.16±0.87**
Ursolic acid (0.05 mg/kg)	4.66±0.21	8.83±0.60	53.67±3.38	217.16±14.10**	59.16±9.03**	7.33±1.08**
Ursolic acid (0.1 mg/kg)	6.00±0.36**	10.00±0.57	49.67±9.87	139.83±16.36**	42.16±16.96**	4.83±0.40*
Ursolic acid(0.2 mg/kg)	4.66±0.33	7.83±0.60*	59.76±1.39*	230.50±8.31**	51.16±6.28**	7.83±0.70**
F value	5.72	2.99	3.186	34.12	47.63	10.73

showed significant reduction in duration of immobility. Ursolic acid (0.05, 0.1&0.2mg/kg) treated rats showed a significant ($P<0.01$) reduction in the duration of immobility whereas increased number of bright chamber entries, time spent in bright chamber and number of rears in bright chamber only at higher dose (0.2 mg/kg).

Table 3. Effect of chronic administration of diazepam and ursolic acid on behaviour of rats in bright and dark arena

Drug groups (n=6)	Number of bright chamber entries	Time spent In bright chamber(Sec)	Number of Rears in bright chamber	Duration of Immobility (Sec)
14%DMSO (10.0 ml/kg)	1.00±0.00	4.83±0.30	0.50±0.50	158.16±2.05
Diazepam (1.0 mg/kg)	2.66±0.21**	15.00±1.77**	2.00±0.44*	62.00±1.15**
Ursolic acid (0.05 mg/kg)	1.50±0.22	9.16±1.64	1.13±0.65	45.83±1.42**
Ursolic acid (0.1 mg/kg)	1.50±0.22	8.50±1.05	1.16±0.40	52.83±1.85**
Ursolicacid (0.2 mg/kg)	1.83±0.30*	10.33±1.20*	1.87±0.36*	42.66±1.68**
F value	7.96	7.92	2.82	85.01

(All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$ **$P < 0.01$)

DISCUSSION

The two experimental models of anxiety, elevated plus maze and bright and dark arena, are based on the assumption that unfamiliar**,** non-protective and brightly lit environmental stress provokes inhibition of normal behaviour. This normal behavioural inhibition is further augmented in the presence of fear or anxiety like state.

In the elevated plus maze, the open arms are more fear provoking than the closed arms. The ratio of entries, time spent and rearing behaviour in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms (Pellow G et al., 1985). The reduction in entry, time spent, rearing in open arms, ratio of open arm to total arm entries and increased defecation are the indications of high level of fear or anxiety. Anxiolytic drugs increase the proportion of entries, time spent and rearing in open arms. They also increase the ratio of open arm to total arm entries. In the light and dark box paradigm, the brightly lit environment is a noxious environment stressor that inhibits the exploratory behaviour of rodents. Reduction in the number of entries, time spent and rearing behaviour in the light chamber are regarded as markers of anxiety (Costall B et al., 1988). Rearing reflects an compound, ursolic acid on chronic administration, increased the number of entries, time spent and rearing in open arms and also increased the percentile ratio of open arm to total arm entries in the elevated plus maze paradigm. The anti-anxiety effects of ursolic acid in the elevated plus maze were comparable with those following the administration of diazepam.

In bright and dark paradigm, ursolic acid at higher dose i.e. 0.2mg/kg was significantly increased the time spent in light arena, rears in both light and dark arena and transition between chambers. All these behavioural changes in both paradigms are suggestive of decreased fear, decreased aversion to bright light and increased exploratory behaviour of the animal. A possible mechanism by which ursolic acid acts have been postulated: through its GABAergic properties, through inhibition of gamma-aminobutyric acid transaminase (GABA-T) activity and increases GABA levels in the brain (Award et al., 2007) . Awad et al in 2009 also reported in-vitro assays of ursolic acid inhibited GABA-T by 20% at 100ug/ml. Because the increase in GABAergic neurotransmission was associated with reduced anxiety, the behavioral study that has been described in this report was aimed at determining the antianxiety effects of ursolic acid. Further studies are required on ursolic acid to elucidate the possible mechanism involved and its use in human beings.

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