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# Exploratory and anxiety potentials of aqueous extract of *Phragmanthera capitata*

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## Abstract

**Aim:** To evaluate the exploratory and anxiety potentials of aqueous extract of *Phragmanthera capitata* (AEPC) in Wistar rats using hole-board paradigm. **Materials and Methods:** Healthy rats were randomized into 5 groups. Group I (control) received 10 ml/kg saline, Group II (standard) received 0.2 mg/kg Diazepam, Groups III-V (tests) received 100, 200 and 400 mg/kg AEPC respectively. Changes in the emotional state of rats were assessed through changes in exploratory activities. Hole exploration consisted of point-sniff, circular-sniff and central sniff while inside hole exploration consisted of static-dip, rapid-dip and active-dip. Latency of the first head-dipping, number and duration of rearing, number of crossing from one compartment to another were also evaluated. Data were analyzed using analysis of variance (ANOVA) with the test used as post hoc. **Results:** In head dipping, there was a significant increase for 200 and 400 mg/kg AEPC as compared to control. In sniffing, 200 and 400 mg/kg AEPC also showed significant increase in point sniffing and central sniffing respectively. Rearing, duration and mean number of crossing were significantly decreased by 400 mg/kg AEPC. Latency of the first head-dipping was significantly decreased by 400 mg/kg AEPC. **Conclusion:** The results suggest that *Phragmanthera capitata* has bioactive molecules that act in the central nervous system to lower anxiety.

**Keywords:** *Phragmanthera capitata*, Exploratory, Anxiety, Hole-board, Dipping, Sniffing.

## Introduction

Anxiety is an unpleasant state of inner turmoil which causes nervous behavior like fear, apprehension and worries. [1] It can lead to feelings of dread over something unlikely to happen, such as a feeling of imminent death. [2] Anxiety is often accompanied by muscular tension[3], restlessness, fatigue and problems with concentration. Anxiety is a normal human emotion that everyone experiences at times, but when it is too much and continues too long, the individual may acquire anxiety disorder. Anxiety disorders include panic, social anxiety, specific phobias and generalized anxiety.[4-6] Symptoms of anxiety disorders depend on the type of disorder, but general symptoms include: feelings of panic, fear and uneasiness; problems sleeping, cold or sweaty hands and/or feet, shortness of breath, heart palpitations, an inability to be still and calm, dry mouth, numbness or tingling in the hands or feet, nausea, muscle tension and dizziness.[4, 6]

The exact cause of anxiety disorders is unknown, but like other forms of mental illness, the disorders are not the result of personal weakness, a character flaw, or poor upbringing, but might be caused by problems in the functioning of brain circuits that regulate fear and other emotions and environmental stress. [4, 7]

Allopathic medicine used in treating anxiety disorders include: selective serotonin reuptake inhibitors (SSRIs), selective serotonin and norepinephrine inhibitors (SNRIs), tetracyclic antidepressants that are noradrenergic and specific serotonergic antidepressants (NaSSAs), dopaminergic drugs, monoamine oxidase inhibitors (MAOIs) and Emsam.[7] However, using some of these medicines continuously for more than two weeks has a risk of a withdrawal and rebound syndrome, tolerance and dependence.[8] There is also the added problem of accumulation of drug metabolites and adverse effects.[9]

It has been estimated that about 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents approximately 88% of the world's population that

rely mainly on traditional medicine for their primary health care.[10] On top of that, herbal drugs are regarded to have a relatively higher therapeutic window, fewer side-effects and are economic than synthetic drugs.[11] The mistletoe plant, *Phragmanthera capitata*, belongs to loranthaceae family[12] with woody shrub and pendent branches to 2 m, often associated with ants' nests.[13] It is found in West-Central Africa including Cameroon, West Tropical Africa including Nigeria and Angola in South Tropical Africa.[14, 15] Aqueous extract of *P. capitata* administered orally to rats has shown to have anti-diarrheal properties[16]; anti-secretory, gastroprotective and anti-ulcer activities[17]; ant-pyretic and analgesic potentials[18], but no significant adaptogenic effect has been reported.[19] Infusion of leaves treats diabetes, Chlamydia infection, cancer, arthritis, epilepsy, gynecological problems and cardiovascular diseases in Cameroon folkloric medicine.[20-22]

## Materials and Methods

### Plant material and preparation of extract

The mistletoe plant, *Phragmanthera capitata* or Ntsalar, as it is called in Babadjou dialect, was plucked uniquely from avocado trees in Konka, Baligham village in North West Region of Cameroon in February 2014. Plant authentication and preparation of the extract were as reported previously. [16]

### Subjects and housing

Healthy Wistar rats of either sex weighing between 180-200 g were randomly selected from the Animal House Unit, Department of Pharmacology, University of Calabar and used for the experiment. The animals were housed in polyvinyl cages of at least 4 animals per cage and maintained under standard laboratory conditions of temperature (28 ± $2^0$C), relative humidity (50 ± 5%), a normal 12 h light/dark cycle and received standard pellet diet (Agro Feed, Calabar) and tap water *ad libitum*. This animal experimentation was strictly carried out in accordance with the guidelines of the CPCSEA.[23]

### Phytochemical and Acute toxicity tests

Preliminary phytochemical analysis of bioactive agents in the extract and acute toxicity tests had been described previously.[16]

### Experimental Apparatus

The apparatus is a horizontal enclosed board (50 x 50 cm) of white opaque Plexiglas having a raised floor of 5 cm above a white opaque Plexiglass sub-floor. The floor is divided into low, narrow wall compartments (10 x 10 cm) and each compartment having a central hole of 3.5 cm.

### Experimental procedure

Healthy rats were randomized into 5 groups of 6 rats per group. Group I (control) received 10 ml/kg saline; Group II (standard) received 0.2 mg/kg diazepam s.c; Group III-V (tests) received 100, 200, 400 mg/kg AEPC respectively. At 60 min post treatment, each subject was placed in the arena center of the hole - board and allowed to freely explore for 10 min. After each observation, the hole-board apparatus was cleaned with alcohol (70%) to remove scent cues left from the preceding subject. Hole exploration consisted of sniffing and inside-hole exploration. In sniffing, parameters assessed were point-sniff, circular-sniff and central sniff. In inside-hole exploration, parameters evaluated were Static-Dip (StD): the rat puts and maintained its head into the hole, the body is immobile; Rapid-Dip (RaD): the rat rapidly puts into and removed its head from the hole; Active-Dip (AcD): the rat puts its head into the hole; body movements were produced. A 'rearing' was scored when the rat raised itself onto the hind legs and the fore-paws rested on a wall, and 'crossing' was scored when the animal climbed over the wall dividing one compartment from another. Latency of the first head dipping and duration of rearing were also recorded. All behavioral data were scored by two independent observers in a blind process with more than 90% agreement between them.[24, 25]

### Statistical analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple range test for post hoc. Each value represented the Mean ± SEM. $P$ <0.05 was fixed at the designed stage of the experiment.[26]

## Results

Hole exploratory activities of rats after 10 min observation in a hole-board test are shown in Table 1. In static dipping, 0.2 mg/kg diazepam and 100 mg/kg AEPC significantly ($P$ <0.05) reduced the mean number of static dipping as compared to control. In rapid dipping, 200 mg/kg and 400 mg/kg AEPC respectively increased significantly ($P$ <0.05) the mean number of rapid dipping. In active dipping, 0.2 mg/kg diazepam and 200 mg/kg AEPC significantly ($P$ <0.05) increased mean number of active dipping while 400 mg/kg AEPC significantly increased dipping at $P$ <0.05. In sniffing, 100 mg/kg AEPC significantly ($P$ <0.05) reduced the mean number of positions sniffing while 200 mg/kg significantly reduce it to $P$ <0.05 level. In central sniffing, only 400 mg/kg AEPC showed a significant increase ($P$ <0.05).

The latency of the first head-dipping of rats after 10 min of exploratory activities is presented in Fig. 1, where 0.02 mg/kg diazepam significantly ($P$ <0.05) increase mean latency time while 200 mg/kg extract only significantly increased it by $P$ <0.05 level.

The mean number of crossing of rats from one compartment to another after 10 min of exploration is presented in Fig. 2. Diazepam significantly ($P$ <0.05) increased mean number of crossing while 400 mg/kg significantly ($P$ <0.05) reduced the mean number of crossing.

The mean number of rearing, duration of rearing, urination and defaecation of rats after 10 min of exploration is presented in Fig. 3. Diazepam significantly ($P$ <0.05) increased mean number of rearing while at the same time reduced the duration of rearing by the same significant level. 100 mg/kg extract equally reduced duration of rearing while 400 mg/kg reduced it to $P$ <0.05 level.

**Table 1:** Hole exploration of rats after 10 min observation in a hole-board test

| Experimental group | StD | RaD | AcD | PoS | CeS | CiS |
|---|---|---|---|---|---|---|
| I (control) 10 ml/kg saline | 6.21±0.05 | 3.01±0.01 | 2.78±0.01 | 7.56±0.03 | 4.15±0.05 | 1.02±0.01 |
| II (standard) 0.2 mg/kg diazepam | 1.11±0.01[b] | 4.12±0.02 | 5.12±0.03[a] | 4.21±0.02 | 5.12±0.04 | 0.45±0.01 |
| III (test) 100 mg/kg AEPC | 1.10±0.01[b] | 3.65±0.02 | 3.67±0.01 | 2.33±0.01[b] | 4.25±0.02 | 0.23±0.00[a] |
| IV (test) 200 mg/kg AEPC | 4.12±0.02 | 8.21±0.04[a] | 5.17±0.03[a] | 4.81±0.03 [a] | 6.15±0.04 | 0.54±0.01 |
| V (test) 400 mg/kg AEPC | 5.14±0.04 | 10.61±0.41[b] | 10.26±0.42[b] | 6.15±0.05 | 8.18±0.14 [a] | 0.71±0.01 |

StD = static dipping, RaD = rapid dipping, AcD = active dipping, PoS = point sniffing, CeS = central sniffing and CiS = circular sniffing.

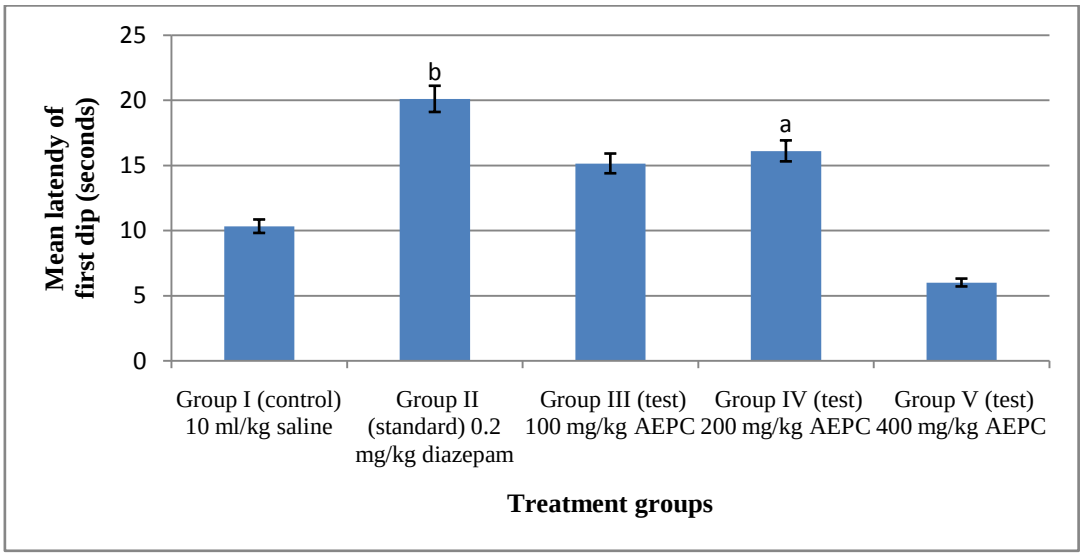


**Figure 1:** Mean latency of first head-dipping of rats after 10 min of exploration in hole board test

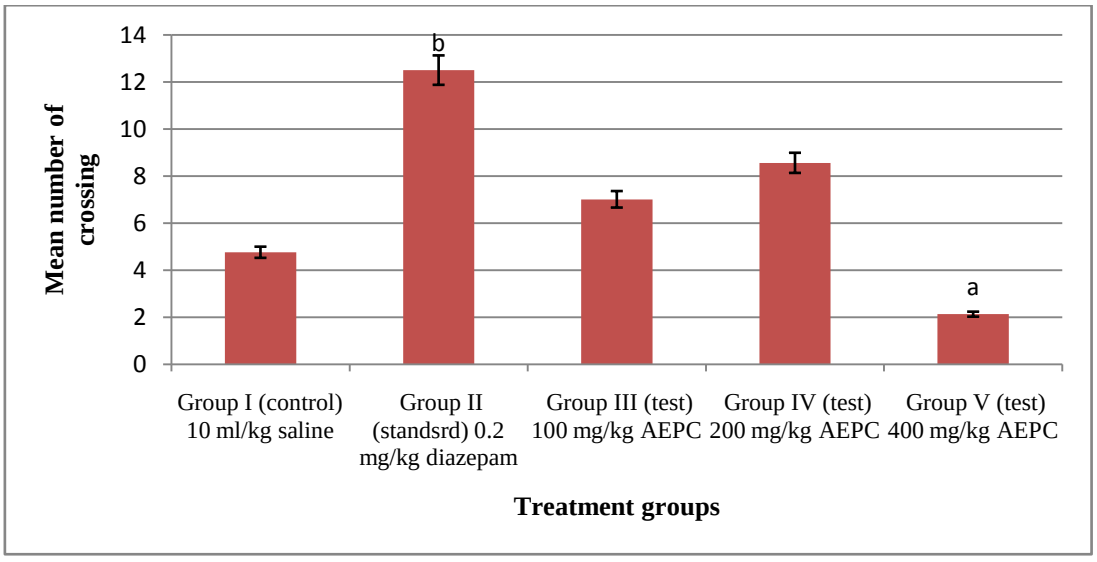


**Figure 2:** Mean number of crossing of rats from one compartment to another after 10 min of exploration in hole board test

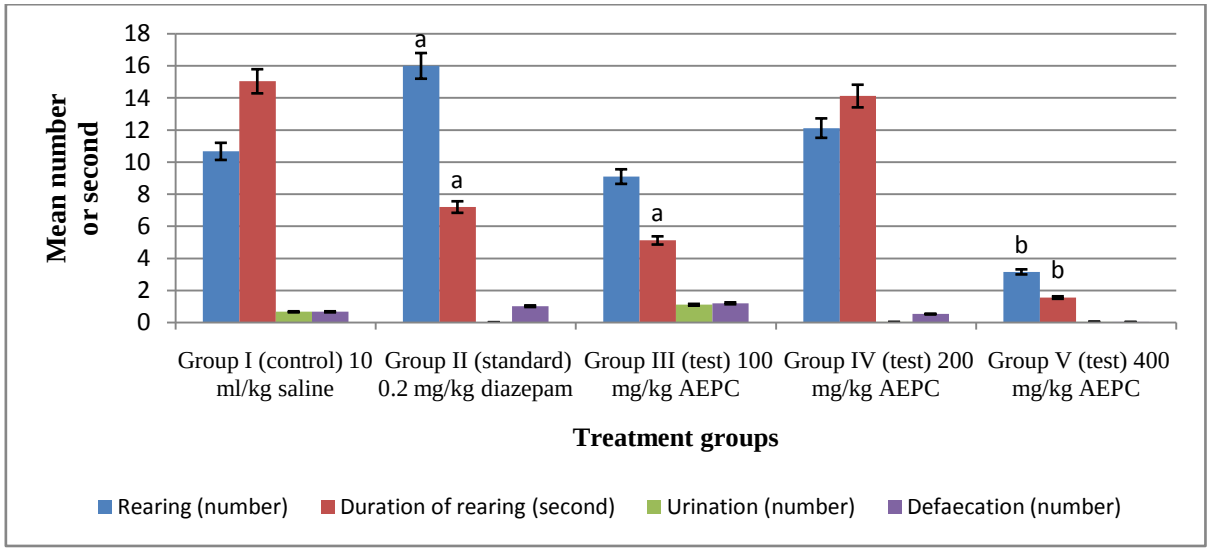


**Figure 3:** Mean number of rearing, duration of rearing, urination and defaecation of rats after 10 min of exploration in hole board test

## Discussion

Hole-board experimental assay is widely used to study anxiety-related behavioral effects produced by a variety of drugs in rodents. It is reported that an increase in rearing is caused by dopaminergic transmission through inhibition of $D_3$ receptors mainly found in the striatum and limbic systems. This inhibition results in: increase dopamine synthesis and release, and increased firing of dopaminergic neurons due to accumulation of dopamine metabolites in these parts of the brain probably by blocking neuronal feedback pathway.[27]

In the present study, rearing was significantly reduced by AEPC as compared to control, suggesting that the extract acted as an agonist to $D_3$ receptors in the brain. Meanwhile, diazepam used as standard increased rearing and this is in agreement with the finding of Davies and Wallace.[25]

Other catecholamines and serotonin have also been reported to increase exploratory activity through inhibition of monoamine oxidase which degrades them in the brain. This, therefore, implicates noradrenergic and serotonergic pathways in shaping and modulating electrical activity involved not only in wakefulness and alertness, but in exploratory activity of an organism. [27]

In the present study, mean number of crossing from one compartment to the other was significantly reduced by 400 mg/kg AEPC as compared to control and significantly increased by diazepam. This suggests that the extract does not antagonize monoamine oxidase. However, diazepam is reported to be commonly used to treat anxiety amongst other psychiatric disorders where it enhances the effect of the neurotransmitter GABA by binding to the benzodiazepine site on the GABAA receptor via the constituent chlorine atom, leading to central nervous system (CNS) depression.[28] In the present study, CNS depression was not obvious as there was increase hole dipping and hole sniffing by extract and diazepam.

## Conclusion

Therefore, aqueous extract of *Phragmanthera capitata* increased rat exploratory activity in a holed board paradigm. It is known that increased exploratory activity of rats is inversely related to anxiety. This means that the extract has anxiety lowering potentials in rat models.

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