

## Neuropharmacological effects of *Peperomia pellucida* leaves in mice

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### ABSTRACT

**Background and the purpose of the study:** Local people of Lakshmipur district of Bangladesh use leaves of *Peperomia pellucida* in the treatment of excited mental disorder but its neuropharmacological effect has not yet been explored. In the present study depressant effects of leaves of this plant was investigated.

**Methods:** Petroleum ether and ethyl acetate soluble fractions of ethanol extract of *Peperomia pellucida* leaves were administered to mice intraperitoneally and their effects on duration of diazepam-induced sleep, nikethamide-induced toxicity, light-dark test and force swimming test were determined.

**Results and major conclusion:** The duration of diazepam-induced sleep was extended by administration of these fractions. Nikethamide at high doses caused death of mice and administration of these fractions delayed the time that nikethamide caused the death of animals. In light-dark test and force swimming test these fractions showed diazepam type effects. The changes in doses (50-200 mg/kg) resulted in modification of efficiency of these effects. These results suggest that both fractions of *P. pellucida* leaves have dose dependent depressant effects.

**Keywords:** *Peperomia pellucida*, Leaves, Mice, CNS depression.

### INTRODUCTION

*Peperomia pellucida* (L.) Kunth (Fam. Piperaceae), locally known as Luchi Pata, is an annual herb (1) that is widely distributed in many South American and Asian countries (2-5). The plant is refrigerant, and its leaves are used in the treatment of headache, fever, eczema, abdominal pains and convulsions (1). In different region of Lakshmipur district of Bangladesh, the leaves of the plant are used by local people in the treatment of excited mental disorder (information gathered from local herbalist Mr. Monto herbalist, Ramganj, Lakshmipur, Bangladesh). According to Manila Medical Society, *Peperomia pellucida* is used to relieve arthritic pains, but it may causes CNS depression (6). Antibacterial, anti-inflammatory and analgesic activities (2, 7-8) and isolation of antifungal and anticancer constituents (9, 10) from this plant have also been reported. Although the plant is widely used for remission of several ailments related to central nervous system, its CNS potential has not yet been explored. Therefore, in the present study an attempt was made to evaluate the CNS effects of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the leaves of *P. pellucida*.

### MATERIALS AND METHODS

#### Plant materials

The leaves of the plant from various part of Lakshmipur district of Bangladesh was collected with the help of local herbalist (Mr. Monto herbalist, Ramganj, Lakshmipur, Bangladesh) and identified by Prof. A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh where its voucher specimen (No. KM6543) was deposited. The leaves were air-dried and ground into powder.

#### Preparation of petroleum ether and ethyl acetate fractions of ethanol extract

The powder materials (450 g) were extracted with ethanol (3 L) in a Soxhlet apparatus (Quickfit, England) at 65<sup>o</sup> C for 72 h (11). The extract was filtered through filter paper and the filtrate was concentrated under reduced pressure at 50<sup>o</sup> C in a rotary vacuum evaporator to afford a blackish green mass (32 g). The resulting mass was further extracted (12) with petroleum ether (3 x 50 ml), ethyl acetate (3 x 50 ml) and methanol (3 x 50 ml) and solvents were evaporated under reduced pressure to afford residues of 9 g from petroleum ether, 7 g from ethyl acetate and 11 g from methanol fractions, respectively.

#### *Preparation of sample solutions*

The working solutions of different fractions of plant extract were prepared by dispersing each dried fraction of plant extract in distilled water with few drops of Tween 80. Preparation of solution was made in such way that each 0.2 ml solution contained 1.107 mg of the dried fraction of plant extract (for the dose of 50 mg/kg of the body weight) and 2.213 mg of the dried fraction of plant extract (for the dose of 100 mg/kg of the body weight). To maintain the dose of 200 mg/kg of body weight, 0.4 ml of second solution was used. Solutions were kept frozen until they were used (13).

#### *Standards and their solutions*

Diazepam as Sedil injection (contains 10 mg diazepam) was obtained from the local market of Square Pharmaceutical Ltd, Bangladesh. Its solution was prepared by dilution in distilled water in such a way that each 0.2 ml solution contained 0.12 mg of diazepam for the dose of 5 mg/kg of the body weight and 0.045 mg diazepam for the dose of 2 mg/kg of the body weight.

Nikethamide as Nikethamide injection (contains 500 mg nikethamide) was obtained from local market of Jayson Pharmaceutical Ltd, Bangladesh. Its solution was prepared by dilution in distilled water in such way that each 0.2 ml of solution contained 6.7 mg nikethamide for the dose of 300 mg/kg of the body weight and 0.45 mg nikethamide for the dose of 20 mg/kg of the body weight. All solutions were kept frozen until they were used (13).

#### *Animals*

The experiment was carried out on Albino mice (Swiss strain) of 2-3 months old of both sexes weighing between 20 -27 g (average weight was 22.13 g). They were obtained from the International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B) and housed in iron cages (considering group) under temperature and light controlled conditions (14). Animals were fed with balanced diet (15) and tap water for 25 days before the experiment in order to adjust with food and environment. Food and water were withdrawn 2 h prior to the experiment (16). Number of mice in each group was six and for all administration intraperitoneal route was used.

#### *Acute toxicity study*

Acute toxicity study was carried out by using graded doses of each fraction on albino mice. Both petroleum ether and ethyl acetate fractions were administered intraperitoneally in graded doses (200 to 1600 mg/kg body weight). Animals were observed continuously during first 2 h for toxic symptoms and up to 24 h for mortality (17).

#### *Treatment protocol to determine effects of extracts on duration of diazepam-induced sleep*

Total eight groups of animals were used for this test and animals were subjected to pretreatment and treatment (18, 19). Pretreatment was carried out 30 min prior to treatment (19). In pretreatment, 0.2 ml distilled water was given to each animal of a control group and 0.4 ml distilled water was administered to each animal of another group. In pretreatment for each fraction, sample solutions were given to animals of three remaining groups in doses of 50, 100, 200 mg/kg of body weight, respectively (Table 1).

In treatment group, 0.2 ml of diazepam solution for the dose of 5 mg/kg of body weight was given to each animal of all groups. Each mouse was observed and duration of sleep was recorded. Sleeping times in all cases were measured as the time interval between the loss and regaining of righting reflex (19).

#### *Treatment protocol to determine the effects of extracts on nikethamide-induced toxicity*

Totally eight groups of animals were used for this test. The animals were subjected to pretreatment 30 min prior to treatment (19, 20). In pretreatment, 0.2 ml of distilled water was administered to each animal of the control group and each animal of remaining control group was given 0.4 ml of distilled water. In pretreatment with each fraction, sample solutions were given to animals of three remaining groups at doses of 50, 100, 200 mg/kg of body weight, respectively (Table 2).

In treatment groups, 0.2 ml of nikethamide solution at the dose 300 mg/kg of body weight was given to each animal of all groups. Each mouse was observed and the time interval between the treatment and death were recorded (13, 20).

#### *Treatment protocol for light-dark test*

Total nine groups of animals were used for this test. Each animal of the control group received 0.2 ml distilled water, diazepam group received 0.2 ml of diazepam solution at the dose of 2 mg/kg body weight and nikethamide group received 0.2 ml of nikethamide solution at the dose of 20 mg/kg body weight. For each fraction, sample solutions were given to animals of three remaining groups at doses 50, 100, 200 mg/kg body weight, respectively (Fig. 1).

The apparatus used consisted of a Plexiglas box with two compartments (20 x 20 cm each). One of them was illuminated with white light and other stayed in dark (21). Thirty minutes after drug administration, each animal was placed at the center of the illuminated compartment, facing toward dark area (21). The time that animals spent in the illuminated and dark areas were recorded

for 5 min (21). Depressed mice spent longer time in dark area and excited mice spent longer time in light areas (21).

#### *Treatment protocol for force swimming test*

This test was carried out using Porsolt et al method (22). Total nine groups of animal were used for this test. Each animal of the control group received 0.2 ml of distilled water, of diazepam group received 0.2 ml of diazepam solution at the dose of 2 mg/kg of the body weight and of nikethamide group received 0.2 ml of nikethamide solution at the dose of 20 mg/kg of body weight. For each fraction, sample solutions were given to animals of three remaining groups at doses of 50, 100, 200 mg/kg of body weight, respectively (Fig. 2).

The apparatus used consisted of a clear Plexiglas cylinder (20 cm high x 12 cm diameter) filled to a 15 cm depth with water ( $24 \pm 1^\circ \text{C}$ ). After 30 min of drug administration, each animal was placed individually into the cylinder for 15 min and observed for climbing (upward movement along the side of the swim chamber) and swimming (movement throughout the swimming chamber) behavior and immobility (by keeping the head of the mice above water in the way that animal made no further attempts to escape). The criteria for effectiveness of extracts on mice were based on the principles that the mobility of excited mice was higher than depressed mice (22).

#### *Statistical analysis*

The results were presented as mean  $\pm$  standard error mean (Mean  $\pm$  SEM) where  $n = 6$ . Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test and  $p < 0.05$  was considered to be statistically significant.

## RESULTS

#### *Acute toxicity study*

In acute toxicity study, both petroleum ether and ethyl acetate fractions were found to be safe and no mortality was observed at doses as high as 1200 mg/kg for petroleum ether fraction and 1000 mg/kg for ethyl acetate fraction.

#### *Effects on diazepam-induced sleep*

At doses of 50, 100 and 200 mg/kg of the body weight, both petroleum ether and ethyl acetate fractions extended the duration of diazepam-induced sleep and at higher doses the duration of sleep were more extended (Table 1). These findings indicate dose dependency of the CNS depressant effect of *P. pellucida* leaves.

#### *Effect on nikethamide-induced toxicity*

At doses 50, 100 and 200 mg/kg of the body weight, both petroleum ether and ethyl acetate

fractions delayed the latency of the death caused by nikethamide and it was also observed that at higher doses the latency of death were more delayed (Table 2). These results indicate dose dependency of the CNS depressant effects of the leaves of *P. pellucida*, which interfere with CNS stimulant effects and as a result delayed deaths caused by nikethamide.

#### *Effect of light-dark test*

Mice, which were given nikethamide, spent most of their times in light illuminated space. Mice, which were given petroleum ether fraction, ethyl acetate fraction and diazepam were moved toward dark space and spent most of their times in this space (Fig. 1). These results indicate the petroleum ether and ethyl acetate fractions of ethanol extract of the plant have diazepam type effects.

#### *Effect of force swimming test*

While decreased times of immobility were observed for mice which were administered nikethamide, increased times of immobility were observed for remaining mice (Fig. 2). These results indicate that petroleum ether and ethyl acetate fractions of ethanol extract of the plant have diazepam like effects.

## DISCUSSION

The acute toxicity results reveal that this plant might be considered non-toxic. The data presented in tables 1 and 2, figures 1 and 2 suggest that both petroleum ether and ethyl acetate fractions of ethanol extract of *P. pellucida* leaves contain psychoactive substances which are CNS depressant in nature. In all experiment it was observed that petroleum ether fraction is more active than ethyl acetate fraction and at the same doses, effects of petroleum ether fraction on the duration of diazepam-induced sleep and latency of the death caused by nikethamide toxicity is better. In light-dark test, it was observed that for the same doses, mice which were administered petroleum ether fraction spent longer time in dark areas than mice which were administered ethyl acetate fraction. In force swimming test, mice which were administered petroleum ether fraction showed lesser mobility than mice administered ethyl acetate fraction. The neuropharmacological effects of methanol fraction was insignificant and hence its data was not represented.

The uses of *P. pellucida* against convulsions (1), in treatment of excited mental disorder (information gathered from local herbalist) and its CNS depressant effects as reported by Manila Medical Society (6) are in agreement with our experimental results. Isolation of styrene,

**Table 1.** Effect of petroleum ether and ethyl acetate fractions of ethanol extract of *P. pellucida* leaves on duration of diazepam (5 mg/kg)-induced sleep in mice.

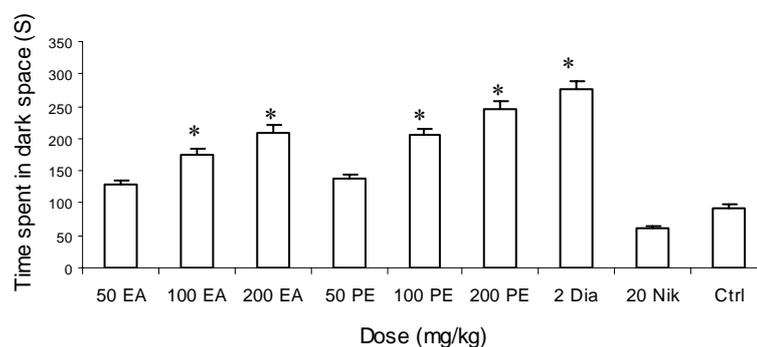
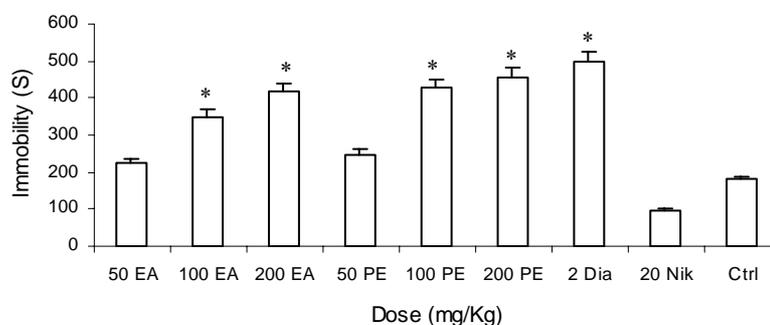
Pretreatment	Duration of sleep (Min) Mean $\pm$ S.E.M.
Distilled water, 0.2 ml	54.56 $\pm$ 2.19
Distilled water, 0.4 ml	53.04 $\pm$ 2.10
Petroleum ether fraction, 50 mg/kg	61.45 $\pm$ 2.51
Petroleum ether fraction, 100 mg/kg	74.18 $\pm$ 1.07*
Petroleum ether fraction, 200 mg/kg	86.54 $\pm$ 1.52*
Ethyl acetate fraction, 50 mg/kg	59.13 $\pm$ 1.29
Ethyl acetate fraction, 100 mg/kg	70.35 $\pm$ 2.38*
Ethyl acetate fraction, 200 mg/kg	76.14 $\pm$ 1.54*

\* P &lt; 0.05 significant compared to control (solvent).

**Table 2.** Effect of petroleum ether and ethyl acetate fractions of ethanol extract of *P. pellucida* leaves on nikethamide (300 mg/kg)-induced toxicity of mice.

Pretreatment	Latency of Death (Min) Mean $\pm$ S.E.M.
Distilled water, 0.2 ml	48.02 $\pm$ 1.42
Distilled water, 0.4 ml	48.58 $\pm$ 2.23
Petroleum ether fraction, 50 mg/kg	56.12 $\pm$ 2.13
Petroleum ether fraction, 100 mg/kg	65.57 $\pm$ 1.14*
Petroleum ether fraction, 200 mg/kg	72.38 $\pm$ 1.44*
Ethyl acetate fraction, 50 mg/kg	55.11 $\pm$ 1.37
Ethyl acetate fraction, 100 mg/kg	62.32 $\pm$ 1.54*
Ethyl acetate fraction, 200 mg/kg	67.41 $\pm$ 1.17*

\* P &lt; 0.05 significant compared to control (solvent).

**Figure 1.** Effect of petroleum ether (PE) and ethyl acetate (EA) fractions, and diazepam (Dia) and nikethamide (Nik) in light-dark test. Mean  $\pm$  S.E.M. of each group expressed in figure and time expressed in second. Ctrl = control. \* P < 0.05 significant compared to control (solvent).**Figure 2.** Effect of petroleum ether (PE) and ethyl acetate (EA) fractions, and diazepam (Dia) and nikethamide (Nik) in force swimming test. Mean  $\pm$  S.E.M. of each group expressed in figure and time expressed in second. Ctrl = control. \* P < 0.05 significant compared to control (solvent).

campesterol, stigmasterol and  $\beta$ -sitosterol from this plant has been reported (1). Slowing of sensory nerve and nervous conduction velocity as well as CNS depression has been reported as the neurotoxic effects of styrene (23). Increased sitosterol and campesterol levels reduce cholesterol biosynthesis (24) and stigmasterol by inhibiting sterol Delta-22-reductase (25) and cholesterol absorption (26). Consequently, campesterol, stigmasterol and  $\beta$ -sitosterol reduce cholesterol levels in animal body. Since CNS synaptogenesis is promoted by cholesterol (27) it seems that styrene, campesterol, stigmasterol and

$\beta$ -sitosterol of *P. pellucida* might be responsible for the depressant action of this plant. However, further studies are warranted to understand the mechanism of depression and to isolate other constituents responsible for this action.

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