



GABA mediated response of aqueous, ethanol and ethyl acetate extracts of *Dicranopteris linearis* leaf in Swiss Albino mice

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ABSTRACT

Introduction: The objective of the study was to assess the potential of the leaf of *Dicranopteris linearis* in altering the CNS functions with three different extracts; aqueous, ethanol and ethyl acetate.

Methods: To evaluate and compare the activities Morris maze, elevated plus maze (EPM), open field, hole cross and head dip tests were performed and many behavioral parameters were observed. The forced swim in Morris water maze analyzed the depression of rodents in terms of inability to self-rescue. Alongside, hole cross and open field tests assessed the inhibition of locomotor activities. Moreover, EPM test screened the anxiolytic potential while the head dipping hole board test supported the previous experiments by evaluating both sedative, depressive and anxiolytic potentials of the extracts.

Results: The results showed that the ethanol extract significantly suppressed CNS activity by reducing number of locomotor activities and increasing the stability phase (in EPM and Morris maze) supporting mild sedation, depression and anxiolysis. Furthermore, the ethyl acetate extract also possessed moderate to high potential in reducing locomotor activities depending on gradient doses. Results were compared with control group and found statistically significant.

Conclusion: As this plant mimic the activity of a gamma-aminobutyric acid (GABA) agonist, it can be concluded that the plant may have GABA mediated involvement in central nervous system. However, the responsible compounds for these activities are yet to be investigated and this may potentiate a new source of drug development.

Implication for health policy/practice/research/medical education:

Dicranopteris linearis mimics the activity of a gamma-aminobutyric acid (GABA) agonist and the plant may have GABA mediated involvement in central nervous system. This may potentiate a new source of drug development. The research work can be a lead for fellow researchers to investigate in depth pharmacology of the compounds present in this plant leaf extract.

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Introduction

Dicranopteris linearis is a medicinal plant used by tribes in different localities of Bangladesh for various ailments. The plant belongs to Gleicheniaceae family and is characterized as forking fern by its segmented leaves in linear shape, up to 7 centimeters long and a few millimeters wide. The undersides are hairy and sometimes waxy. The young leaf juice has also been reported to be used by the Garo tribes

of Haluaghat (Mymensingh) for cough, allergic symptoms and respiratory troubles (1). Many other pharmacological responses including analgesic, antipyretic, anti-inflammatory (2), antibacterial, antioxidant (3), hepatoprotective (4), gastroprotective (5) and cytotoxic (6) activities have been reported in scientific literatures and other potential activities are under investigation. However, no CNS activity has been reported in literatures

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and no comparison study on different extracts was found. Thus, the aim of this study was to investigate the CNS activity of the leaf of this plant, to support the scientific basis of its medicinal use and to find most potent extract by comparison. To assess the potentials, three different solvents were selected to extract the maximum potent compounds depending on the polarity of the solvents. The study was designed accordingly to compare the activity of these three extracts.

Materials and methods

Collection and preparation of leaf samples

The plant leaves were collected from the district of Mymensingh of Bangladesh in June, 2014 (Accession Number: DACB 42009). The leaves were processed by washing, drying and crushing into powder. The powdered material was soaked for 7 days in water (DLAQ), ethanol (DLET) and ethyl acetate (DLEA) solvents in separate closed vessels intended to extract the pharmacological active components. The solution was then filtered and condensed through a rotary evaporator to get the viscous extracts.

Drugs and Chemicals

Diazepam, ethanol and ethyl acetate obtained for the experiments were of analytical grades.

Grouping of animals

Swiss albino mice were used for all the experiments. Animals were obtained from the university animal house and selected based on their age (2.5 months), weight (25-30 gm) and capability of free movement. For each experiment, mice were divided into eight groups ($n = 5$) for different treatments; control (dH₂O), standard (diazepam 1 mg/kg), DLAQ (200 mg/kg), DLAQ (400 mg/kg), DLET (200 mg/kg), DLET (400 mg/kg), DLEA (200 mg/kg) and DLEA (400 mg/kg) as per body weight. For handling and treating animals, institute adopted animal research standards were followed (7).

Morris maze test

The test was performed according to the method established by Morris (8) and as described by Alikatte et al (9) with slight modifications. A round bowl measuring 70 cm diameter and 14 cm height was filled with water where there was only one crystal clear glass stand at water level intended to make it almost completely invisible. The glass stand was subjected to avoid forced swim. The animals were trialed for their response in forced swimming the day before the experiment for 5 minutes and the mice able to rescue themselves were selected for the experiment. On day of experiment, 30 minutes after oral gavages of control, standard diazepam and test samples the mice were placed in the water away from the stand and were observed for the time being they find the place to rescue themselves from the induced stress. Five minutes were considered as

the withdrawal time. The drug induced depressed groups were compared to the control group which served as the model of natural behavior.

Elevated plus maze test

Elevated plus maze (EPM) test is a well-established method to analyze the anxiolytic potential of samples. A cross sectional four armed (two closed and two open) apparatus was used for this assessment. One hour after drugs and samples treatment of respective groups the animals were placed in the centre of maze to observe and record their tendency to entry in the open or closed arms and their duration spent in those arms. The 5 minutes observation of this parameter identifies their behavioral changes (10).

Hole cross test

A specially designed rectangular shaped (30×20×20 cm) apparatus having a mid-partition with an integrated hole (3 cm diameter) which divided the box into two equal compartments was used for this test. Animals were placed in the apparatus 20 minutes after treatment of drugs and samples and observed for the number of hole crossed and number of rearing by them for next 10 minutes (11).

Open field test

The open field apparatus was designed as a square floor divided into several small squares colored alternatively black and white. Like the hole cross tests, 20 minutes after oral treatments animals were placed in the apparatus and observed accordingly for next 10 minutes for their behavior of distance travelled (number of square crossing) and number of rearing (12).

Head dipping test

This method was described by Clark et al (13). A specially designed square board containing uniformly distanced 4X4 holes and having in-between walkways for the animals was used for this experiment. The animals after being treated with standard and samples were placed in the board where they were observed for 20 minutes for their activity of dipping the head into the hole (14).

Statistical analysis

Statistical analysis for animal experiments was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests using SPSS 20 for windows. The results obtained were compared with the vehicle control group. P values < 0.05 , 0.01 and 0.001 were considered to be statistically significant.

Results

Morris maze test

The results showed that the ethanol extract in higher dose significantly increased ($P < 0.01$) the time for the animals to find the rescue object which was reluctance of the animal and the indication for depressive pattern

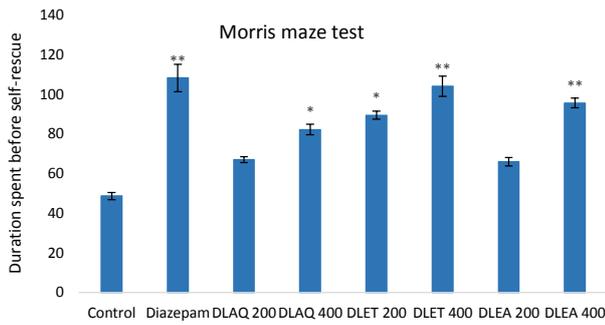


Figure 1. Effect of different extracts of *D. linearis* on duration spent before self-rescue (swimming time in seconds) in Morris maze test. Data are presented as the mean \pm SEM (n = 5). ** $P < 0.01$, * $P < 0.05$

of behavior (Figure 1). In comparison, the control group showed a rapid recognition of the rescue object (glass stand). Ethyl acetate extract also increased the duration compared to the standard ($P < 0.01$).

Elevated plus maze test

Figure 2 compared the parameters of number of entry in open arm and duration spent in open arm exhibited by the extracts to control group. This indicates the development of confidence level. The result showed firm activity of the standard to increase both the parameters as an indication of anxiolytic activity. Only ethyl acetate and ethanol extract exerts their potential to mimic the standard. The result was statistically significant.

Hole cross test

Two parameters (number of hole crossing and rearing) regarding the pattern of depressive behavior was studied and the result found standard ($P < 0.001$), DLET 200, DLET 400 ($P < 0.001$) and DLEA 400 significantly reduced

the locomotor activities which was the indication of depressive behavior (Figure 3).

Open field test

Like hole cross test, open field test also produced similar results observing the depressive parameters of distance travelling and rearing (Figure 4). Both parameters exhibit that DLET 400 suppressed the CNS functions compared to the standard ($P < 0.001$). However, DLET 200 and DLEA 400 also showed moderate activity in reducing locomotor function.

Head dipping test

Like all the above experiments DLET 400 mimics the activity of standard in reducing the CNS functions significantly in head dip test. The number of head dip into the holes were significantly reduced compared to control and was statistically significant at $P < 0.01$ (Figure 5).

Discussion

The use of three different solvents in extraction procedure rationalized for the presence of maximum potent compounds. Water served to extract the polar compound, ethanol for slightly polar to nonpolar compounds while ethyl acetate obtained the nonpolar compounds. Both sedative, depressive and anxiolytic potentials were assessed by the performed experiments.

Morris water maze test is widely used method for evaluating the memory retention behavior of experimental animal (15). However some literature suggests that the forced swimming in Morris maze can also assess the depression in rodents (16). Thus method was slightly modified and designed accordingly to evaluate depression in mice. Mice with natural behavior tends to reach the rescue object quickly than that of the mildly sedated or depressed mice.

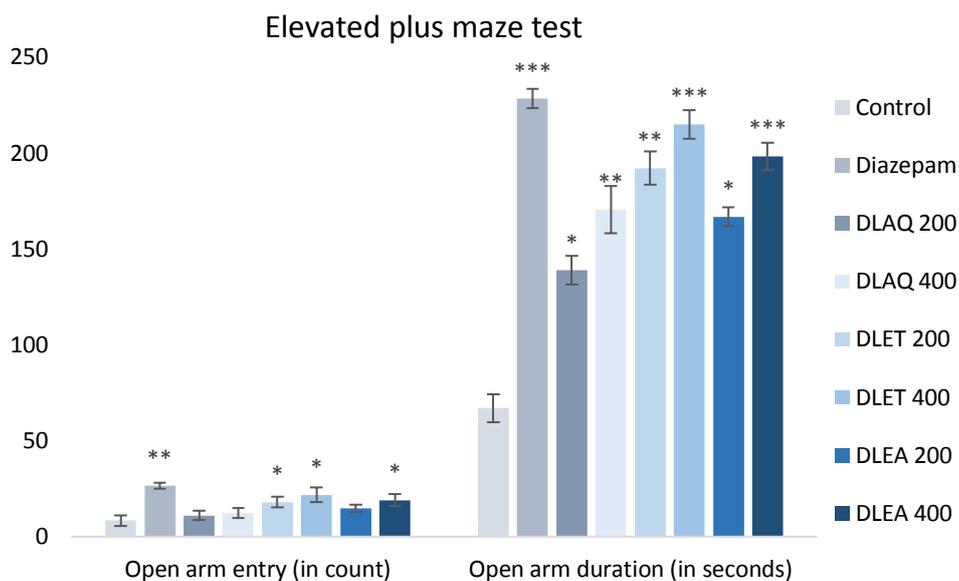


Figure 2. Effect of different extracts of *D. linearis* on number of open arm entry and duration spent in seconds at open arm in elevated maze apparatus. Data are presented as the mean \pm SEM (n = 5). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

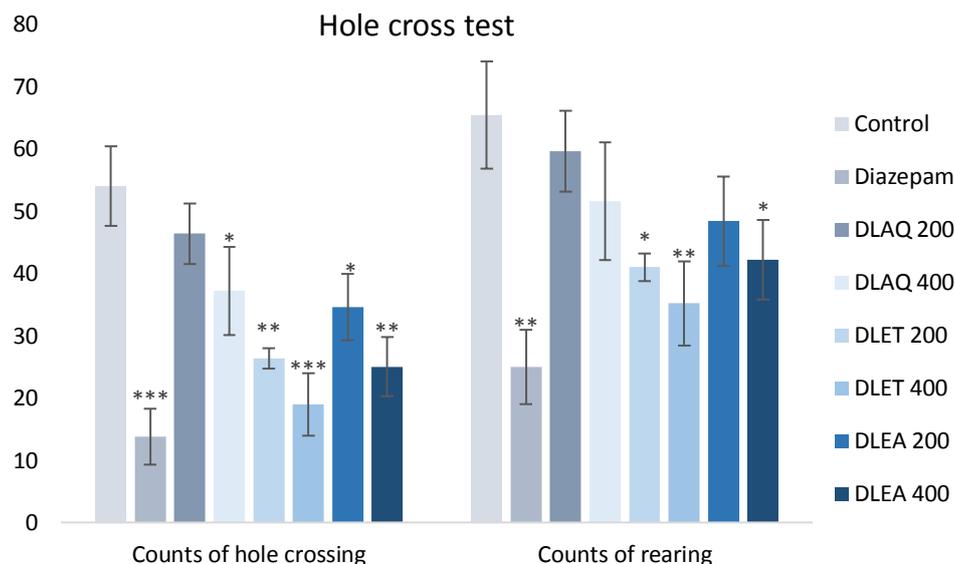


Figure 3. Effect of different extracts of *D. linearis* on number of hole crossed and number of rearing in hole cross apparatus. Data are presented as the mean ± SEM (n=5). ***P < 0.001, **P < 0.01, *P < 0.05.

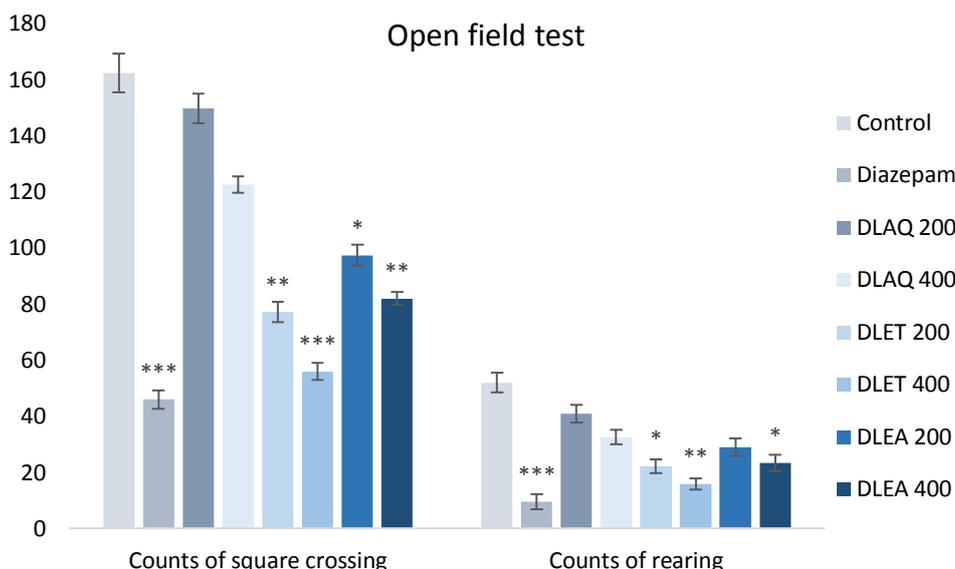


Figure 4. Effect of different extracts of *D. linearis* on number of square crossed (distance travelled) and number of rearing in open field. Data are presented as the mean ± SEM (n=5). ***P < 0.001, **P < 0.01, *P < 0.05.

Therefore, increase in swimming time indicates mild sedation or depression for the experimental subject. The ethanol extract significantly increased the swimming time compared with standard suggesting mild sedation or hypnosis whereas ethyl acetate extract at higher dose also increased swimming time proposing a mild depression by inhibiting neurotransmission.

EPM screened the anxiolytic potential by observing different parameters (17). The frequency of open arm entry and time spent in open arm of maze apparatus are the measures to assess emotional aspects and psychomotor performance of the rodents. The effect of the extract was possibly due to the action on gamma-aminobutyric acid

(GABA) benzodiazepine receptor complex, stimulation of glucocorticoid production and release in the adrenal cortex (18). Alongside, Head dipping test on a hole board also suggests the measure of anxiolytic as well as sedative properties being assessed (19). Head dipping is considered as the sign of exploratory behavior. The ethanol and ethyl acetate extracts confirmed both the anxiolytic and depressive properties via responding to these tests.

Hole cross and open field tests examine the Inhibitory activities of locomotor functions of mice (20,21). The ethanol extract found to reduce the exploratory activities of mice. Since GABA is the major inhibitory neurotransmitter of the central nervous system (22) and

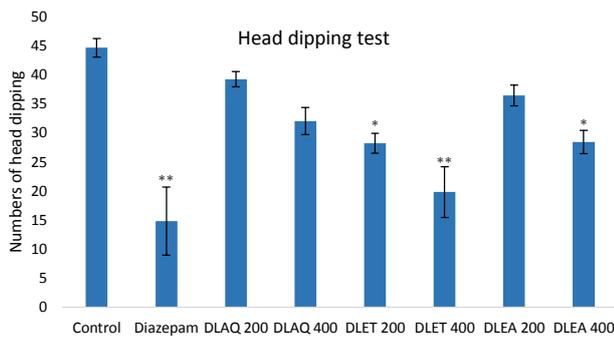


Figure 5. Effect of different extracts of *D. linearis* on number of head dip in hole board apparatus. Data are presented as the mean \pm SEM (n = 5). ** $P < 0.01$, * $P < 0.05$.

most of the anxiolytic as well as CNS depressant drugs like diazepam act through GABA receptor (23), it can be hypothesized that the extracts showing similar activities acted similarly by membrane hyperpolarization to potentiate GABAergic inhibition. This was led by either decrease in the firing rate of critical neurons in the brain or by direct activation of GABA receptor (24).

Conclusion

The ethanol leaf extract of *Dicranopteris linearis* possess significant neuropharmacological potential. Alongside the ethyl acetate extract also produced moderate to significant activities. Behavioral alteration in regard of depression was observed through the different experiments. Therefore it can be concluded that the plant leaf is a good source for new drug development. However, the sample is yet to be investigated for the responsible compounds. Moreover, it can be declared that the use of this plant in medicinal purpose has scientific basis and is safe and nontoxic.

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Authors' contributions

This work was carried out in collaboration between all authors. MMB and KN performed the neuropharmacological tests and prepared the manuscript. KTA designed the methodology and coordinated the whole project. MSU Javed prepared the plant extract and assisted in tests. MNI designed and made the apparatuses and assisted in tests. MMU conducted the statistical analysis of data and assisted in tests.

Conflict of interests

Authors declare no conflict of interests.

Ethical considerations

All ethical issues regarding animal and lab works were fully considered.

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