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Research Article

Evaluation of CNS Effects of the Methanolic Extract of *Desmodium triflorum* DC in Swiss Albino Mice

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Abstract

In Sri Lanka, *Desmodium triflorum* Linn (Fabaceae) is a well-known medicinal plant known by the Sinhala name "Heen-Undupiyaliya". In this work, Swiss albino mice were used to determine the central nervous system effects of the methanolic extract of *Desmodium triflorum.* The methanolic extract of *Desmodium triflorum* was used to assess the CNS activity using the force swimming, tail suspension, and thiopental sodium induced sleeping time tests. Five groups of five mice each were created out of the animals. In CNS activity testing, conventional medications such as thiopental sodium (40 mg/kg) and imipramine hydrochloride (30 mg/kg) were utilized. The plant extract was dissolved in deionized water at concentrations of 50, 100, and 200 mg/kg body weight to create the sample. Thirty minutes before to the experiment, the test groups were given the medication orally, whereas the control group was given 0.1 mL/mouse of deionized water by gavage. The experimental mice were given the medicines intraperitoneally (i.p.) fifteen minutes after the treatments were administered. In the forced swimming and tail suspension tests, the extract significantly reduced the amount of time that subjects were immobile. Furthermore, a significant reduction in the duration of sleep and an increase in latency time were seen with the crude extract (*$p < 0.05$). The findings imply that *Desmodium triflorum* methanolic extract may have a CNS stimulating effect that warrants investigation for possible therapeutic use as a substitute therapy for ailments. Therefore, the current study supported the use of the plant in the treatment of depression; nevertheless, more research on the phytochemicals in the plant is required to prove its central nervous system effects.

Keywords: *Desmodium triflorum; Forced Swimming; Tail Suspension*

Background

Drugs or medications known as central nervous system effects are prescribed to treat narcolepsy and attention deficit hyperactivity disorder [1]. Some CNS drugs like modafinil are used to treat excessive somnolence that might arise owing to shift work problem, narcolepsy and obstructive sleep apnea [2]. Psychomotor stimulants and hallucinogens are the two main pharmacological types that are utilized to excite the central nervous system. Psychomotor stimulants often increase motor activity, reduce sensations of exhaustion, and provide feelings of exhilaration and euphoria. The effects of hallucinogens on mood and thinking patterns are reflected, but effects on the brainstem and spinal cord are very little affected [3]. Additionally, individuals with depression linked to the human immunodeficiency virus (HIV) and post-stroke depression have been successfully treated with CNS drugs [4]. Nevertheless, there is a risk of addiction and misuse of CNS drugs due to their associated recreational effects. The unmonitored use of CNS drugs and herbal recreational substances damages human neuropsychobiological integrity. By acutely increasing certain emotional states and then impairing them during the

recovery period after the drug's influence has worn off, they upset the psychological homeostasis of the human body. This ambiguity in mood states may serve as a barometer for more drastic shifts in psychological states [5]. Caffeine, Yerba Mate (*Ilex paraguariensis*), Ginkgo, *Centella asiatica*, *Panax ginseng*, Ephedra, Tea (*Camellia sinensis*), Coffee (*Coffea robusta/arabica*), Cocoa (*Theobroma cacao*), Cola Nut (*Cola nitida/acuminata*), Guarana (*Paullinia cupana*), and Yerba Mate (*Ilex paraguariensis*), Ephedra, Khat (*Catha edulis*), and Ginkgo are some of the herbs that are widely used globally for their potential as CNS tonics [6].

Because herbal medicine is more culturally acceptable, more suited to the human body, and has fewer side effects, it continues to be the major source of primary healthcare for around 75 - 80% of the world's population, primarily in poor nations. But in the industrialized world, their use has significantly increased during the past few years. According to estimates, 80% of the world's population, or around 4 billion people, cannot afford the goods made by the Western Pharmaceutical Industry and must instead rely on traditional remedies, the majority of which are made from plants [7]. It is important to look for strong, efficient, and generally safe plant medicines given the heavy dependence on conventional medicinal plants for the treatment of illnesses and the possibility for drug development.

In Sri Lanka, *Desmodium triflorum* Linn (Fabaceae) is a well-known medicinal plant known by the Sinhala name "Heen-Undupiyaliya". Many other tropical nations, such as Taiwan, Java, the Philippines, and India, carry the plant. The plant is often found in the low area of Sri Lanka [8,9]. The plant's leaves, roots, and entire body are its valuable portions. Ursolic acid, Vitexin, Genistin, Fucosterol, and the uncommon diholosylflavane, 2-0 Glucosylvitexin, are among the chemical components found in *Desmodium triflorum* [10,11]. Total alkaloid, 0.01 - 0.015%, α-Phenethylamine (primary alkaloid), Indole-3-acetic acid, Tyramine, Trigonelline, Hypaphorine, and Choline are all present in *Desmodium triflorum* leaves. The root of *Desmodium triflorum* includes the following total alkaloids: N, N-Dimethyl tryptophan betaine, choline, and hypaphorine (major alkaloid) [12,13]. The leaves are used as a galactagogue, for diarrhea, and for convulsions [14]. It is used to treat wounds and abscesses that are typically difficult to cure using the plant's fresh leaves. On occasion, the paste is applied to itch sores. It is also advised to utilize the plant's fresh juice as a laxative and for dysentery. When consumed on an empty stomach, dried powder of the whole plant *Desmodium triflorum* helps heal bone fractures [15]. *Desmodium triflorum* root infusion is used to induce labor and cure vertigo [16]. To reduce a high fever, external leaf paste made from *Desmodium triflorum* leaves diluted in water is applied to the forehead [17]. The roots are employed in bilious symptoms and are said to have carminative, tonic, and diuretic properties. The leaves are provided every day in the morning and are ground with cow's milk. Antispasmodic, sympathomimetic, central nervous system stimulant, curare-mimetic activity, and diuretic are among the primary activities. A decoction is also used as an expectorant and mouthwash in the Philippines. The whole plant is used as a thrust quencher and antipyretic in Thailand. Because of the plant's purported antibacterial qualities, crushed leaves are topically administered as a poultice to wounds, ulcers, and skin conditions in general in Indonesia, Malaysia, the Philippines, Laos, and India [18]. *Desmodium triflorum* has several extracts that have been shown to have anti-inflammatory and analgesic properties [19], as well as antioxidative and antiproliferative properties [20,21]. The antinociceptive properties of *Desmodium triflorum* cold water extract in rats [22] and the antioxidant properties of phenolic components from different Desmodium species plants were investigated [23]. *Desmodium triflorum*'s ethanolic leaf extract was recently evaluated for anticonvulsant efficacy in mice, and the results showed that it has extremely good anticonvulsant action [24].

A review of the literature found that there hasn't been any systematic research on the CNS action of *Desmodium triflorum* on various portions of the plant. The goal of the current investigation was to determine if the methanolic extract of *Desmodium triflorum* in Swiss albino mice might excite the central nervous system.

Methods

Drugs and chemicals

Chemicals and medications such as methanol (Merck, Germany), Imipramine hydrochloride (Sandoz, Norvartis Bangladesh Ltd.), and thiopental sodium (Gonoshastha Pharmaceuticals Ltd., Bangladesh) were utilized in the tests that are being discussed. All of the groups received the medicine and samples by gavage. Each and every additional chemical and reagent was of analytical grade and extremely pure.

Collection of plant materials

Desmodium triflorum was purchased in its whole from a nearby vendor of herbal remedies, and Bushra Khan, the Principal Scientific Officer of the Bangladesh National Herbarium in Mirpur, Dhaka, Bangladesh, recognized it. For future reference, a reference specimen has been placed in the Herbarium.

Preparation of extraction

Fresh leaves of *Desmodium triflorum* were dried at room temperature. The dried leaves were used to make the powder. Using a sterile glass rod, 280g of powdered materials were steeped in 1000 mL methanol in a beaker for 72 hours at 25 ± 2ºC. Stirring was done every 18 hours. The filtrate was collected three times using a sterile cotton bed and Whatman 102 filter paper. A rotary evaporator was used to extract the solvent, producing 25.20g of extract (Yield 9%). The extract was used in studies on CNS effects, phytochemical screening, and acute toxicity.

Test animals

One hundred adult Swiss albino mice weighing twenty to twenty-five grams were collected by the Animal Research Branch of the International Center for Diarrheal Disease and Research in Bangladesh. Mice were housed in standard conditions, with a 12-hour light/dark cycle, a temperature of 25ºC, and a humidity range of 55 - 65%. Mice were acclimated to the lab setting for 14 days before the tests began. All of the experimental mice adhered to the Ethical Principles and Guidelines for Scientific Experiments with Animals, which were created in 1995 by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee of Stamford University Bangladesh approved all experimental protocols.

Treatment schedule

CNS activity tests were conducted using the conventional medications thiopental sodium (40 mg/kg) and imipramine hydrochloride (30 mg/kg). *Desmodium triflorum*'s methanolic extract was dissolved in deionized water at concentrations of 50, 100, and 200 mg/kg body weight to create the sample. Thirty minutes before to the studies, the test groups were given the medication orally, whereas the control group was gavaged with 0.1 mL/mouse of deionized water. The experimental mice were given the medicines intraperitoneally (i.p.) fifteen minutes after the treatments were administered. Each medication was manufactured afresh before to each trial.

Acute toxicity test

Twenty-five adult mice in good health were split up into four test groups and one control group (n = 5). Deionized water was given to the control group (0.1 mL/mouse). Oral extract dosages of 500, 1000, 2000, and 3000 mg/kg were administered to the test groups. The first four hours after the drug were spent observing the animal to look for any behavioral changes. To check for any mortality, they were nonetheless kept under observation for 72 hours after injection [25].

Phytochemical screening

The methanolic extract of *Desmodium triflorum* included the following: alkaloids, flavonoids, saponins, tannins, cardiac glycosides, carbohydrates, reducing sugars, proteins, terpenoids, and steroids [26].

CNS activity tests

Forced swimming test

One of the most used pharmacological models for assessing the effectiveness of antidepressants is the forced swimming test. With few modifications, Porsolt., *et al*. [27] technique was used. Three sets of twenty-five healthy mice each were assigned: test sample, positive control, and control. Five mice were present in every group. For 15 minutes (pre-test session), mice were put individually in a glass cylinder (height 45 cm, diameter 20 cm) that was filled with water to a depth of 17 cm at 25 ± 1°C. Three oral doses of the extract solution were given to the mice between the pre-test and main sessions, and the mice were then left to undergo the same conditions for five minutes. A mouse, with the exception of tiny movements to keep its head above water, was considered inactive. It was observed by observers for five minutes, from 1 to 3 p.m.

Tail suspension test

One easy, fast, and reliable technique to look for antidepressant effects is the tail suspension test [28]. This method is based on the finding that a mouse pulled by its tail alternately agitates and immobilizes [29]. Based on Steru., *et al*. methodology, the tail suspension test was described in detail with a few minor modifications [30]. Three sets of twenty-five mice each were created: test sample, positive control, and control. Five mice were present in every group. There were two stands with a clamp located 22 cm from the floor, spaced 23 cm apart. Mice were held passively on a stand 5 cm from the tip of their tail for 6 minutes before being deemed immobile. The examination took place from 1 to 3 p.m. Spectators graded how long the immobility lasted.

Thiopental sodium-induced sleeping time test

Ferrini., *et al*. [31] offered a method for carrying out this assessment. Thirty minutes after ingesting the methanolic extract of *Desmodium triflorum*, the test groups were administered thiopental sodium (40 mg/kg; i.p.). The length of sleep was measured as the interval between the loss and recovery of the righting reflex. The standard group was given imipramine hydrochloride (30 mg/kg; i.p.), whereas the control group was given deionized water (0.1 mL/mouse; p.o.).

Statistical analysis

Standard error of the mean (SEM) or mean ± SEM was the data's format. The statistical analysis was performed using one-way analysis of variance (ANOVA) and Dunnett's post hoc test, if necessary, using SPSS 18.00 software. The differences between the groups were considered significant at the *$p < 0.05$ significance level.

Results

Phytochemical screening

Phytochemical screenings revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, terpenoids, and steroids in the crude extract of *Desmodium triflorum* (Table 1).

Acute toxicity test

Following oral MEDT delivery at doses ranging from 500 to 3000 mg/kg, there was no mortality. On the other hand, anomalies in behavior were seen over a full 72 hours. MEDT is therefore believed to have a low toxicity profile, with an LD_{50} above 3000 mg/kg.

Plant constituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Cardiac glycosides	+
Carbohydrates	-
Reducing sugars	-
Proteins	-
Terpenoids	+
Steroids	+

Table 1: *Preliminary qualitative phytochemical screening of methanolic extract of Desmodium triflorum (MEDT).*

+: Presence; -: Absence.

Forced swimming test

The length of immobility time was significantly shortened (*p < 0.05) by the *Desmodium triflorum* extract at doses of 50, 100, and 200 mg/kg when compared to the control. The standard drug imipramine hydrochloride (30 mg/kg, i.p.) dramatically shortened the immobility period in the mouse model (*p < 0.05). *Desmodium triflorum*'s maximal antidepressant effect was seen at a dose of 200 mg/kg (Table 2).

Treatment	Dose (mg/kg)	Immobility time (s)
Control	0.1 mL/mouse	122.60 ± 1.56
Imipramine hydrochloride	30	41.00 ± 1.41*
MEDT	50	99.60 ± 1.77
MEDT	100	74.80 ± 1.11*
MEDT	200	51.40 ± 1.03*

Table 2: *Effects of Desmodium triflorum extract and Imipramine hydrochloride on forced swimming test in mice.*

Values are presented as mean ± SEM (n = 5). MEDT = Methanolic Extract of Desmodium triflorum.

**p < 0.05, vs. control (Dunnett's test).*

Tail suspension test

Desmodium triflorum (50, 100, and 200 mg/kg) effects in the tail suspension test are displayed in table 3. The results demonstrated a significant reduction in immobility time (*p < 0.05) when compared to the control group. Similar to this, the standard drug imipramine hydrochloride (30 mg/kg, i.p.) significantly reduced immobility time (*p < 0.05).

Treatment	Dose (mg/kg)	Immobility time(s)
Control	0.1 mL/mouse	99.40 ± 1.63
Imipramine hydrochloride	30	28.60 ± 0.92*
MEDT	50	78.20 ± 2.74
MEDT	100	55.80 ± 1.88*
MEDT	200	35.40 ± 0.81*

Table 3: *Effects of Desmodium triflorum extract and Imipramine hydrochloride on tail suspension test in mice.*

Values are presented as mean ± SEM (n = 5). MEDT = Methanolic Extract of Desmodium triflorum.

**p < 0.05, vs. control (Dunnett's test).*

Thiopental sodium induced sleeping time test

In the thiopental sodium induced hypnosis test, the extract of *Desmodium triflorum* at dosages of 100 and 200 mg/kg increased the latency time at an earlier stage. Additionally, the same extract at doses of 100 and 200 mg/kg had a dose-dependent effect on the duration of the thiopental sodium induced sleep. Additionally, all doses reduced the amount of time test animals spent resting compared to the control (Table 4).

Treatment	Dose (mg/kg)	Onset of action (Seconds)	Duration of sleeping time (min)
Control	0.1 mL/mouse	13.32 ± 0.71	94.20 ± 1.85
Imipramine hydrochloride	30	67.30 ± 0.94*	38.60 ± 1.03*
MEDT	50	16.32 ± 1.39	76.00 ± 1.00
MEDT	100	32.60 ± 0.92*	64.40 ± 1.36*
MEDT	200	54.40 ± 1.50*	46.00 ± 1.61*

Table 4: *Effects of Desmodium triflorum extract and imipramine hydrochloride on thiopental sodium-induced sleeping time test in mice.*

Values are presented as mean ± SEM (n = 5). MEDT = Methanolic Extract of Desmodium triflorum.

**p < 0.05, vs. control (Dunnett's test).*

Discussion

All of the nervous system's control centers are located in the central nervous system [32]. The majority of medications that affect the central nervous system also affect specific receptors that control synaptic transmission. Certain receptors on the central nervous system are acted upon by drugs, and these actions alter synaptic transmission [33]. Convulsions, elevated anxiety and uneasiness, and modest enhancement of attentiveness are some of the behaviors associated with CNS stimulation. The delicate balance between excitatory and inhibitory forces that is ordinarily maintained in the central nervous system is often altered by any hyper excitability related to medication treatment [34].

The methanolic extract of *Desmodium triflorum* was tested using three different methods to assess its CNS effects. Mice are forced to swim in a constrained area as part of the forced swim test paradigm, which measures CNS activity. Eventually, the mice give up swimming and become motionless. While all antidepressants lessen immobility in the forced swim test, pharmacologically specific antidepressants result in the production of two unique active behavioral patterns. Without influencing swimming, antidepressants that specifically block norepinephrine absorption lessen immobility and promote climbing. On the other hand, serotonin reuptake inhibitors promote swimming rather than ascending while simultaneously decreasing immobility [35].

Desmodium triflorum leaves have been researched in this project. The extract was found to significantly reduce immobility time in a dose-dependent manner in force swimming and tail suspension tests when compared to control at dosages of 100 and 200 mg/kg. As anticipated, the animals given 30 mg/kg of Imipramine hydrochloride also saw a notable reduction in their period of immobility. According to earlier research by Rénéric and Lucki [36], when an animal is given medication that raises serotonin, norepinephrine, and dopamine levels in the nerve terminals, the animal's swimming and climbing behaviors in the force swimming test are said to rise. One possible explanation for an increase in all three neurotransmitters is a reduction in brain monoamine oxidase activity. More and more research suggests that depression may be caused by a variety of different pathophysiological processes in addition to the depletion of catecholamine and serotonin neurotransmitters. According to research, depression may prevent hippocampal neurogenesis [37]. The observation that antidepressants can stimulate neurogenesis lends credence to this theory [38].

The thiopental sodium-induced sleeping duration and forced swim tests are commonly employed to evaluate the depressant/antidepressant or sedative/hypnotic qualities of test compounds. It is clear that the $GABA_A$ receptor complex's barbiturate binding site is bound by CNS depressant barbiturates, which enhance GABA-mediated hyperpolarization of postsynaptic neurons in rats [39]. When developing anxiolytic and antidepressant medications, the GABA receptor is a key target [40]. A major inhibitory neurotransmitter, GABA is essential for brain function and development as well as for preserving the balance between neuronal activation and inhibition in the central nervous system [41]. Conversely, the force swimming test is also referred to as the behavioral despair test. It is frequently used to assess how well a wide range of test compounds antidepressant activity works and how vulnerable animals like mice and rats are to the risk of drowning [42]. Imipramine hydrochloride has been shown to have a central nervous system stimulant effect in every instance, which may be related to the drug's stimulating effects in animals.

Early phytochemical research indicates that the contents of *Desmodium triflorum* include proteins, lipids, carbohydrates, amino acids, phenols, terpenoids, glycosides, and tannins [43]. *Desmodium triflorum* in a water solution verified the presence of proteins, alkaloids, phenols, steroids, and saponins. Methanolic extract from *Desmodium triflorum* reveals the presence of amino acids, carbohydrates, proteins, steroids, flavonoids, alkaloids, and phenol [44]. Upon phytochemical analysis, *Desmodium triflorum* was found to contain proteins, tannins, alkaloids, steroids, and flavonoids. There have been several reports of phytochemicals with CNS activity. Premature ejaculation has been treated by delaying ejaculation using drugs that raise serotonin levels in the central nervous system, such as selective serotonin reuptake inhibitors (SSRIs) [45,46]. The human body experiences anxiolytic and antidepressant effects when serotonin levels are up [47]. The underlying reasons for the plant's anxiolytic and antidepressant effects may be the rise in serotonin levels, which is comparable to SSRIs and SNRIs. This is because the majority of antidepressant medicines work to restore serotonin levels and brain function. Furthermore, the CNS effects of the crude extract of *Desmodium triflorum* reported in this experiment may be attributed to the phytoconstituents in the plant.

Conclusion

The findings imply that *Desmodium triflorum* methanolic extract may have a CNS stimulating effect that may be investigated for therapeutic use as a substitute treatment for illnesses linked to sedation, sleepiness, and dizziness. Alkaloids, flavonoids, phenols, saponins, and tannins are present and are linked to a range of pharmacological activity, according to the phytochemical profiling. Therefore, the current study supported the use of the plant in the treatment of depression; nevertheless, more research on the phytochemicals in the plant is required to prove its central nervous system stimulating impact.

Ethics Approval and Consent to Participate

There is no personal information about any individual in the paper. Therefore, this knowledge is irrelevant. All of the experimental mice adhered to the Ethical Principles and Guidelines for Scientific Experiments with Animals, which were created

in 1995 by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee of Stamford University Bangladesh approved all experimental protocols.

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Competing Interests

The authors have disclosed no conflicts of interest. The writers alone are in charge of the writing and substance of the publication.

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