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# CNS DEPRESSANT ACTIVITY OF ETHANOL EXTRACT OF *DERRIS TRIFOLIATA*

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## ABSTRACT

In the present study, we investigated neurobehavioral activity of ethanol extract of the aerial parts of *Derris trifoliata* (Lour.) in mice as a part of a psychopharmacological screening of this plant. The effects of the plant extract on CNS were studied by using potentiation of phenobarbitone-induced sleeping time and exploratory behavior in mice. The crude extract (500 mg/kg body weight) significantly ($P<0.001$) potentiated onset of sleep and prolonged the total sleeping time in mice. In tests for exploratory behavior, the plant extract (1000 mg/kg body weight) showed significant effect on ambulation and emotional defecation and also in the open field score in case of staying in single area. The overall results suggest that the ethanol extract of *D. trifoliata* contains some active principles which may be CNS depressant in nature.

**INTRODUCTION:** *Derris trifoliata* (synonyms: *D. uliginosa*, *Pongamia uliginosa*; local name: pan lota, kali lota, goali lata, kirtana) is an important mangrove plant of the Fabaceae (alternatively Leguminosae) which is known as the legume family, pea family or pulse family. It is probably the only common climber that grows in mangroves, especially in Sundarban (mangrove forest) of India and Bangladesh. It is a perennial climber, or a much branched climbing evergreen shrub, reaching a length of 8 meters or less. The plant has 12.5-20 cm long odd-pinnate compound leaves with 3-7 leaflets (5.7-10 cm by 3.2-5 cm) and 7.5-15 cm long white flowers that are fascicled in axillary racemes.

The principal secondary metabolite of *D. trifoliata* is rotenone which has low toxicity to mammals, but is extremely toxic to fish [1]. Some species belonging to the genera including *D. elliptica*, are commercially cultivated as a source of insecticidal rotenoids [2]. The plant is also used as stimulant, antispasmodic and counter irritant, and against rheumatism and dysmenorrhoea [3]. Stem bark contains tannic acid, resins, wax, colouring matters, alkaloidal substance, rotenone, saponin-like glycosides, gum, glucose and minerals and roots contain rotenone, dehydrorotenone, deguelin, lupeol, toxicarol and a ketone [4].

Several rotenoids [5, 6] and glycosidic compounds [7] have been isolated from aerial parts of *D. trifoliata*. Previously we reported diuretic [8] and antidiarrhoeal [9] activities of the plant. Besides the synthetic drugs acting on CNS, many plant materials possessing action on CNS have been detected [10-13]. Since *D. trifoliata* is a poisonous plant, it would have an effect on the CNS.

In the present study, we aimed to correlate antidepressant-like activity of ethanol extract of the aerial parts of *D. trifoliata* against several neuro-

pharmacological models, such as phenobarbitone-induced sleeping time and exploratory behavior in mice.

## MATERIALS AND METHODS:

**Plant Material*:*** The selected plant *D. trifoliata* was collected from the Koromjol area of Sundarbon Mangrove Forest during the middle of the July, 2005. The identification of the plant material was confirmed by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and also by the authorities of Botanical Garden, Mirpur, Dhaka. The collected aerial parts of the plant were washed repeatedly with water, sliced into small pieces and sun-dried for one week. The plant parts were powdered with a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place prior to extraction process.

**Preparation of Extracts:** The dried powered sample **(**500 gm) was extracted in 1300 mL of 80% ethanol for 7 days accompanying occasional shaking and stirring. The whole mixture was filtrated through a funnel plunged with white cotton and also using Whatman filter paper number 1. The resultant filtrate was concentrated by using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK). The concentrated extract was then air dried to solid residue (15.5 g) which was treated as the ethanol extract and stored in refrigerator until further investigation.

**Animals and their Treatment:** White albino mice (*Swiss-webstar* strain, body weight = 20-25 gm) of both sexes were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for assessing biological activity. The animals were kept in standard environmental conditions for at least one week for adaptation and had free access to standard laboratory food and water. The test animals were divided into two groups consisting of six mice in each group. The animals of one group (control) received vehicles only (distilled water containing 0.1% Tween-80). The animals of another group (test group) were treated with the test sample prepared by mixing the ethanol extract of *D. trifoliata* in distilled water and Tween-80. All animal experiments were conducted on an isolated and noiseless condition.

**Phenobarbitone-induced Hypnotic Test:** The plant extract was administered intraperitonially (i.p.) to each animal of the test group at a dose of 500 mg/kg body weight. An interval of 30 minutes was given to assure proper absorption. Then sodium phenobarbitone (50 mg/kg body weight) was administered (i.p.) to all animals of both control and test groups and the time for onset of sleep and total sleeping time were recorded. The time for onset of sleep was the time elapsed between administration of phenobarbitone and the loss of righting reflex, while total sleeping time was measured as the duration between the loss and regain of righting reflex [14].

**Hole board and Open Field Test*:*** In hole board experiment [15], each animal was placed carefully in the center of the board and the number of holes passed, head dipping and the number fecal boluses excreted in 2 minutes were recorded. The number of squares traveled by the animal was recorded for 2 minutes in open field experiment [16]. In both tests, the observations were made at the time of administration (i.p.) of the plant extract at the doses of 1000 mg/kg body weight and 30, 60, 120, 240 minutes after the administration.

**Phytochemical Screening:** The preliminary phyto-chemical studies were performed to detect the presence of different chemical groups in 10% (w/v) solution of the plant extract. Qualitative chemical tests for steroids, flavonoids, reducing sugars, tannins, gums, alkaloids and saponins were carried out using standard procedures [17, 18].

**Statistical Analysis:** All the data obtained were expressed as the mean ±standard error of mean (SEM). Statistical differences between the treatments and the controls were examined by SPSS 11.5 software for Windows followed by the student's t-test. A difference in the mean values of $P<0.05$ was considered to be statistically significant.

## RESULTS:

**Phenobarbitone- induced Hypnotic Test:** The experimental findings from the phenobarbitone

induced sleeping time test showed that the ethanol extract of *D. trifoliata* (500 mg/kg body weight) significantly (P<0.001) potentiated the time for sleeping induction by phenobarbitone in mice (**Table 1**). The crude extract also exhibited significant effect on the duration of sleep. Compared to test group, the plant extract (500 mg/kg body weight) significantly (P<0.001) prolonged the total sleeping time, causing approximately 65 % increase in sleeping time.

**TABLE 1: EFFECT OF *D. TRIFOLIATA* ON PHENOBARBITONE INDUCED ONSET OF SLEEP AND TOTAL SLEEPING TIME**

| Treatment* (i.p.) | Onset of sleep (min.) | Total sleeping time (min.) | % decrease in onset of sleep | % increase in sleeping time |
|---|---|---|---|---|
| Control | 85.83 ± 1.87 | 76.00 ± 1.91 | - | - |
| Extract (500 mg/kg) | 43.33 ± 2.16** | 125.50 ± 2.262** | 49.51 | 65.13 |

* Phenobarbitone sodium at 50 mg/kg body weight was administered (i.p.) to all mice. All values are expressed as mean ± SEM (n=6) and **P<0.001 vs. control

**Hole board and Open Field Test:** The hole board test was performed evoking a pattern of behavior characterized by exploration (head dipping through the holes), locomotion (ambulation past the holes) and emotional defecation. The plant extract did not show any significant effect on head dipping but it caused a significant (P<0.001) effect on ambulation and a moderate (P<0.05) effect on defecation in mice at a dose of 1000 mg/kg body weight (**Table 2**). The animals treated with the plant extract showed a significant (P< 0.05) reduction in the open field score in case of staying in single area from 30 minutes to 240 minutes at the same dose (**Table 3).** These results furthermore support the central sedative properties of the extract [19].

**TABLE 2: EFFECT OF *D. TRIFOLIATA* ON HOLE BOARD TEST**

| Treatment (i.p.) | Response after | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 240 min |
| | | | **Ambulation** | | |
| Control | 19.33±3.8 | 9.33±2.47 | 9.167±1.30 | 12.00±3.7 | 12.17±3.67 |
| Extract (1000 mg/kg) | 23.42±3.12 | 5.50±1.72 | 1.00±0.36** | 3.00±0.97 | 3.33±1.56 |
| | | | **Head dipping** | | |
| Control | 8.50±2.21 | 4.17±2.79 | 2.50±1.56 | 1.83±1.27 | 2.00±1.00 |
| Extract (1000 mg/kg) | 9.66±2.47 | 3.16±1.92 | 0.67±0.49 | 2.17±0.54 | 7.83±3.93 |
| | | | **Defecation** | | |
| Control | 1.83±0.40 | 0.50±0.50 | 0.33±0.21 | 0.33±0.33 | 1.17±0.48 |
| Extract (1000 mg/kg) | 1.17±0.48 | 0.33±0.21 | 0.00±0.00 | 0.33±0.33 | 0.00±0.00* |

All values are expressed as mean ± SEM (n=6); *P<0.05, **P<0.001 vs. control

**TABLE 3: EFFECT OF *D. TRIFOLIATA* ON OPEN FIELD TEST**

| Treatment (i.p.) | Response after | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 240 min |
| | | | **Area crossed** | | |
| Control | 59.50±9.83 | 49.63±8.55 | 26.75±6.84 | 19.87±6.01 | 47.00±4.51 |
| Extract (1000 mg/kg) | 57.13±8.29 | 39.75±11.17 | 13.25±4.99 | 9.62±3.16 | 40.37±10.51 |
| | | | **Stay in single area** | | |
| Control | 0.37±0.26 | 7.37±3.50 | 16.00 ±5.85 | 27.62±12.62 | 2.13±0.93 |
| Extract (1000 mg/kg) | 0.63±0.62 | 52.12±17.36* | 71.87±6.65*** | 74.75±8.46** | 6.00±1.41* |
| | | | Stool | | |
| Control | 0.38±.26 | 0.38±.18 | 0.00±0.00 | 0.38±0.18 | 0.38±0.18 |
| Extract (1000 mg/kg) | 0.12±0.13 | 0.00±0.00 | 0.123±0.12 | 0.38±0.26 | 0.25±0.16 |
| | | | **Number of move in the middle** | | |
| Control | 0.63±0.37 | 1.00±0.33 | 0.00±0.00 | 0.00±0.00 | 0.63±0.37 |
| Extract (1000 mg/kg) | 0.25±0.16 | 0.63±0.26 | 0.13±0.12 | 0.38±0.26 | 0.13±0.12 |

All values are expressed as mean ± SEM (n=6); *p<0.05, **p< 0.01, ***p<0.001 vs. control.

**Phytochemical Screening:** Preliminary phytochemical screening of ethanol and chloroform extracts of aerial parts of *D. trifoliata* revealed the presence steroid, reducing sugar, gum, saponin, tannin and flavonoids as major active constituents (**Table 4**). However, saponin was absent in chloroform extract and alkaloids were absent in both extracts.

**TABLE 4: QUALITATIVE ANALYSIS OF THE EXTRACTS OF *D. TRIFOLIATA***

| Phytoconstituents | Ethanol extract | Chloroform extract |
|---|---|---|
| Steroid | + | + |
| Alkaloid | - | - |
| Reducing sugar | + | + |
| Gum | + | + |
| Saponin | + | - |
| Tannin | + | + |
| Flavonoid | + | + |

"+" means presence and "-" means absence.

**DISCUSSION:** The enhancement of barbital hypnosis is a good index of CNS depressant activity [20]. The results obtained in this study suggest the CNS depressant activity [21] and the probable tranquilizing action [22] of ethanol extract of *D. trifoliata*. Prolongation of sleeping time may be due to enhancement of γ-aminobutyric acid (GABA) in the brain as it is known to have depression action [23].

The preliminary phytochemical screening of the extract of *D. trifoliata* showed the presence of steroids, flavonoids, reducing sugar, tannin, gum and saponin. CNS depressant activity of flavonoid glycosides were reported in several studies [10, 11, 24]. Many flavonoids were found to be ligands for the GABA type A receptors in the CNS; which led to the hypothesis that they act as benzodiazepine like molecules. This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion [25]. It has also been reported that the saponins show a potent sedative activity when tested in similar models and also inhibit spontaneous motor activity in mice [26].

Therefore, the observed CNS depressant effect of the plant extract may be due to its glycoside and/or saponin content.

**CONCLUSION:** The results of activity of the ethanol extracts of *D. trifoliata* in the several neuropharmacological models justified its central depressant effect and possible application in anxiety condition. However, extensive biological and chemical investigations are required to characterize the active CNS depressant principle(s).

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