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Ameliorative Effect of Ethanolic Extract of *Peperomia pellucida* on Anxiety in Pentylenetetrazol-Induced Epileptic Seizure in Mice

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Abstract

Although *Peperomia pellucida* has been traditionally used to manage central nervous system disorders, its anxiolytic properties remain insufficiently characterized. This study aimed to evaluate the anti-anxiety potential of ethanolic extract of *Peperomia pellucida* in a pentylenetetrazol (PTZ)-induced epileptic mouse model. Forty male Swiss albino mice (21–35 g) were randomly divided into four groups (n = 10 per group): Group A (control), Group B (PTZ 35 mg/kg, intraperitoneally), Group C (PTZ 35 mg/kg + *P. pellucida* 2 mg/kg, orally), and Group D (PTZ 35 mg/kg + Diazepam 0.001 mL/kg, orally). Epilepsy was induced via PTZ, confirmed by characteristic jerky movements progressing to generalized convulsions. Treatment was administered for 14 days prior to behavioral assessments. Anxiolytic activity was evaluated using the elevated plus maze and light/dark transition test. Behavioral data were analyzed using IBM SPSS Statistics version 20, with significance set at $p < 0.001$. Mice treated with *P. pellucida* exhibited significant reductions in anxiety-like behavior compared to the PTZ-only group, demonstrating increased time spent in the open arms of the elevated plus maze and in the light compartment of the light/dark box. These effects were comparable to those observed in the Diazepam-treated group. The findings suggest that ethanolic extract of *Peperomia pellucida* possesses notable anti-anxiety activity in PTZ-induced epileptic mice, supporting its potential as a complementary therapeutic agent for anxiety-related conditions.

Keywords: *Peperomia pellucida*; Pentylenetetrazol; Epilepsy; Anxiety; Anxiolytic activity.

INTRODUCTION

In individuals with epilepsy, anxiety has been characterized as the forgotten mental illness (Kanner *et al*., 2011). In individuals with epilepsy, anxiety disorders were long thought to be the second most common mental illness aftsser depression; however, it is now known that anxiety is more common than depression in this population (Jansen et al., 2018; Gandy *et al*., 2013; Sheehan *et al*., 1998; Beyenburg *et al*., 2005).

Anxiety in epilepsy has a substantial impact on a number of critical outcomes, such as mortality, seizure status, and quality of life (Scott *et al*., 2017). According to reports, anxiety accounts for 27% of the variance in lower quality of life among individuals with epilepsy, compared to 12% for depression and 3% for seizure frequency (Choi-Kwon *et al*., 2003).

According to Munir *et al*., (2019), anxiety disorders are the most prevalent mental disorders, characterized by disruptions in mood, thinking, behavior, and physiological activity, as well as accompanying disruptions in sleep, concentration, social functioning, and/or occupational functioning. They are also linked to restlessness, a sense of unease, tenseness in the muscles, and irritability.

Social phobia, which is characterized by fear and anxiety in social situations and results in avoiding social interactions, might affect people with epilepsy (Ballenger *et al*., 1998). The fact that epileptic seizures can happen anywhere and at any moment may contribute to this social anxiety.
The world has recently been much more concerned about these issues, particularly in developing and underdeveloped nations where plants have been used as medicine to treat basic medical conditions.

The plant *Peperomia pellucida* is thought to have cooling qualities and is edible (Melo *et al*., 2017; Termote *et al*., 2010). The entire plant is used to cure measles in Ogun State, Nigeria (Sonibare *et al*., 2017). In Nigeria, it is also used to treat bone fractures, convulsions, hypertension, and hemorrhoids (Chukwuma et al., 2015). According to Gini and Jothic (2013), Raina and Hassan (2016), Egwuche *et al*., (2011), and others, the plant contains terpenoids, tannins, flavonoids, alkaloids, saponins, steroids, and glycosides. According to research by Enene *et al*., (2025), *Peperomia pellucida's* ethanolic extract has been considered to possess anti-epileptic property and can also relieve pain in a pentylenetetrazol induced epileptic seizure in mice.

METHODOLOGY

Procurement of Test Substances

1. **Chemicals:** The Pentylenetetrazol was purchased from Sigma-Aldrich Limited, Canada while Diazepam was purchased from Bez Pharmacy, Calabar, Cross River State. All reagents and chemicals used for this study were of analytical grade.

2. **Extraction of the Ethanolic extract of *Peperomia pellucida***

   Peperomia pellucida leaves were obtained from the botanical garden, University of Calabar, Calabar. A botanist authenticated a sample of the plant (Voucher number - Bot/Herb/UCC/0994). The leaves were washed under running tap water and made free from dirt and sand. The fresh air-dried leaves was powdered in an electric kitchen blender and 300g of the powdered plant material was obtained. Preparation of the ethanolic extract was by the method of Klyushnichenko *et al.,* (1996). 300g of the powered leave extract of *Peperomia pellucida* was completely immersed in 30% of ethanol and shaken vigorously. It was allowed to stand for (24) twenty four hours at room temperature and stirred at intervals. After 24 hours, the extract was filtered severally especially using NO1 What Mann filter paper of pore size 0.45 micrometer and funnel, the filtrate was then allowed to concentrate using rotatory evaporator and Astell Hearson oven was further used to dry the concentrate at 40 – 45^{0}c. This was to ensure complete evaporation of the extract to a pasty black residue. The leave extract of *peperomia pellucida* was collected with the aid of a spatula into a container and was measured using an electric weighing balance. 35g of *peperomia pellucida* paste was obtained at the end of preparation and was kept in a cool dry place at room temperature.

3. **Laboratory Animals:** Forty (40) Swiss male mice of 10 weeks weighing 21 to 35kg were used for this study. The animals were housed in the Department of Physiology Animal House, University of Calabar, Nigeria. Standard animal cages (435 x 290 x150mm) with wood shavings as bedding were used in housing the animals (5 mice per cage). They were given *ad libitum* access to feed (Flourmill Calabar, Cross River State, Nigeria) and fresh water, and exposed to 12/12-hr light/dark phase. The animals were acclimatized for a period of one week and kept in line with laid - down ethics for animal care approved by the National Committee for Research Ethics in Science and Technology (NENT), 2018. Before the commencement of this research, ethical approval was obtained from the University of Calabar animal ethics committee, which aligned with the standard guidelines for the use of laboratory animals outlined by the World Health Organization. The study was permitted with ethical clearance with approval number (Approval No.FARE C/PA/ [UC/050]/181PHY318).
4. **Experimental Design**

   The animals were randomly allotted into 4 different groups (n=10). At the expiration of the one week of acclimatization, *peperomia pellucida* 2mg/kg and Diazepam 0.001ml/kg was administered orally to treatment groups C and D for 14 days. Thereafter, Pentylenetetrazol 35mg/kg intraperitoneally was administered to Groups B and C. The animals were considered epileptic after administration of pentylenetetrazole few minutes after with a jerky movement of the tail and limb progressing to the entire body.

   (Dose per mice outlined in Table 1), once daily, to animals in treatment groups B and C using the doses outlined in Table 1. Whereas the control group was given feed and 0.5ml normal saline as a vehicle throughout the experimental duration.

   Thereafter, the animals were subjected to behavioral testing to assess changes at the expiration of the treatment.

Table 1. Study Design and Drug and Extract administration

GROUPS	NO. OF MICE	TREATMENT
GROUP A	10	Feed + 0.5ml of normal saline as a vehicle throughout the experimental period
GROUP B	10	35mg/kg bw of Pentylenetetrazole
GROUP C	10	35mg/kg bw of Pentylenetetrazole + 2mg/kg bw of *peperomia pellucida*
GROUP D	10	35mg/kg bw of Pentylenetetrazole + 0.001ml/kg bw of Diazepam

5. **Induction of epilepsy**

Epilepsy was induced by intraperitoneal injection of 35mg/kg of pentylenetetrazole, freshly prepared in normal saline before laboratory testing.

The animals were considered epileptic after administration of pentylenetetrazole few minutes after with a jerky movement of the tail and limb progressing to the entire body. The parameters scored included:

a. Onset of seizures: This is when the seizures starts in the brain
b. Frequency of jerks: This is the brief shock-like jerks of a muscle or a group of muscles that occur during seizures in epilepsy
c. Duration of seizures: is how long the seizure lasted
d. Onset of tonic seizures

6. **Protocol test for accessing anxiety**

Light/dark transition box

The light-dark box apparatus has two compartments. The light compartment is 2/3 of the box and is brightly lit and open. The dark compartment is 1/3 of the total box and is painted black and dark. A door of (7.5 x7.5 cm) connects the two compartments (Bourin *et al.,* 2003). The floor is divided into 9 x 9 cm squares and is covered with Plexiglas. Both compartments are covered with lids of clear Plexigals. The mouse is allowed to explore the apparatus for 5 minutes and the behaviors of the mice in the box were recorded. After this, the mice are removed and the box is cleaned using a cotton wool and 70% ethyl alcohol and allowed to dry between tests.

Behaviors scored include:

i. **Transitions:** Number of times the animal passes into the opposite compartment. All four paws of the mouse must have moved into the new compartment for a transition to be scored and for that compartment to be considered entered.

ii. **Line crosses:** Number of times the animal crossed a line drawn on the floor.

iii. **Rearing:** Frequency with which the animals stands on hind legs or leans against the walls of the box with front paws.

iv. **Stretch-attend postures:** Frequency with which the animal demonstrates forward elongation of head and shoulders followed by retraction to original position.

v. **Grooming:** Duration of grooming the body.

vi. **Dark box duration:** Length of time the animal spent in the light side of the box.

vii. **Light box duration:** Length of time the animal spent in the light side of the box

RESULTS

1. Comparison of Rearing

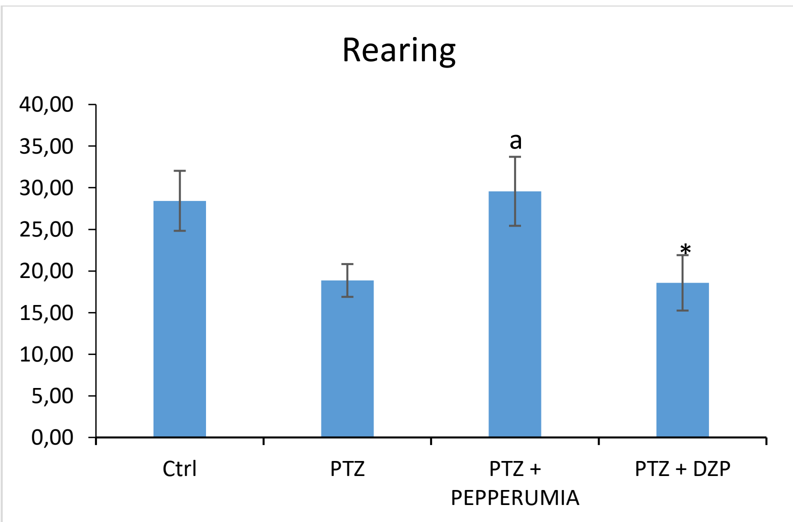

Figure 1: Frequency of rearing of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA followed by *LSD post hoc* *= $p<0.05$ when compared to control; a=$p<0.05$ when compared to PTZ group. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

2. Comparison of Transition

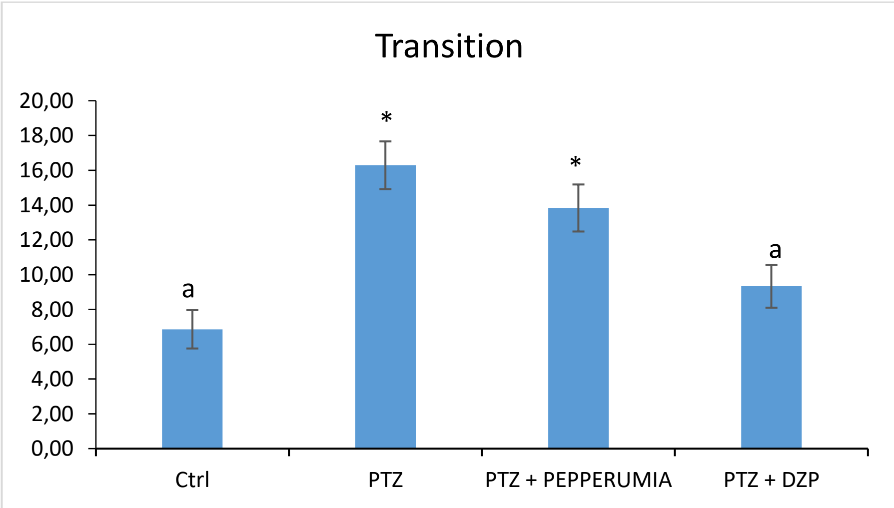

Figure 2: Transition of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA followed by *LSD post hoc* *= $p<0.05$ when compared to control; a=$p<0.05$ when compared to PTZ group. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

3. Comparison of Stretch Attend Postures

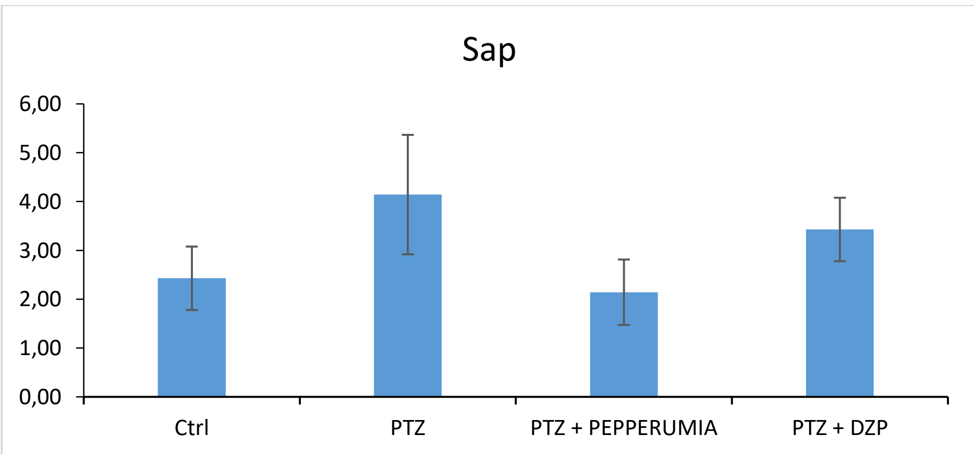

Figure 3: Sap of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA P>0.05 **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

4. Comparison of Grooming

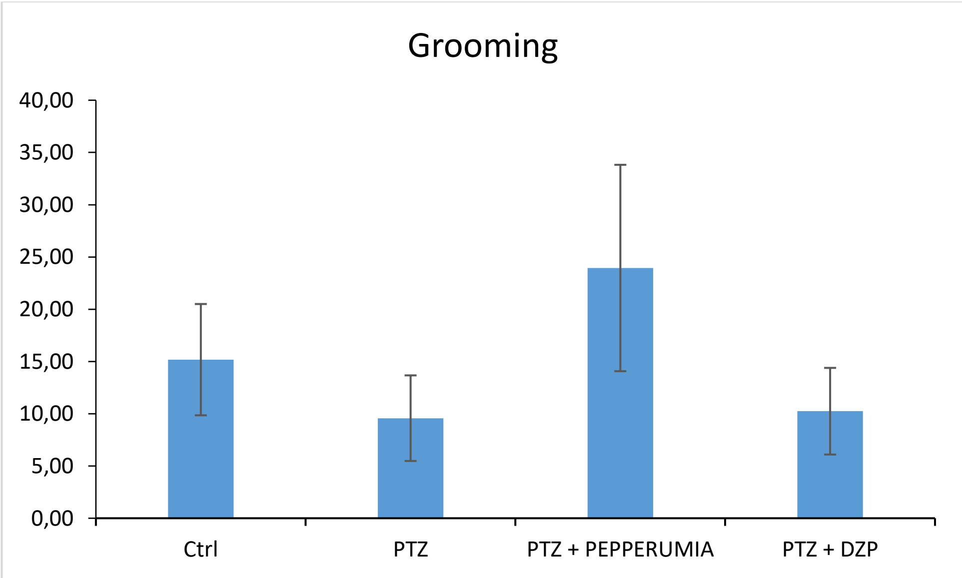

Figure 4: Grooming of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA P>0.05 **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

5. Comparison of Dark duration

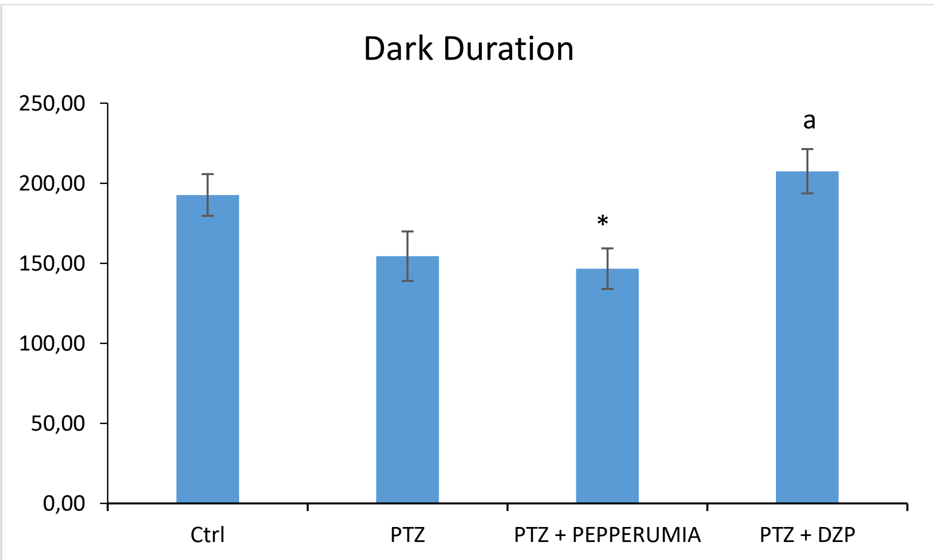

Figure 5: Dark duration of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA followed by *LSD post hoc* *= p<0.05 when compared to control; a=p<0.05 when compared to PTZ group. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

6. Comparison of Light Duration

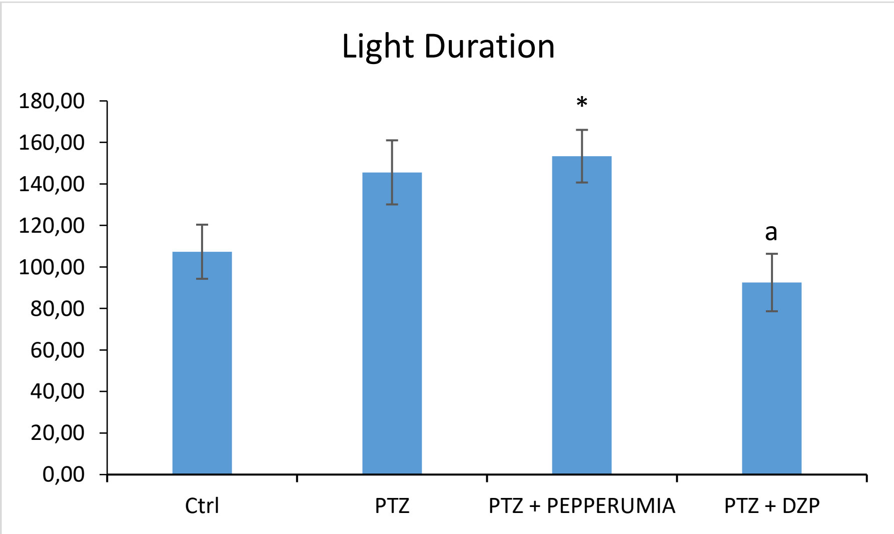

Figure 6: Light duration of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA followed by *LSD post hoc* *= p<0.05 when compared to control; a=p<0.05 when compared to PTZ group. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

7. Comparison of Urination

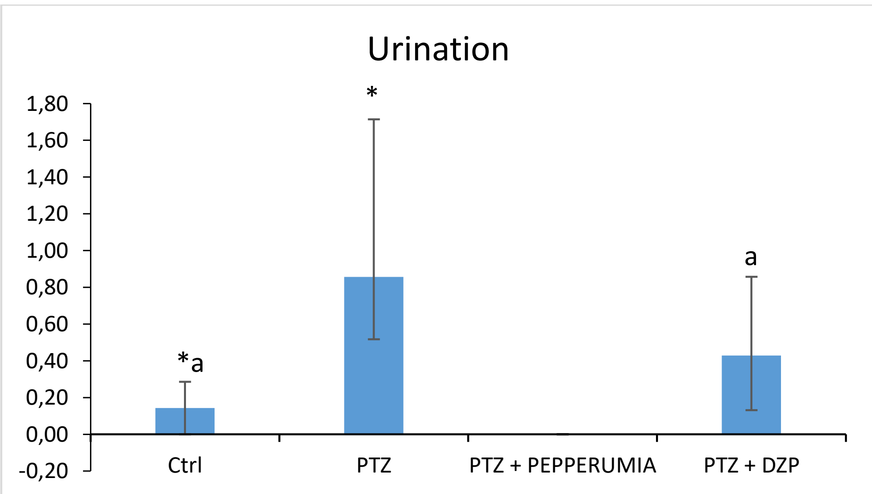

Figure 7: Urination of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA followed by *LSD post hoc* *= $p<0.05$ when compared to control; a=$p<0.05$ when compared to PTZ group. **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

8. Comparison of Defecation

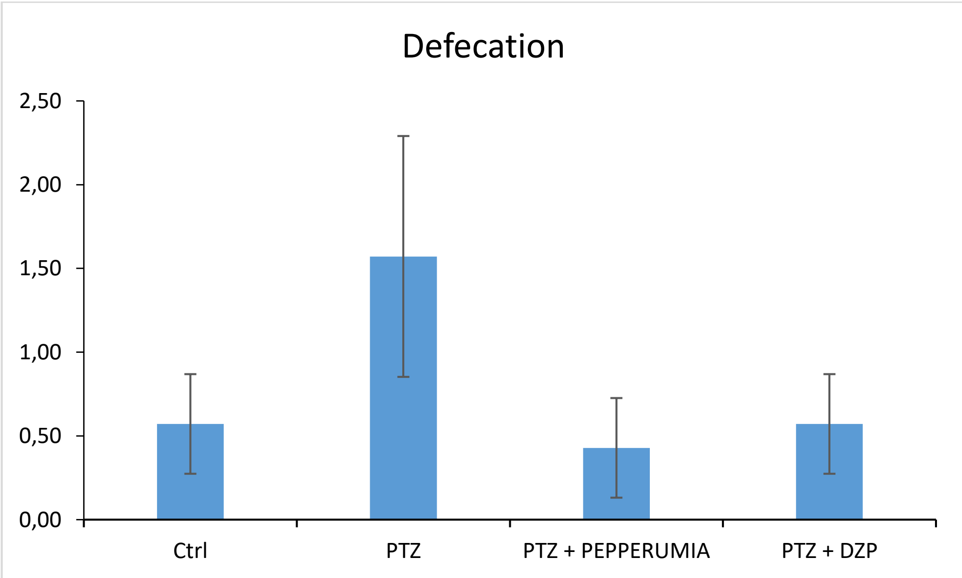

Figure 8: Defecation of albino mice during LD BOX

n=7; mean ± SEM one-way ANOVA $P>0.05$ **Ctrl**= Control; **PTZ**= Pentylenetetrazol; **DZP**= Diazepam

DISCUSSION

The research was aimed at assessing the anti-anxiety potentials of *Pepperomia pellucida* in pentylenetetrazol-induced epileptic seizure in mice.

The light dark transition box was used to assess anxiety in the animals. In the light dark transition box, it is observed that all the animals had increased duration in rearing (fig 1), stretch attend posture (fig 3), grooming (fig 4) and defecation (fig 8). These was an indication that all the animals were anxious though not significant. This may be due to exposure to a new environment and the animals trying to acclimatize.

Duration the transition (fig. 2) which is the number of times the animal passes into the opposite compartment, it was observed that the PTZ+ *Peperomia pellucida* had a significant shorter count in traversing between the opposite compartments (P>0.05). And also during the dark box duration (fig. 5), the length of time the animals spent in the dark side of the box, it was also observed that the PTZ+ *Peperomia pellucida* had a significant shorter duration time in the dark side of the box (P>0.05) when compared to the other experimental groups which spent longer time because there were anxious. Similar trend also followed during the light box duration (fig. 6), which is the length of time the animals spent in the light side of the box, it was also observed that the PTZ+ *Peperomia pellucida* had a significant longer duration time in the light side of the box (P>0.05) when compared to the control and other experimental groups.

All these parameters is an indication that as the experimental procedure progresses, the PTZ+ *Peperomia pellucida* became less anxious when compared to the other experimental groups. This reduction in anxiety in the PTZ+ *Peperomia pellucida* may be attributed to the isolated components styrene, campestrol, stigmasterol and β-sitsterol in the plant. Isolation of styrene, campesterol, stigmasterol and β-sitsterol from the plant has been reported (Ghani, 1998). This is consistent with the findings of (Salen *et al*., 1996) where increased sitosterol and campesterol levels reduce cholesterol biosyntheseis and stigmasterol by inhibiting sterol Delta-22-Reductase (Fernàndez *et al*., 2002) and cholesterol absorption (Hajjaj *et al*., 2005). Consequently, campesterol, stigmasterol and β-sitsterol reduce cholesterol levels in animal body. Since central nervous system synaptogenesis is promoted by cholesterol (Mauch *et al*., 2001) it seems that styrene, campestrol, stigmasterol and β-sitsterol of *peperomia pellucida* might be responsible for this reduction of anxiety of this plant.

CONCLUSION

This study provides compelling evidence that the ethanolic extract of *Peperomia pellucida* exhibits significant anxiolytic activity in a pentylenetetrazol (PTZ)-induced epileptic mouse model. Behavioral assays, including the light/dark transition test and the elevated plus maze, demonstrated a marked reduction in anxiety-like behaviors among mice treated with *P. pellucida*, evidenced by increased time spent in the light compartment and open arms, as well as reduced locomotor transitions indicative of diminished anxiety levels. These effects were statistically significant and comparable to those observed in the diazepam-treated group, a recognized standard for anxiolytic efficacy.

The anxiolytic potential of *P. pellucida* may be attributed to its bioactive phytoconstituents—styrene, campesterol, stigmasterol, and β-sitosterol—which have been previously implicated in modulating cholesterol metabolism and, by extension, central nervous system function. Given that cholesterol plays a critical role in synaptogenesis, the observed reduction in anxiety may be mechanistically linked to the regulation of neuronal plasticity via these compounds.

Overall, the findings support the traditional use of *P. pellucida* in managing central nervous system disorders and suggest its promise as a complementary therapeutic agent for anxiety, particularly in the context of epilepsy. Future studies involving molecular pathway elucidation and clinical validation are warranted to further characterize its mechanism of action and therapeutic applicability.

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