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**Original Research Article**

# Attenuation of Fear and Anxiety Related Behaviours by Ethanolic Leaf Extract of *Gongronema latifolium* in Swiss Albino Mice

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## Abstract

Anxiety disorders are serious medical illnesses that affect approximately 19 million American adults. To cope with anxiety, people employ potentially harmful strategies like alcohol, cigarettes, medication, withdrawal etc. which may cause panic disorder to worsen. *Gongronema latifolium* (GL) is one of the medicinal plants used in the treatment of ailment including mental disorders. This study was therefore aimed to elucidate effects of administration of ethanolic leaves extract of fear and anxiety using light/dark transition box. Thirty (30) adult male Swiss white mice were assigned into three groups of ten mice each, thus: control, low and high dose groups placed on 0.9% normal saline, 200 mg/kg and 400 mg/kg of ethanolic leaves extract of GL respectively. All the animals were allowed food and water *ad libitum*. Results showed significant ($p<0.001$) increase in the frequency of line crosses, rearing activities in the extract treated groups when compared to the control. There was a corresponding decrease ($p<0.001$) stretch attend posture, freezing, grooming frequency and grooming duration in the test groups when compared to the control. These indices show that the extract treated groups of mice exhibited decreased fear and anxiety behaviours when compared to the control group. In conclusion, extract of *Gongronema latifolium* could be used as an anxiolytic for anxiety related disorders due to its ability to attenuate fear and anxiety related behaviours in mice.

**Key words:** *Gongronema latifolium*, fear, anxiety, behaviour, mice.

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## INTRODUCTION

Fear and anxiety act as a signal of danger, threat, or motivational conflict, and to trigger appropriate adaptive behavioural responses (Borrsini *et al.*, 2002). The phenomenon of fear is controlled by a neural circuit involving the amygdala that has been designed to keep the organism alive in dangerous situations (Steimer, 2002).

Anxiety, as a feeling of apprehension and fear is characterized by physical symptoms such as palpitations, sweating and feelings of stress. Anxiety disorders include: panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, social phobia (or social anxiety disorder), specific phobias and generalized anxiety disorder (Muech and Hamer, 2010).

Anxiety disorders are serious medical illnesses that affect approximately 19 million American adults. Unlike the relatively mild brief anxiety caused by a stressful event such as a business presentation or a first date, anxiety disorders are chronic, relentless and can grow progressively worse if not treated (Scott, 2011).

To cope with anxiety, people employ potentially harmful strategies like alcohol, cigarettes, medication, withdrawal etc. Although alcohol and sedatives initially help ease panic disorder symptoms, medium or long term alcohol and sedative abuse can cause panic disorder to worsen (Terra *et al.*, 2004).

*Gongronema latifolium* (Asclepiadaceae) is among the medicinal plants used in the treatment of ailment. Phytochemical screening of *G. latifolium* showed the presence of iron, zinc, vitamins, proteins and amino acids. The leaf extract contains alkaloids, saponins, tannins, flavonoids and glycosides (Schneider *et al.*, 2003; Eleyinmi and Bressler, 2007; Atangwho *et al.*, 2003). Its roots contain polyphenols in abundance,

alkaloids, glycosides and reducing sugars (Antai *et al.*, 2009). It has been used to manage gastrointestinal disorders such as diarrhea, ulcers and dyspepsia, hyperglycaemia (Ugochukwu and Babady, 2003). It has antioxidant (Ugochukwu and Babady, 2003), blood boosting (Antai *et al.*, 2009), hepatocellular healing, anti-inflammatory, antimicrobial (Osuala *et al.*, 2005), anti-diabetic (Edet *et al.*, 2011) activities. It is used in the traditional folk medicine for mental disorders.

This study was therefore designed to investigate the effect of *G. latifolium* on fear and anxiety behaviour in Swiss albino mice.

## MATERIALS AND METHODS

### Experimental animals

Adult male Swiss white mice (15g-33g) used for the study were purchased from the animal house of the department of Physiology, University of Calabar. They were kept in the animal house of the department of physiology, Abia State University, Uturu for two weeks for acclimatization before actual experimentation commenced. The temperature of the animal house was 26 ± 2.0$^{o}$c. The mice were exposed to natural day light cycles, and allowed access to rodent laboratory chow obtained from Vital Feeds Nig. Ltd., Lagos, Nigeria and clean drinking water *ad libitum.*

### Experimental Design

Thirty Swiss albino mice weighing between 17g and 33g were assigned into three groups of ten mice each. The control, the low dose and the high dose groups. Identification of the animals was simply done using identification cards attached to each cage. The research was approved by the ethical committee of the Department of Physiology, Abia State University, Uturu.

### Preparation of ethanolic leaves extract of *Gongronema latifolium*

The leaves of the plant, *Gongronema latifolium* were purchased in a local market in Calabar, Cross River State, Nigeria. The leaves were washed and dried under shade, then blended into coarse powder and stored in a cool dry place away from light until required for use. 400 g of the grounded leaves was dissolved in 1250 mL of ethanol (BDH Ltd Poole, England) and allowed to stay overnight. The mixture was then centrifuged and the supernatant collected. The supernatant was suction filtered, first, using Whatmann no. 1 filter paper, and then a second time using cellulose filter paper. The filtrate was evaporated to dryness at 30$^{0}$c using a vacuum rotatory evaporator (Caframo, VV2000, Ohio) and water bath (Caframo, WB2000). This extraction gave a percentage yield of about 4.3%. The dry ethanol extract was reconstituted to a stock of 500mg/ml in 0.9% saline from which various dose concentrations were obtained. Doses of 200 mg/Kg and 400 mg/Kg representing the low dose and high dose respectively were obtained and administered on the mice at the rate of 0.1 ml/10g body weight orally. The control mice were given normal saline via gavaging.

This dosage of administration was adopted from earlier published works of Edet *et al.*, (2011); Owu *et al.*, (2012).

### Assessment of fear and anxiety

The light/dark transition box was used to assess anxiety and fear. The light/dark transition box (LD BOX) is a test of unconditioned anxiety and exploratory behaviour. It is based on the conflict between exploring in a novel environment and avoidance of bright light (Bourin and Hascoët, 2003). The LD BOX (45x27x27cm) is an apparatus made of plywood and consisting of two chambers connected by an opening (7.5x7.5cm) located at floor level in the centre of the dividing wall. The floor is divided into 9x9cm squares and covered with Plexiglas. The small chamber (18x27cm) is painted black and the larger chamber (27x27cm) is painted white (Bourin and Hascoët, 2003). The apparatus was located in a room (2x5m) and lit by a 60 watt red lamp for background lighting.

Procedure**:** Mice were administered the test drug orally, five minutes before experimenting on the LD box. The procedure is based on that of Constall *et al.*, (1989). Mice were scooped up using a plastic container from their home cage and placed in the centre of the white compartment facing the door and allowed to explore the apparatus for five minutes, while the experimenter sitting 1m away, recorded the behaviour of the animals.

After five minutes, mice were removed from the LD box by the base of their tails and returned to their home cages. The number of urinations and defecations were then recorded. The procedure was repeated for each mouse. The maze was cleaned with a solution of 70% ethyl alcohol between tests for each mouse to eliminate olfactory cues.

### Behaviours scored include

- Transitions: Number of times the animal passes into the opposite compartment (all four paws of the mouse must have moved to the new compartment for a transition to be scored).
- Line crosses: Number of times the animal crosses a line drawn on the floor.
- Rearing: frequency with which the animal stands on hind legs or leans against walls of the box with front paws.
- Stretch-attend postures: Frequency with which the animal demonstrates forward elongation of head and shoulders followed by retraction to original position.
- Grooming: Duration of grooming the body while stationary.

- Dark box duration: Length of time the animal spends in the dark side of the box.
- Light box duration: Length of time the animal spends in the light side of the box.
- Defaecation: Number of fecal boli produced (light v. dark).
- Urination: Number of puddles or streaks of urine (light v. dark) (Podhorna and Brown, 2002).

## DATA ANALYSIS

All data were analyzed using StatView 5.0.1 (SAS Institute) for PC or Mac, Microsoft office excel, 2007 version (Microsoft Inc.), and primer of Biostatistics (version 3.01), an MS Dos based statistical package (McGraw-Hill, Inc.). The analysis of variance (ANOVA) was used to test for variability within and among groups. Results were expressed as mean ± SEM (standard error of the mean) and probability level $P<0.05$ was accepted as significant.

## RESULTS

### Frequency of transitions

The frequency of light-dark transitions in the control, low dose, and high dose groups were 17.5 ± 1.14, 21.4 ± 0.90, and 31.7 ± 0.42 (/5min) respectively. The result showed a significant ($p<0.001$) increased frequency of transitions in the extracted treated groups compared to control. The high dose group in turn had significantly ($p<0.001$) higher transitions than the low dose group, fig. 1.

### Frequency of line crosses

The frequency of line crosses in the control, low dose, and high dose groups were 73.2 ± 2.65, 137.1 ± 12.63, and 144 ± 1.31 (/5min) respectively. The frequency of line crosses was significantly ($p<0.001$) higher in the extract treated groups compared with the control. This is shown in fig. 2.

### Frequency of rearing

The frequency of rearing in the control, low dose, and high dose groups was 14.9 ± 0.60, 21.1 ± 0.57 and 30.8 ± 0.49 respectively. There was significant increased ($p<0.001$) rearing activity in extract treated groups compared with the control. This is shown in fig. 3.

### Stretch attend posture

The frequency of stretch-attend posture in the control, low dose, and high dose group was 2.30 ± 0.30, 2.10 ± 0.23, and 1.10 ± 0.10 respectively. The result showed decreased stretch-attend posture in the extract treated groups compared with the control. The high dose group had a significantly ($p<0.001$) lower stretch-attend posture compared to control and the low dose group. This is shown in fig. 4.

### Frequency of grooming

The frequency of grooming in the control, low dose, and high dose group was 5.50 ± 0.40, 5.20 ± 0.49, and 2.80 ± 0.32 respectively. The result shows a significant ($p<0.001$) decreased grooming activity in the high dose group compared with the control and low dose groups. This is shown in fig. 5.

### Light box duration

The light box duration in the control, low dose and high dose groups was 1.33 ± 0.11, 1.63 ± 0.11, and 3.34 ± 0.03 respectively. The high dose extract treated group had a significantly ($p<0.001$) higher time spent in the light chamber of the light-dark box compared with the control. This is shown in fig. 6.

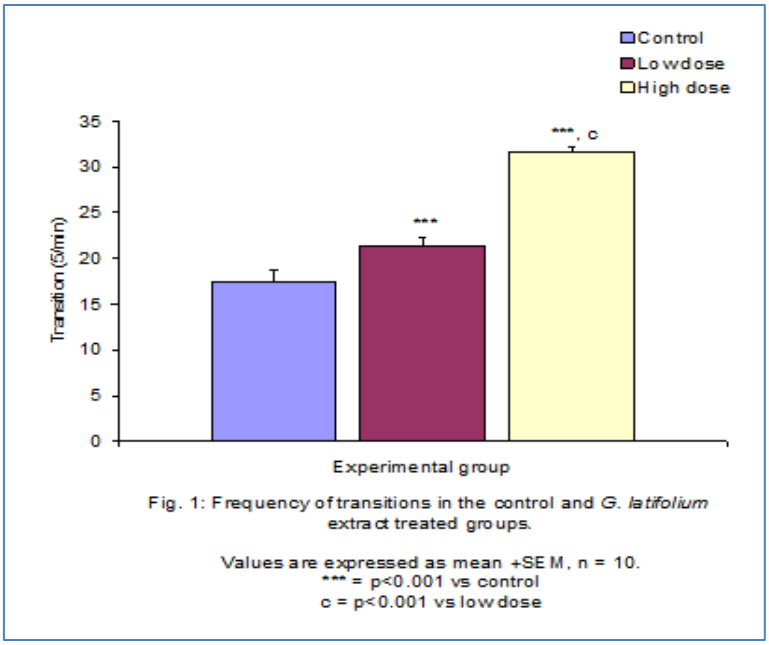


Fig. 1: Frequency of transitions in the control and *G. latifolium* extract treated groups.

Values are expressed as mean +SEM, n = 10.
*** = p<0.001 vs control
c = p<0.001 vs low dose

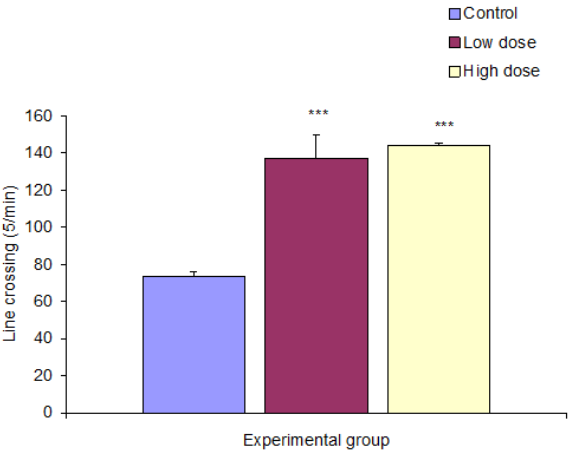


Fig. 2: Frequency of line crossing in the control and *G. latifolium* extract treated groups.

Values are expressed as mean +SEM, n = 10.
*** = p<0.001 vs control

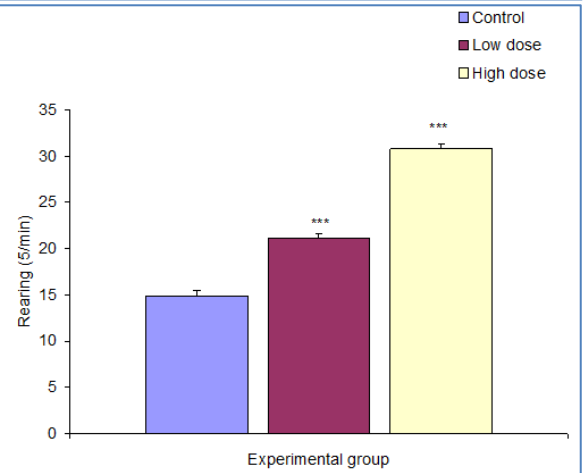


Fig. 3: Rearing frequency in the control and *G. latifolium* extract treated groups.

Values are expressed as mean +SEM, n = 10.
*** = p<0.001 vs control

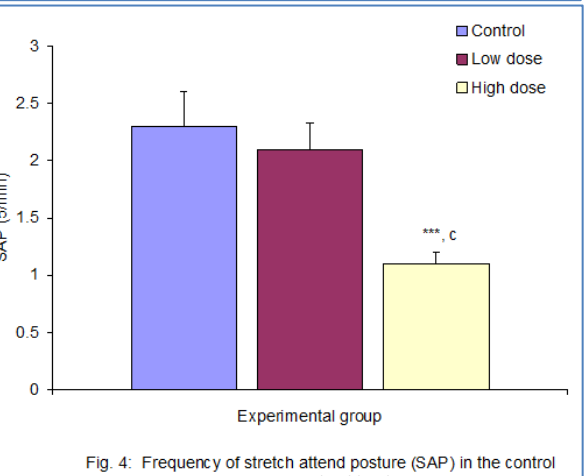


Fig. 4: Frequency of stretch attend posture (SAP) in the control and *G. latifolium* extract treated groups.

Values are expressed as mean +SEM, n = 10.
*** = p<0.001 vs control
c = p<0.001 vs low dose

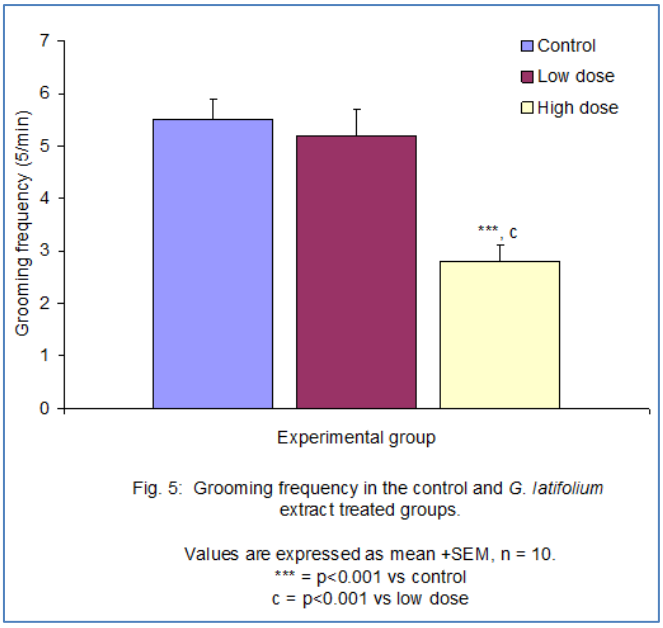


Fig. 5: Grooming frequency in the control and *G. latifolium* extract treated groups.

Values are expressed as mean +SEM, n = 10.
*** = p<0.001 vs control
c = p<0.001 vs low dose

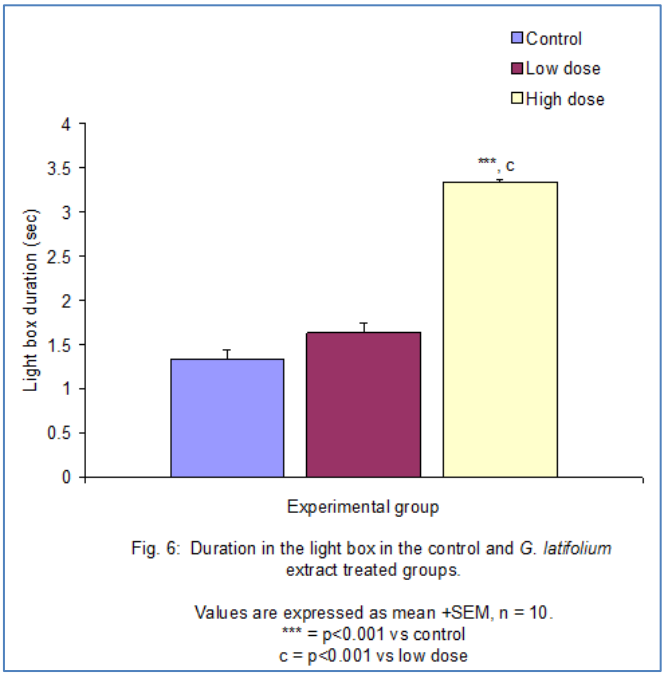


Fig. 6: Duration in the light box in the control and *G. latifolium* extract treated groups.

Values are expressed as mean +SEM, n = 10.
*** = p<0.001 vs control
c = p<0.001 vs low dose

## DISCUSSION

This study was aimed to investigate the effect of administration of ethanolic leaves extract of *Gongronema latifolium* on fear and anxiety behaviours in Swiss white mice using the light/dark transition box.

Brown *et al.* (1999); Rogers (1999) and Stengel (1954) had reported the use of the light dark transition box as a measure of unconditioned anxiety. The physiological basis of this test is centered on the conflict between exploring in a novel environment and avoidance of bright light (Bourin and Hascoët, 2003; Bisong *et al.*, 2010; Erigbali *et al*, 2017). The light chamber of the light/dark transition box is the aversive chamber. So, mice would normally spend more time in the dark chamber of the apparatus. However, an increase in exploratory behaviour in the light chamber of the apparatus indicates decreased fear. This decreased fear (anxiolytic effect) could be caused by anxiolytic drugs/agents (Costall *et al.*, 1989). Since the amygdala is the brain area that controls fear and anxiety, stimulation of, which would induce anxiety and fear (Guyton and Hall); anxiolytic drugs would act by inhibiting it.

The frequency and duration of transition between the two chambers is used as a guide for proper judgment of fear related behaviour in the light/dark transition box. This was significantly increased in the high dose extract treated group compared to the control group. An increase in the frequency of light/dark transition is thought to be an indication of decreased anxiety (Bourin and Hascoët, 2003).

The frequency of rearing in the light/dark box was significantly increased in the extract treated group. This result indicates that *G. latifolium* decreases anxiety in mice. Increased activity such as rearing is associated with non-anxious behaviour (Kim *et al.*, 2002; Bourin and Hascoët, 2003), although baseline activity in other apparatuses such as the open field should be taken into consideration (Bourin and Hascoët, 2003).

The frequency of stretch-attend postures was significantly decreased in the extract treated groups compared to the control group, indicating that root extract of *G. latifolium* decreases anxiety. Stretch-attend postures are "risk assessment" behaviours. These behaviours indicate that the animal is hesitant to move from its present location to a new one (Blanchard *et al.*, 2001). Therefore, a high frequency of this behaviour indicates a higher level of anxiety.

## CONCLUSION

The ethanolic leaves extract of *Gongronema latifolium* decreases anxiety and fear related behaviour in mice. It could therefore be used as an anxiolytic for anxiety related disorders.

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