

Anxiolytic Effect of Chronic Administration of Gallic acid in Rats

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ABSTRACT

Anxiety is a cardinal symptom of many psychiatric disorders and an inevitable component of many medical and surgical conditions. It is a universal human emotion, closely allied with appropriate fear presumably serving psycho biologically adaptive purposes. Anxiety is a normal emotional behaviour. When it is severe and/or chronic, 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. Gallic acid has been identified as active ingredient found in gall nuts, sumac, witch hazel, tea leaves and oak bark. In the present study, we have attempted to evaluate the anti-anxiety- activity of Gallic 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 Gallic 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, Gallic acid exhibited anxiolytic like activity comparable to diazepam.

INTRODUCTION

Anxiety is a cardinal symptom of many psychiatric disorders and an inevitable component of many medical and surgical conditions. Anxiety is a universal human emotion, closely allied with appropriate fear, presumably serving psycho biologically adaptive purposes (Ross *et al.*, 2006). Anxiety is a normal emotional behaviour. When it is severe and/ or chronic, it is usually pathological and can precipitate or aggravate cardiovascular and psychiatric disorders.

Although many medicines are available in modern medicine to treat anxiety disorders, these produce various systemic side effects or exhibit tolerance upon chronic use. In Ayurveda, many plant products have been claimed to be free from side effects and less toxic than synthetic drugs (Pari *et al.*, 1999). Gallic acid is a trihydroxybenzoic acid found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants.

Various plants having Gallic acid as an active ingredient has shown antiviral, antimicrobial and cytotoxic action against cancer (Oozccelik *et al.*, 2011).

Gallic acid has been implicated in attenuation of platelet activation and platelet- leukocyte aggregation. It also has anti-leukemic effects on human leukemia K562 cells as well as cardio protective effects in diabetes induced myocardial dysfunction in rats (Reddy *et al.*, 2012).

Gallic acid is reported to exhibit antioxidant property and anti inflammatory action (Sohi *et al.*, 2003). We have reported the antianxiety, anticataleptic and anti depressant activities of NR-ANXC16, *Ocimum sanctum* and *Emblica officinalis* (Sudhakar *et al.*, 2007).

The property of altering brain biogenic amines, and antioxidant properties of the constituents by polyherbal products prompted us to study antianxiety activity of a dietary phenolic compound Gallic acid, by employing two validated experimental models; Elevated plus maze and Bright and dark arena in rats (Costall *et al.*, 1988).

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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±2°C with 12 hrs light and dark cycle and free access to food and water *ad libitum*. Animals were kept in experimental lab for seven days prior to experiment for laboratory acclimatization. Each rat was used only once. Experiments were conducted between 9:00 to 14:00 hrs.

The experimental protocol was approved by Institutional Animal Ethics 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, Gallic acid (Sigma Aldrich Chemicals Pvt. Ltd, United Kingdom) and standard anxiolytic drug Diazepam (Ranbaxy Ltd, India) were suspended in 14% Dimethyl sulfoxide (DMSO). Each drug solution was prepared freshly just before the administration. Drugs and vehicle were administered orally 60 minutes prior to the experiment. The doses of each drug were selected on the basis of earlier findings with *Ocimum sanctum* (Sudhakar P *et al.*, 2007). Drugs, dosage and number of animals used per treatment are shown in table 1.

Table. 1: The rats groups for experimentation.

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

METHODS

Elevated plus maze

The wooden maze consisted of two open arms (length 50 cm x 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 dim red light and a bright chamber (30 x 30 x 35 cm) painted white and brightly illuminated with 100W white light source, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 x 5 cm) situated on the floor level at the centre of the partition.

Behavioural 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 during 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 separately.

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 exposure, the apparatus was cleaned with hydrogen peroxide to mask the odour left by the animal in the previous experiment. Hand operated counters and stop watch were used to score the behaviour of animals.

STATISTICAL ANALYSIS

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

RESULTS

Elevated plus maze

Table 2 shows that there was significant increase in diazepam (1.0mg/kg) treated rats in terms of 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. Gallic 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).

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

Drug groups (n=6)	Number of open arm 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 (10.0ml/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 (1.0mg/kg)	5.50±0.34*	9.13±0.61	58.20±1.15*	238.66±14.35**	60.00±11.33**	6.19±0.87**
Gallic acid (0.05mg/kg)	4.63±0.21	9.81±0.60	53.67±3.38	217.16±14.10**	58.16±8.03**	7.33±1.06**
Gallic acid (0.1mg/kg)	6.00±0.39**	9.90±0.57	49.67±7.86	135.83±14.39**	42.16±16.96**	4.83±0.40**
Gallic acid (0.2mg/kg)	4.62±0.33	7.80±0.60*	59.72±1.39*	230.50±8.31**	51.16±6.28**	7.89±0.70**
F value	5.83	3.20	3.32	35.60	48.01	10.86

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

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

Drug groups (n=6)	Number of bright chamber Entries	Time spent In bright chamber (in sec)	Number of Rears in bright chamber	Duration of mobility(in sec)
14%DMSO (10.0ml/kg)	1.00±0.00	4.80 ±0.30	0.50 ±0.50	157.13±2.02
Diazepam(1.0mg/kg)	2.61±0.24**	15.03±1.75**	2.00±0.44**	62.00±1.17**
Gallic acid (0.05mg/kg)	1.70±0.22	9.16±1.64	1.13±0.65	46.85±1.44**
Gallic acid (0.1mg/kg)	1.70±0.22	8.50±1.05	1.16±0.40	52.63±1.76**
Gallic acid (0.2mg/kg)	1.85±0.33**	10.33±1.20*	1.87±0.36*	42.35±1.66**
F value	7.90	7.86	2.82	83.04

Bright and dark arena

In table 3 diazepam (1.0mg/kg) treated rats showed significant rise in the number of bright chamber entries, time spent and the rears in bright arena, and significant reduction in duration of immobility.

Gallic 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 (0.2mg/kg) .

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 and anxiety states.

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. 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 is regarded as markers of anxiety. Rearing reflects the compound, Gallic 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 Gallic acid in the elevated plus maze were comparable with those following the administration of diazepam and 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 Gallic acid acts have been postulated to be through its GABAergic properties; through inhibition of gammaaminobutyric acid transaminase (GABA-T) activity; and nitriergic modulation

involving only inducible NOS and not neuronal NOS (Gilhotra N *et al.*, 2010).

Because the increase in GABAergic neurotransmission was associated with reduced anxiety, the behavioural study that has been described in this report was aimed at determining the anxiolytic effects of Gallic acid. However further studies are needed with Gallic acid to elucidate the possible mechanism involved and its use in humans.

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