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Original Research Article

Anxiolytic activity of hydro-alcohol extract of *Trema guineensis* and its effect on behavioral activities of mice

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ABSTRACT

Background: Objective was to study the anxiolytic activity of hydro ethanolic extract of *Trema guineensis*.

Methods: Male swiss albino mice were used. Hydro ethanolic extract of *Trema guineensis* was administered in the doses of 5, 10 and 20 mg/kg IP hole board (HB), open field (OF), elevated plus maze (EPM) tests were used for determination of anxiolytic activity.

Results: The hydro ethanolic extract of *Trema guineensis* significantly increased the number and duration of head poking in HB test. The extract also significantly increased the time spent and the number of entries in open arm in EPM. In OFT, the extract showed significant increase in number of rearing, assisted rearing and the squares crossed.

Conclusions: In the present study, *Trema guineensis* exhibited anxiolytic activity which might be attributed to its phytoconstituents like alkaloid, steroid and triterpenes. Since *Trema guineensis* is ubiquitous and abundantly grown, it could be a fairly economical therapeutic agent for management of anxiety disorders.

Keywords: *Trema guineensis*, Anxiety, Mice

INTRODUCTION

Anxiety-related disorders such as generalized anxiety, panic, obsessive-compulsive disorder, phobias or post-traumatic stress are the most common mental illness and a major cause of disability in the world.¹ Anxiety disorders are among the most common psychiatric disorders that affect all age groups of the general population.² Approximately 450 million people suffer from a mental or behavioral disorder.³ Although benzodiazepines are among the first line of anxiolytic drugs with well-known benefits, their side effects are prominent, including sedation, muscle relaxation, anterograde amnesia and physical dependence. Therefore, finding novel therapeutic agents with fewer complications in the treatment of anxiety disorder, is of

major interest to researchers.⁴ Despite a phenomenal development of modern drug industry, medicinal plants with traditional background of use in neurological diseases could be good candidates to find new anxiolytic agents. Plants of the *Trema guineensis*, from Ulmaceae family are among important medicinal plants in Ivorian traditional medicine. They have long been used in the treatment of health problems and diseases. *Trema guineensis* leaves are empirically used for sore throat, cough and hypertension treatment.⁵ Literature survey revealed a variety of pharmacological actions such as anticonvulsant, analgesic, anti-inflammatory, anti-plasmodial activities for these plants.⁶⁻⁸ Its phytochemical analysis indicated the presence of several secondary metabolites such as polyphenols, alkaloids, flavonoids, saponosids and tannins. The presence of these compounds could confer to the plant, these several

pharmacological activities. No scientific report regarding the in vivo anxiolytic activity of *Trema guineensis* extract (TG) has been published. That's why, the present study was undertaken to assess the possible anxiolytic effects following single administration of hydro ethanolic extract of leaves from *Trema guineensis* in mice. For this purpose, we used the elevated plus-maze, hole board and open field tests.

METHODS

Plant material

Fresh leaves of the plant were collected from Daloa, Cote d'Ivoire in October, 2019. The plant was identified and verified by botanist Professor from Jean Lorougnon GUEDE university of Daloa, Cote d'Ivoire. The collected leaves were dried under a shade during two weeks and pulverized using the crushing assistance (IKAMAG RCT®). The powder of leaves obtained, constituted our sample to be analyzed.

Extract preparation

100 g of *Trema guineensis* powder were extracted in one liter (1 ltr) of ethanol-water mixture (70/30 v/v). The mixture obtained was then homogenized using a mixer during 24 hours. The homogenate obtained is filtered successively twice on absorbent cotton then once on Wattman No. 1 filter paper. The filtrate was carried thereafter to evaporation in a drying oven with 50°C during 48 hours.

Animals

Healthy adult male swiss albino mice weighing (20-30 g) were obtained from the animal house of Jean Lorougnon GUEDE University, Daloa. These animals were housed under standard environmental conditions. The mice were fed with FACI® (Fabrication d'Aliments de Cote d'Ivoire) pellets, groundnuts and dried fish. They had free access to drinking water ad libitum.

Drugs and chemicals

The standard drugs diazepam was collected from square pharmaceuticals Ltd., Cote d'Ivoire. Saline water which was used for dilution purpose was prepared was obtained from Jean Lorougnon GUEDE university of Daloa (Cote d'Ivoire).

Behavioral parameters used to test anxiolytic activity elevated plus-maze test

The elevated plus-maze (EPM) test consisted of two open arms (30×5×0.25 cm) and two closed arms (30×5×15 cm) emanating from a common central platform (5×5 cm). Two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 40 cm above floor level. At the beginning of the session, a

mouse was placed at the centre of the maze, its head facing an open arm and allowed to explore the maze for 5 minutes, and the following parameters were scored: the time spent and number of entries in each type of arms.⁹ The plus maze was carefully cleaned with a wet towel after each animal test. The mice were divided into five groups (5 mice/group). The control group received vehicle (saline water 0.1 ml/mice). Diazepam (1 mg/kg BW, IP) was used as the positive control or standard group and *Trema guineensis* extract at doses of 5, 10 and 20 mg/kg body weight, in the three remaining groups. After each trial, the EPM apparatus was wiped clean with alcohol 70% solution.

Open field test

Locomotor activity and exploratory behavior were assessed in an open field. The apparatus consisted of a wooden box (60×60×30 cm³) with the floor divided into 16 squares (15×15 cm²). The apparatus was illuminated with a 40-W lamp suspended 100 cm above. Mice were treated with *Trema guineensis* (5, 10 and 20 mg/kg, i.p.), diazepam (1 mg/kg, IP) was used as the positive control drug or vehicle (IP). After 30 minutes, they were placed individually in one of the corner squares. The number of rearing, assisted rearing (forepaws touching the wall of the apparatus) and squares traveled were counted for 5 minutes.¹⁰

Hole board test

The hole board apparatus consisted of a wooden chamber (40×40×25 cm³) with 16 holes (each of 3 cm diameter) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the mice could peep through the holes. The mice were treated with *Trema guineensis* (5, 10 and 20 mg/kg, IP), diazepam (1 mg/kg, IP) or distilled water (IP) 30 minutes prior to test and kept in the apparatus. The numbers and the duration of head poking were recorded during the 5 minutes observation period

Statistical analysis

Results are expressed as mean±SEM. The statistical analysis of data was done using the one-way analysis of variance (ANOVA) followed by Dunnett's test. A probability level less than 0.05 was considered statistically significant.

RESULTS

Elevated plus maze test

The saline-treated mice spent 28.8±1.2 s in the open arm and 247±2.6 s in the closed arm, with 9.3±4.5 entries into the open arm and 11.7±2.6 entries into the closed arm. *Trema guineensis* (5 and 10 mg/kg) and diazepam (1 mg/kg) induced significant (p<0.01) increase in the occupancy in the open arm.

Table 1: Effect of *Trema guineensis* on animals' stay in the open and enclosed arms of the elevated plus-maze in mice (n=5).

Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	Entries into open arm	Entries into enclosed arm
NaCl 10 ml	28.8±1.2	247±2.6	9.3±4.5	11.7±2.6
DZP 1 mg/kg	104±6.4**	159±4.5**	20.5±1.9**	8.7±1
RV5 mg/kg	115±1.2**	138.2±2.4**	18.5±1.7**	9.5±2.1
RV10 mg/kg	90.8±12**	219.8±1.8	8.3±4.1	10.5±2.4
RV20 mg/kg	30.5±2.6	220.7±2.1	10.2±4.2	9.7±2.2

Values are expressed as mean ±SEM. * - p<0.05, ** - p<0.01

Table 2: Effect of *Trema guineensis* on rearing and locomotion in open field test model (n=5).

Treatment	Rearing	Assisted rearing	Number of square traversed
NaCl 10 ml	3.5±1.2	10.8±1.2	85.2±1
DZP 1 mg/kg	14±6.4**	20.7±6.4**	154±3.4**
RV5 mg/kg	29.5±2.6**	19.5±2.6*	115.2±1.2**
RV10 mg/kg	20.8±5.2**	18.8±2.2*	135.8±2.2**
RV20 mg/kg	11±2.2	12.3±1.2	147.5±2.6**

Values are expressed as mean ±SEM. * - p<0.05, ** - p<0.01

Trema guineensis in the dose of 10 and 20 mg/kg did not cause a significant decrease in the time spent in the closed arm, whereas *Trema guineensis* at a dose of 5 mg/kg and diazepam brought about a significant (p<0.01) decrease in the time spent in the closed arm. The animals treated with diazepam and *Trema guineensis* (5 mg/kg) showed a decreased preference for the closed arm and significantly (p<0.01) increased entries into the open arm. *Trema guineensis* at 10 and 20 mg/kg did not produce any significant increase in open arm entries (Table 1).

Open field test

The saline-treated mice traversed 85.2±1 square and showed 10.8±1.2 assisted rearing and 3.5±1.2 self-rearing during the test interval of 5 minutes. *Trema guineensis* at 5 and 10 mg/kg and diazepam brought about a significant (p<0.01) and dose-dependent increase in the number of squares traversed. The assisted rearing and self-rearing were significantly (p<0.05 and p<0.01, respectively) increased by *Trema guineensis* (5 and 10 mg/kg) and diazepam; *Trema guineensis* at 20 mg/kg did not produce significant effect (Table 2).

Hole board test

Each mouse was placed individually in the hole-board apparatus and the number of head pokes and the duration of head poking were noted. With the dose of 20 mg/kg, IP, of *Trema guineensis* there was no significant increase in number of head pokes when compared with vehicle. *Trema guineensis* at 5 and 10 mg/kg, IP, increased the number of head pokes significantly (p<0.01) and dose dependently. The duration of head poking was also significantly (p<0.01) increased by *Trema guineensis* at all doses.

The reference standard (diazepam, 1 mg/kg, IP) treated group showed significant increase in exploratory activity (p<0.01) (Table 3).

Table 3: Effect of *Trema guineensis* on hole board (n=5).

Treatment	Duration on head poking (s)	Number of head poking
NaCl 10 ml	28.8±1.2	26.8±1.2
DZP 1 mg/kg	70±6.4**	74±6.4**
RV5 mg/kg	50±1.2**	48±1.2**
RV10 mg/kg	74.8±12**	76.8±12**
RV20 mg/kg	90.5±2.6**	27.1±2.6

Values are expressed as mean ±SEM. * - p<0.05, ** - p<0.01

DISCUSSION

The benzodiazepines (BZDs) are relatively safe and are widely used anxiolytic agents. These agents are known to act through the BZD-GABA receptors. The role of GABA in anxiety is well established.¹¹ The EPM is one of most popular animal tests for research on behavioral pharmacology of anxiety. It involves spontaneous or natural aversive stimuli, i.e., height, unprotected opening, and novelty.¹² Several plants that are used in folk medicine to diminish anxiety are reported to bring about an increase in the exploration of the open arms in the EPM test.¹³ In EPM, NAOVE mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by fear of open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics.¹⁴ In our study, we noticed that *Trema guineensis* (5 and 10 mg/kg) induced significant increases in the both the

number of entries and time spent in the open arms. The number of entries and the time spent in the closed arms were reduced in the extract-treated group as compared to the control group. The open-field apparatus provides information on anxiety-related behavior characterized by natural aversion of rodents to an open brightly lit area.¹⁵ Animals are thus afraid of the centre and spend more time in the protective corners and in freezing state. Anxiolytics increase total locomotive activity resulting in a reduction of time spent in corners, an increased time spent in the center and a decreased time spent in freezing state. The results obtained in the open field test showed that *Trema guineensis* administration significantly increased rearing, assisted rearing, and number of squares traversed, which supports the anxiolytic-like activity of *Trema guineensis*. The anxiolytic activity of some agents has been assessed by using the hole-board test.¹⁶ A significant increase in the exploratory head-dipping behavior was observed after treatment with 5 and 10 mg/kg of *Trema guineensis* extract, thus reinforcing the hypothesis that it has anxiolytic-like activity. These results confirm the anxiolytic effects of *Trema guineensis*. They are to be compared with the work of Nsour, who in a similar study showed the anxiolytic effect of *Rauvolfia serpentina*; from Aidee who highlighted the anxiolytic effects of the ethanolic extracts of *Argemone mexicana*; from Carla et al who demonstrated anxiolytic properties of aqueous extracts of *Salvia miltiorrhiza* in rats; Charles and Carnevale, who showed anxiolytic properties of extracts of *Maerua angolensis* in mice and *Griffonia simplicifolia* in rat.¹⁷⁻²¹ The anxiolytic effect of the hydro ethanolic leaves from *Trema guineensis* could be due to the presence of alkaloids among the compounds of *Trema guineensis*. Indeed, Aidee demonstrated that the alkaloids isolated from *Argemone mexicana* extracts increased the percentage of time spent in the open arms of rat EPM, in the same way as diazepam and *Argemone mexicana* extracts.¹⁸

CONCLUSION

In conclusion, the results obtained in our study suggest that the extract of the leaves of *Trema guineensis* possesses anxiolytic activity, which is possibly mediated through the GABA A-BZD mechanism. Thus, *Trema guineensis* has potential clinical application in the management of anxiety disorders. Further investigation of the mechanisms of action of the plant extract, as well as the active substances responsible for its biological actions, is necessary.

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