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# SEDATIVE AND ANXIOLYTIC ACTIVITIES OF METHANOL EXTRACT OF *LYGODIUM PALMATUM* (BERNH.) SW. LEAVES.

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## ABSTRACT

**Background:** *Lygodium palmatum* (Bernh.) Sw. leaves are deliberated as worthy traditional medicine. To give a scientific basis for traditional usage of this medicinal plant, the methanol leaf extract (MELP) was used for its sedative and anxiolytic activities. **Methods:** In this study, the sedative activity was also evaluated using open field test, hole cross test. Besides, elevated plus maze test, hole-board test for exploratory behavior in mice were used to evaluate its anxiolytic activities. The *in vivo* action was done using mice of both sexes. **Result:** The extract showed a dose dependent sedative effect. The number of squares traveled by the mice was decreased significantly ($P<0.01$) from its initial value at 0 to 90 min at the dose of 400 mg/kg body weight of the extract. In the EPM, the behavior of mice model, as observed, confirmed the anxiolytic activity of diazepam as reported previously. The methanol extract of *L. palmatum* leaves (MELP) at the dose of 400 mg/kg, significantly increased the time spent in the open arms. Hole Board test proved that the extract have significant anxiolytic activity. Because, head dipping of mice increased with the tested treatment. **Conclusion:** The obtained results support that *L. palmatum* has well sedative and anxiolytic effects and deserve further investigation to isolate the specific components that are responsible for the sedative and anxiolytic effects. Components from this plant may have a great potential value as medicinal

agents, as leads or model compounds for synthetic or semi synthetic structure modifications and optimization.

**KEYWORDS**: *Lygodium palmatum,* Sedative, open field, Anxiolytic, EPM.

## 1. INTRODUCTION

Herbal medicines have been widely recognized by physicians and patients for their better therapeutic value and fewer adverse effects as compared to modern medicines **(Ansari, Islam, & Sameem, 2012)**. There is an increasing need for multi-component remedies for the treatment of chronic and complicated diseases where drugs with single target action often fail **(Csermely, Agoston, & Pongor, 2005)**. Herbal medicine has already been well established as a valuable source of effective treatment for various human diseases. The phytochemical profiles of herbal remedies can provide hints for designing, screening and developing novel multi-target therapeutics **(Tian & Liu, 2012)**. The therapeutic value of herbal medicines has been attributed to the synergistic effects of a variety of active components; however, most of these active constituents possess insoluble character leading to lower bioavailability and increased systemic clearance **(Bonifacio et al., 2014)**. Huge amounts of phytomedicine used for neuropharmacological disorder and present study also about sedative and anxiolytic effect of *Lygodium palmatum*.

Anxiety disorders are among the most common mental, emotional, and behavioral problems affecting one-eighth of the total population worldwide, and have become a very important area of research interest in psychopharmacology. Anxiety represents a heterogenous group of disorders, probably with no single unifying etiology; various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology of anxiety disorders **(Shri, 2010)**. It is reported to have increasing prevalence in recent cohorts in many countries and to have much earlier ages of onset than other commonly occurring chronic conditions **(Kessler & Greenberg, 2002)**. Anxiety disorders cause performance impairments on numerous tasks and are associated with high rates of medically unexplained symptoms, increased utilization of healthcare, strongly and independently associated with chronic medical illnesses, low levels of quality of life and disability **(Eysenck & Derakshan, 2011; Hoffman, Dukes, & Wittchen, 2008)**. Due to adverse effects associated with the currently available drugs, patients on anxiolytic drugs usually terminate the treatment before full recovery (Gray J. Therapeutic choices. 4. Ottawa, Canada: Canadian Pharmacist Association; 2003). In addition, one-third of patients in controlled studies are

unresponsive to any one of the medications **(Mahe & Balogh, 2000)**. Thus, there is a critical need for development of newer anxiolytic and sedative agents. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models (**Zhang, 2004)**.

*Lygodium palmatum* (Bernh.) Sw. is the only species of its genus native to North America. Unlike most species in the genus, this one, called the American climbing fern *Lygodium palmatum* (Natural Resources Conservation Service PLANTS Database. USDA. Retrieved 25 June 2015) or Hartford fern (after Hartford, Connecticut, is extremely hardy in temperate zones. This fern is on endangered or threatened species lists in several states. It requires constant moisture, high light levels and intensely acid soil to thrive. Its range is essentially Appalachian, ranging from New England down through the Appalachians, Piedmont and Appalachian plateaus into the American south.

But according to the best of our knowledge there is not any scientific detailed report on sedative and anxiolytic activities. So we have selected the methanol extract of leaves of *L. palmatum* to see the sedative and anxiolytic properties.

## 2. MATERIAL AND METHOD

### 2.1 Plant material

Fresh leaves of *L. palmatum* were collected from Bandarban, Chittagong, Bangladesh in the month of March 2015. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

### 2.2 Preparation of Extract

The leaves were dried for a period of 10 days under shade and ground. The ground leaves (450 gm) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then the whole mixture was filtered and the filtrate thus obtained was concentrated using a water bath to get a viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract (yield value, 5.3%). The extract prepared was for pharmacological screening.

### 2.3 Chemicals and equipment

All other chemicals and reagents were of analytical grade. Methanol purchased from Merck (India). Diazepam (Square Pharmaceutical, Bangladesh) and Tween-80(BDH Chemicals, UK) were used.

### 2.4 Animals and experimental set-up

Swiss albino mice, weighing about 28-35 g, were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were furnished with standard lab nourishment and refined water ad libitum and maintained at natural regular day-night cycle having legitimate ventilation in the room. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh. The animals were acclimatized to laboratory condition for 7 days prior to experimentation.

### 2.5 Sedative activity

#### 2.5.1 Open Field Test

The method was adopted as described by **Kulkarni & Reddy, 1996**. In open field test, the animals were divided into control, positive control and test groups containing 5 mice each. The test groups received extract of *L. palmatum* at the doses of 200 and 400 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water). The floor of half square meter open field was divided into a series of squares each alternatively colored black and white. The apparatus had a 40 cm height wall. The number of squares traveled by the animals was counted for 3 min at 0, 30, 60, 90, 120 min after oral administration of both doses of the extract.

#### 2.5.2 Hole cross test

The hole cross test, as described by **Takagi et al., 1971,** was adopted for screening the sedative effect of the methanol extract of *L. palmatum* leaves in mice. A wooden partition having a size of 30×20×14 cm was fixed in the middle of a cage. A hole (diameter 3 cm) was made in the centre of the cage at a height of 7.5 cm. Each mouse was immediately placed in any of the two chambers of the specified instrument after oral administration of the treatments. The number of passages through the hole from one chamber to another was counted on 0, 30, 60, 90, and 120 min for a 3 min test period.

### 2.6 Anxiolytic activity

#### 2.6.1. Elevated plus maze test

The elevated plus maze (EPM) consisted of two open arms (35 × 5 cm) crossed with two closed arms (35 × 5 × 20 cm). The arms were connected together with a central square of 5 × 5 cm. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice ($n$ = 6) were treated with methanol extract of *L. palmatum* leaves (200 and 400 mg/kg, p.o.), diazepam (1 mg/kg, i.p.) or normal saline 30 min before being placed individually in the centre of the EPM, facing a closed arm. The time spent in both the open and closed arms was recorded for 5 min. The numbers of entries into open and closed arms were counted during the test. An entry was defined as having all four paws within the arm **(Pellow & File, 1986)**.

#### 2.6.2. Hole-board test for exploratory behaviour in mice

The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes **(Sonavane, Sarveiya, Kasture, & Kasture, 2002)**. The animals were randomly grouped into four groups each containing six mice. Group one served as the control group and was treated with normal saline 10 mL/kg. Groups two, three were treated with the extract orally at doses of 200 mg/kg and 400 mg/kg respectively; while those in group four received diazepam 1 mg/kg. Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter.

### 2.7 STATISTICAL ANALYSIS

All results are expressed as mean ± standard error of the mean (SEM). The results were statistically analyzed using repeated measures analysis of variance with Dunnett's multiple comparison when compared against negative control in all *in vivo* model of Sedative and Anxiolytic activities. $P<0.05$, $P<0.01$ and $P<0.001$ were considered as statistically significant. Statistical programs used were SPSS (Statistical Package for Social Science, version 22.0, IBM Corporation, NY). GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used for graphical presentation.

## 3 RESULTS

### 3.1 Sedative-hypnotic activity

#### 3.1.1 Open field test

Open field test of *L. palmatum* treated groups (200 and 400 mg/ kg body weight) showed significant and dose-dependent reduction of movement from its initial value at 0 to 120 min

(**Figure 1**). The number of squares traveled by the mice was decreased significantly from its initial value at 0 to 90 min at the dose level of 400 mg/kg body weight (P<0.01) of the methanol extract from the leaves of *L. palmatum* (**Figure 1**).

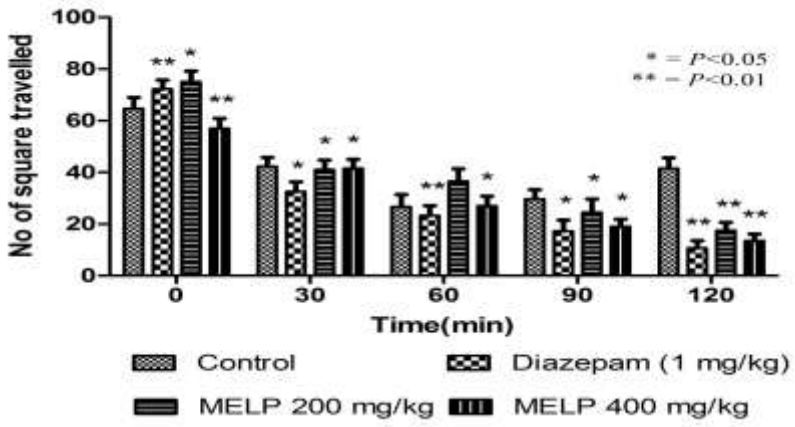


**Figure 1. Effect of methanol extract of *L. palmatum* on exploratory behavior open field test in mice.**

Values are mean±SEM; *P< 0.05, **P< 0.01, Dunnett's test as compared to control (Vehicle=10 ml/mouse).

### 3.1.2 Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 to 120 min (**Figure 2**). Hole cross test of *L. palmatum* treated groups showed decrease of movement from its initial value at 0 to 90 min. But, at doses of 400 mg/kg (P<0.01), maximum suppression of locomotor activity was displayed which was comparable to the reference drug diazepam (**Figure 2**).

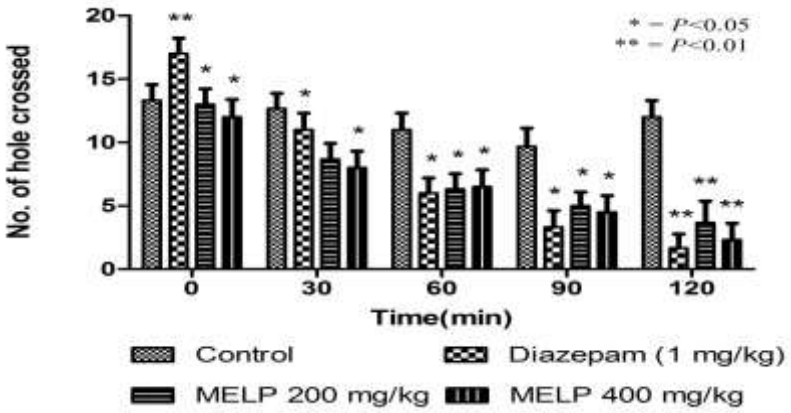


**Figure 2. Effect of methanol extract of *L. palmatum* on exploratory behavior (hole cross test).**

Values are mean±SEM; *P< 0.05, **P< 0.01, Dunnett's test as compared to control (Vehicle=10 ml/mouse).

### 3.2 Anxiolytic activity

#### 3.2.1. Elevated plus maze test

In the EPM, the behavior of mice model, as observed, confirmed the anxiolytic activity of diazepam as reported previously. The methanol extract of *L. palmatum* at the dose of 400 mg/kg (P<0.01), significantly increased the time spent in the open arms of the EPM as shown in **Table 1**. The effects of treatment of mice at the dose of 200 mg/kg on time spent in open arms were dose dependent. The times spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard diazepam. The effect of the methanol extract of *L. palmatum* on EPM in mice is shown in **Table 1.**

**Table 1: Effect of methanol extract of *L. palmatum* on EPM test during 5 min test session.**

| Treatment | Time spent in open arm (min) | Time spent in closed arm (min) |
|---|---|---|
| Control | 9.37±0.95 | 286.17±1.53 |
| Diazepam 1 mg/kg | 43.8±0.62** | 249.27±1.38** |
| MELP 200 mg/kg | 23.45±1.68* | 282.36±1.96* |
| MELP 400 mg/kg | 29.38±1.26** | 265.55±1.84* |

Values are mean±SEM; *$P$< 0.05, **$P$< 0.01, Dunnett's test as compared to control.

#### 3.2.2 Hole board test

The number of head dipping was increased (148.77%) significantly ($P$<0.01) in case of Diazepam treated animals as compared to the control animals. The MEBP at both dose levels showed an increase (58.23% and 94.93% respectively) in the number of head dipping significantly ($P$<0.05) as compared to the control mice. All results are shown in **Table 2**. This test proved that MEBP have significant anxiolytic activity. Because, head dipping of mice increased with all the tested treatment.

**Table 2: Effect of *L. palmatum* leaves extract on head dipping of mice in hole board.**

| Treatment | Number of head dipping | % increase |
|---|---|---|
| Control | 26.33±0.56 | - |
| Diazepam (1mg/kg) | 65.50±1.2** | 148.77 |
| MELP (200mg/kg) | 41.67±1.56* | 58.23 |
| MELP (400mg/kg) | 51.50±1.22** | 94.93 |

*$P$<0.05, **$P$<0.01 as control. Dunnett's test as compared to negative control (1% tween). Statistical representation of number of head dipping of mice by methanol extract of *L.*

*palmatum* leaves, Standard (*Diazepam*, 1 mg/kg) processed by Dunnett's test by using SPSS for windows, version 22.0.

## 4 DISCUSSIONS

The present work has evaluated the anxiolytic activity of various doses of the essential oil of *L. palmatum* in mice employing two behavioral animal models of sedative; open field model and hole-cross model. A another two non-conditioned behavioral animal models of anxiety; EPM model and hole board model. These tests are classic and standard models for screening central nervous system actions providing information about anxiety and psychomotor performance **(Sousa et al., 2004)**. Further, these models can create an anxiety state in normal rodents in a reproducible paradigm while minimizing some of the confounding factors of other conditioned assays **(Eguchi, Inomata, & Saito, 2001)**.

The result of hole cross and open field tests showed that the studied plant decreased the frequency as well as the bountifulness of movements. Since the level of excitability of the CNS is measured by locomotor activity, this reduction in spontaneous motor activity that could be considered as the sedative effect of the plant extracts. The locomotor activity lowering effect was evident at the 2$^{nd}$ observation (30 min) and continued up to the 5th observation period (120 min).

However, the anxiolytic activity of the methanol extract of *L. palmatum* was measured by using EPM suggested when the test drug increases time spending in open arms. Diazepam has been used as a standard anxiolytic and also frequently employed in behavioral pharmacology as a reference compound of potentially anxiolytic-acting substances. And the extract of plant extract at 200 mg/kg body weight in mice also showed significant increase time spent in the open arms of the maze.

In this work anxiolytic effects were screened by hole board test. The number of head dips in hole board test gives an indication of exploratory tendency, an increase in which is an indication of anxiolytic activity. In the present study, there was an increase in the number of head dips on treatment with *B. platyphylla* which indicate anxiolytic activity **(Kabir et al., 2015; Takeda, Tsuji, & Matsumiya, 1998)**. The major inhibitory neurotransmitter in the CNS is Gamma-amino-butyric acid (GABA) **(Rivera, Cid, Zunino, Baiardi, & Salvatierra, 2014)**. Different types of anxiolytics, muscle relaxant; sedative-hypnotic drugs are shown their action through GABA **(LEAVES, 2015)**. This type of effects is analyzed with the drugs

that act on GABA/Benzodiazepine receptor complex. Most of the anxiolytic agents exert their action by opening of activated GABA- chloride channel **(Rezaei, Pashazadeh, Pashazadeh, & Moghadam, 2014)**.

## 5 CONCLUSIONS

Analyzing the results of present study, it can be inferred that the methanol extract of *L. palmatum* leaves possess strong sedative and anxiolytic activity. Therefore, this extract could be considered for the treatment of anxiety and related neuropsychiatric disorders by conducting further pharmacological studies and mechanism of sedative and anxiolytic action, as well as to identify the active compound(s) responsible for this bioactivity in the animal model.

## Competing interests

The authors declare that they have no competing interests.

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