Phytopharmacological Evaluation of *Trapa natans* var. *bispinosa* Roxb. Ethanolic Fruit Extract for Antidepressant Effects

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ABSTRACT:

Depression remains a major mental health challenge, demanding novel therapeutic options. This study evaluated the antidepressant potential of ethanolic fruit extract of *Trapa natans* var. *bispinosa* Roxb. (EETN) in Wistar albino rats. Phytochemical analysis revealed the presence of flavonoids, saponins, glycosides, tannins, amino acids, proteins, and steroids. Rats were divided into five groups: normal control, stress control, standard drug (imipramine, 15 mg/kg), and EETN (250 and 500 mg/kg). Behavioral tests (Forced Swim Test, Tail Suspension Test, Locomotor Activity, Sucrose Preference Test) and serum corticosterone estimation were conducted. EETN significantly reduced immobility, improved locomotion, and restored sucrose preference in a dose-dependent manner, while lowering elevated corticosterone levels induced by acute restraint stress. Findings suggest that EETN exhibits antidepressant-like effects comparable to imipramine, likely via modulation of the HPA axis. Further studies are warranted to isolate bioactive compounds and explore their clinical relevance.

Keywords: Acute restraint stress, Forced swim test, Tail suspension test, Locomotor activity, Sucrose Preference test, Imipramine.

Introduction:

Depression is a pervasive and debilitating psychiatric disorder that affects millions of people worldwide. In 2024, global health statistics revealed that more than 320 million individuals, equivalent to nearly 4% of the population, suffer from depression **(WHO, 2024).** It continues to be a leading cause of disability and contributes significantly to the worldwide burden of disease. Depression is not limited to emotional disturbances but extends its impact to physical health, cognition, social functioning, and quality of life. Persistent low mood, loss of interest in pleasurable activities, guilt, sleep disturbances, and suicidal ideation represent hallmark features of this disorder. In children and adolescents, irritability may predominate, while in adults, feelings of hopelessness and worthlessness are frequently observed.

Clinical Manifestations of Depression

The symptomatic presentation of depression is broad and complex. Core symptoms include a **persistent sad or melancholy mood**, fatigue, and lack of motivation. Individuals often display a **reduced ability to experience pleasure** in daily activities (anhedonia), leading to social withdrawal. Cognitive impairments such as **poor concentration, mental fog, and disorganized thinking** further worsen productivity and daily functioning. Sleep disturbances are particularly characteristic, manifesting either as **insomnia** (difficulty falling or staying asleep) or **hypersomnia** (excessive sleeping). Appetite alterations, ranging from loss of appetite to overeating, often result in noticeable weight fluctuations.

Psychomotor changes also occur, including **agitation** (restlessness, hand wringing, pacing) or **psychomotor retardation** (slowed speech and movements). Emotional symptoms encompass guilt, regret, feelings of worthlessness, and hopelessness. The most alarming consequence of untreated depression is **suicidal ideation and behavior**, which contributes to nearly one million deaths annually **(Kumar et al., 2017).**

Etiological Factors

The etiology of depression is multifactorial, encompassing genetic, biological, psychological, and environmental determinants. Life events such as **childhood adversity, abuse, financial stress, divorce, loss, loneliness, and trauma** are strongly linked to depression onset **(Pillemer et al., 2010; Lindert et al., 2014)**. Similarly, **non-psychiatric illnesses** including infections, nutritional deficiencies, diabetes, neurological disorders, cancer, and chronic pain increase vulnerability **(Saravane et al., 2011).**

From a psychiatric perspective, depression may co-occur with or emerge secondary to conditions such as **major depressive disorder (MDD), bipolar disorder, seasonal affective disorder, borderline personality disorder, and anxiety disorders (Gabbard et al., 2004; APA, 2009).** Epidemiologically, approximately **20% of individuals experience depression during their lifetime**, with a gender ratio of nearly **5:2 (women: men) (Randhawa et al., 2015).**

Neurotransmitter Dysregulation in Depression

The pathophysiology of depression is deeply intertwined with alterations in neurotransmitter systems.

- **Serotonin (5-HT):** Central to mood regulation, sleep, and appetite. Depression is linked to reduced serotonin levels, downregulation of 5-HT1A receptors, and hyperactivity of 5-HT2 receptors (Nemeroff, 2022; Xue et al., 2021). Such imbalances contribute to sadness, anxiety, and disrupted sleep **(Hrdina et al., 1993).**
- **Dopamine (DA):** Essential for motivation, reward processing, and pleasure. Depression is associated with impaired DA transmission, including excessive reuptake, leading to low energy, reduced motivation, and anhedonia (Babaev et al., 2022; Bee et al., 2020).
- **Norepinephrine (NE):** Governs stress responses, vigilance, and attention. Deficiency in NE is linked to fatigue, poor concentration, and persistent low mood **(Thase et al., 2001).**
- **Gamma-Aminobutyric Acid (GABA):** The principal inhibitory neurotransmitter. Reduced GABA levels and receptor dysfunction are implicated in heightened anxiety and depressive symptoms **(Martin et al., 2018; Lener et al., 2017).**

- **Glutamate:** The primary excitatory neurotransmitter. Elevated glutamate levels, NMDA receptor dysregulation, and abnormalities in glutamate signaling contribute to cognitive and mood disturbances. Interestingly, ketamine, a glutamatergic modulator, has shown rapid antidepressant effects through AMPAR activation **(Tomasetti et al., 2019; Kadriu et al., 2019).**

Thus, depression arises from a complex interplay of neurochemical disruptions affecting mood, cognition, and behavior.

Therapeutic Approaches

Conventional treatment strategies for depression include **pharmacotherapy, psychotherapy, and lifestyle modifications**. Antidepressant medications such as SSRIs, SNRIs, tricyclic antidepressants, and MAO inhibitors remain mainstays, but they are limited by **delayed onset, incomplete efficacy, and side effects** (e.g., weight gain, sexual dysfunction, drowsiness).

Non-pharmacological interventions also play a pivotal role. **Exercise** has been shown to elevate serotonin, dopamine, norepinephrine, and endorphin levels, thereby alleviating depressive symptoms (Craft & Perna, 2004). Lifestyle strategies such as **light therapy, meditation, balanced diet, abstinence from tobacco, and adequate sleep hygiene** further enhance recovery **(Alkhatib et al., 2014).**

In recent years, attention has shifted towards **natural therapies and medicinal plants**. Herbal remedies are considered safer, affordable, and culturally acceptable alternatives to synthetic drugs. Several botanicals, such as **Bacopa monnieri (Brahmi), Withania somnifera (Ashwagandha), and Rauwolfia serpentina**, have long been used in traditional systems of medicine for mental health disorders (Kadali et al., 2014). Animal studies and preliminary clinical evidence highlight their antidepressant potential, although comprehensive validation remains necessary.

***Trapa natans var. bispinosa* Roxb. (Singhara/Water Caltrop)**

Among medicinal plants, *Trapa natans var. bispinosa* Roxb., commonly known as Singhara or water chestnut, has gained scientific interest for its therapeutic potential. This aquatic plant is widely distributed across Asia, particularly in India, China, and Southeast Asia, where it is cultivated in wetlands and shallow water bodies **(Salabh et al., 2012).**

Nutritional Profile

Singhara fruits are a rich source of **carbohydrates, dietary fibre, proteins, and essential minerals** including calcium, iron, magnesium, and potassium. They also contain important vitamins such as **thiamine, riboflavin, vitamin C, and vitamin A**. Enzymes like **amylase and phosphorylase** enhance their digestibility and nutritional value.

Traditional Uses

In Ayurvedic medicine, Singhara has been described as an **appetizer, astringent, diuretic, aphrodisiac, and general tonic**. It is believed to provide cooling effects, aid in digestion, and alleviate conditions such as **diarrhea, inflammation, sore throat, bronchitis, urinary disorders, and anemia**. The fruit is also employed as a **wound-healing and anti-inflammatory agent**, while the stem juice has been incorporated in formulations for **ocular diseases (Chatterjee et al., 1995).**

Pharmacological Properties

Modern phytopharmacological investigations suggest that *Trapa natans* possesses a wide array of **bioactive properties**, including:

- **Antioxidant** – scavenges free radicals and reduces oxidative stress.
- **Anti-inflammatory** – mitigates inflammatory processes contributing to chronic diseases.
- **Hepatoprotective** – protects liver function against toxins and infections.
- **Antimicrobial** – inhibits growth of pathogenic bacteria and fungi.
- **Anticancer** – exhibits cytotoxic effects on certain cancer cell lines.

These diverse pharmacological actions, coupled with its nutritional richness, suggest that Singhara may hold potential in the **management of depression**, particularly through its antioxidant, neuroprotective, and adaptogenic mechanisms (Bharthi et al., 2015; Purkayastha et al., 2020).

MATERIALS AND METHODS:

During the study, the selection of chemicals, reagents, equipment, glassware, synthetics, and plant materials was made based on the specific requirements of the research. The necessary equipment and glassware included a Soxhlet apparatus for extraction, a desiccator for drying, and a rat restrainer for safely handling the animals during experiments. A mortar and pestle were used for grinding plant materials or other substances, while beakers of 50ml and 200ml capacities, along with a 100ml measuring cylinder, were essential for measuring liquids and solutions. A China dish was used for evaporating liquids, and a 500ml round-bottom flask served for conducting reactions or distillations. For accurate measurement of liquids, a graduated pipette was employed. An oral gavage was used for the administration of substances to animals, and glass rods were essential for stirring or mixing solutions. Muslin cloth was utilized for filtration or as a fine strainer in some processes.

Drugs and Chemicals:

All drug solutions were freshly prepared before use. Imipramine was sourced from S.S. Drugs Medical Store, Meerut, Uttar Pradesh, India, while petroleum ether, ethanol, and carboxymethyl cellulose were obtained from School of Pharmacy, Bharat Institute of Technology, Meerut, Uttar Pradesh, India. The standard dose of imipramine (15 mg/kg/p. o) was adopted accordance to **Kanase *et al.*, 2020.**

Animals:

The research protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the School of Pharmacy, Bharat Institute of Technology, Meerut, Uttar Pradesh, India, and all experimental procedures were conducted in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India. Healthy Albino Wistar rats weighing 150–200 g were procured from the animal house of the School of Pharmacy, Bharat Institute of Technology, Meerut. The animals were maintained under a controlled environment with a natural 12-hour light/dark cycle and a temperature range of 22–24 °C. They were housed individually in standard cages with free access to food pellets and water ad libitum. A 7-day acclimatization period was provided prior to the commencement of the experimental procedures.

Selection of dose of the ethanolic extract fruit of *Trapa natan var bispinosa roxb*:

The doses of the ethanolic fruit extract of *Trapa natans* var. *bispinosa* Roxb. were adopted from the study reported by Kar et al. (2010). In this study, it was found that the oral administration of *Trapa natans* ethanolic fruit extract at doses of 250 mg/kg and 500 mg/kg was safe.

Preparation of ethanolic fruit extraction of *Trapa Natan var Bispinosa Roxb*:

The extraction was carried out based on the method of **Kar et al. (2010)**. 10 kg of fresh *Trapa natans* var. *bispinosa* Roxb. fruits, purchased from the local market in Meerut, Uttar Pradesh, India in the month of October. According to the method the fruits were cleaned, shade-dried, and cut into pieces before being ground into a coarse powder. The powder was sieved through mesh no. 40 and extracted with petroleum ether (40-60°C) in a Soxhlet extractor. The defatted residue was then extracted with 50% ethanol. The liquid extract was vacuum-concentrated, dried in a desiccator, and suspended in distilled water containing 0.5% sodium carboxymethyl cellulose for oral administration

Behavioral Assessments:

Acute restraint stress procedure:

Rats were kept in separate plastic rodent restraint devices for 12 hours to perform ARS. This prevented the animal from moving physically without hurting it. Food and drink were withheld from the animals for the duration of their stress exposure. The animals were taken out of their enclosure after 12 hours, and 40 minutes later they were tested behaviourally and then given biochemical readings. Rats were housed in the experimental room's animal cage in the usual control group **(Kanase *et al.,* 2019).**

Forced Swim Test (FST):

After the application of ARS on 13th day in the evening hours. On next day (14th day) after 40 minutes rats were placed in a pool of water, and the rat's motoric activity was monitored to calculate the length of time the rats were immobile. Water was poured into a glass cylinder that had a 25 cm diameter and a height of 23 cm. Water had a 23 ± 1 ºC temperature. The appropriate dosage of the extract was administered to each rat orally. The test was administered to the animals after waiting for thirty minutes. The animal was given two minutes to become used to the new surroundings before the six minutes of measurement started. After two minutes, circumstances of increasing motor activity and periods of inactivity were alternated and assessed. For the next four minutes, immobility duration was timed using a stopwatch **(Rath *et al.,* 2009).**

Tail Suspension Test (TST):

After the application of ARS on 13^{th} day in the evening hours. On next day (14^{th} day) after 40 minutes the second method for determining the antidepressant effect of tail-suspension test. The test was performed thirty min after the oral administration of ethanolic fruit extract of *Trapa natan*. The arrangements of tail suspension test were done in between two metal tripods at a height of 70 centimetres, a cord approximately 50 centimetres long was stretched. The sticky tape was used to attach the rat's tails to the cord. After the underlying time of enthusiastic engine action, the rodents turned out to be still and the fixed status time was estimated with a stopwatch, for a complete length of 4 minutes. When rats hung motionless and passively, they were considered immobile **(Rath *et al.,* 2009).**

Locomotor Activity:

After the application of ARS on 13th day in the evening hours. On next day (14th day) after 40 minutes rats were grouped and given medication in order to study the locomotor activity with the use of an actophotometer. After 40 minutes of medication treatment, each animal was put separately in an actophotometer, and the baseline activity score of each animal was recorded for 10 minutes. **(Katolkar *et al.*, 2015)**

Sucrose Preference Test:

After the application of ARS on 13th day in the evening hours. On next day (14th day) after 40 minutes rats were housed alone and trained to drink two bottles of a sucrose solution (1%) for 48 hours prior to the sucrose preference test. After 16 hours of water deprivation, rats were given two pre-weighted bottles, one for each rat. 1% sucrose solution is used in the first, whereas water is used in the second. The two bottle's sides were assigned at random to avoid biases. The bottles were reweighed after an hour, and the weight difference change was calculated. The percentage of the 1% sucrose solution that was drank in relation to the total quantity of liquid consumption was used to assess the desire for sucrose. **(Youssef *et al.*, 2016)**

EUTHANASIA:

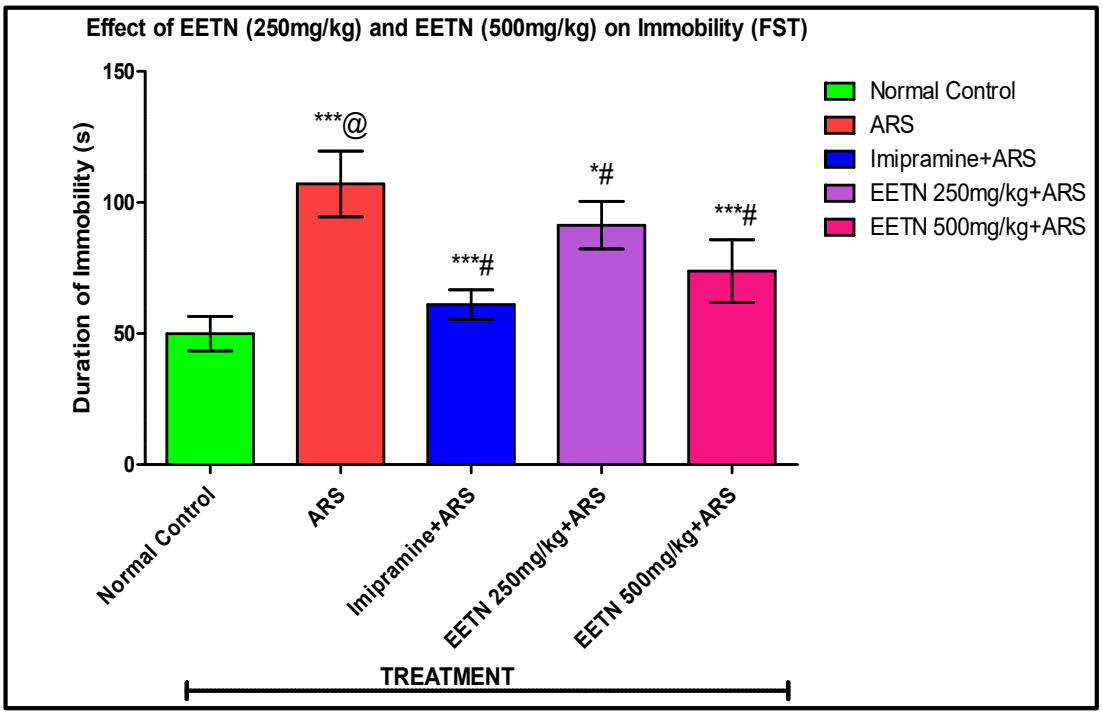
1. Each animal was sacrificed at the end of the 14th day in an antidepressant study by giving the Cervical Dislocation.
2. Set the rat down in a tidy and peaceful spot, preferably on a cushioned or soft surface.
3. Check for reflexes to ensure the rat is unconscious by softly touching the cornea of the eye and observing for a reflexive blink.
4. Support the rat's body while firmly grasping it by the base of the tail.
5. Position the rat vertically with its hand on the ground and its head resting on a hard object like a table or bench. Alternatively, arrange the rat so its head is horizontal and protrudes slightly over the edge of the surface.
6. Ensure the rat's neck is fully stretched. Use your thumb and forefinger to grab the top of the spine and the base of the skull. Apply a quick and firm downward and forward force to dislocate the cervical vertebrae and sever the spinal cord. A distinct crack sound should be heard, indicating successful cervical dislocation.
7. Immediately confirm the rat is dead by checking for the absence of breathing and heartbeat.
8. Dispose of the rat's body properly in compliance with institutional guidelines and regulations **(Carbone *et al.*, 2012).**

Collection of blood sample for serum corticosterone: Blood samples were collected from the retro-orbital plexus of Wistar rats for the evaluation of corticosterone levels in blood serum. The biochemical estimation of serum corticosterone was outsourced to Max Path Lab, which utilized the ELISA method to determine the corticosterone levels in the samples.

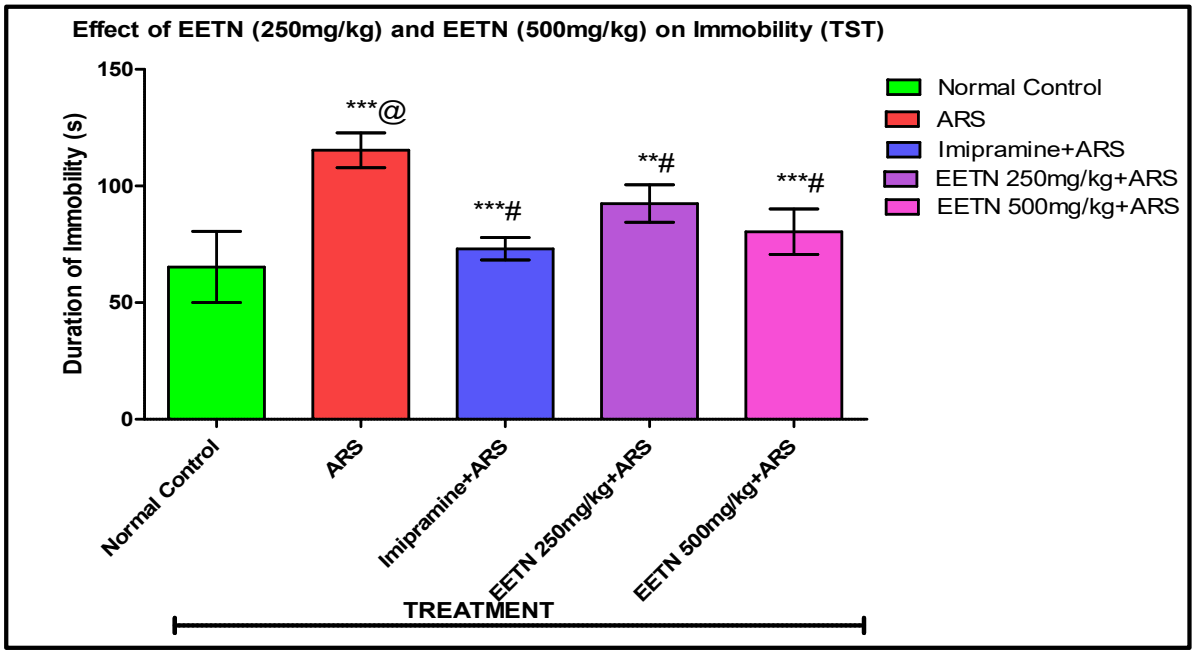
Experimental Design:

The present study was designed to evaluate the antidepressant effects of the ethanolic fruit extract of *Trapa natans* against acute restraint stress (ARS)-induced depression in male albino Wistar rats (150–200 g). Thirty rats were randomly allocated into five groups (n = 6 per group). Group I (Normal Control) received vehicle (0.5% sodium carboxymethyl cellulose [CMC], 2 mL/kg, p.o.); Group II (Stress Control) was subjected to ARS and received the vehicle; Group III (Standard Treatment) was subjected to ARS and administered imipramine (15 mg/kg, p.o.); Group IV (Low-Dose Extract) was subjected to ARS and treated with *T. natans* ethanolic fruit extract (250 mg/kg, p.o., twice daily); and Group V (High-Dose Extract) was subjected to ARS and treated with *T. natans* extract (500 mg/kg, p.o., twice daily). All treatments were given orally for 14 consecutive days using a gavage feeding needle. Depressive behavior was induced using the ARS paradigm, and the efficacy of *T. natans* extract was compared with the reference standard imipramine. The doses of the extract were selected based on prior dose–response studies.

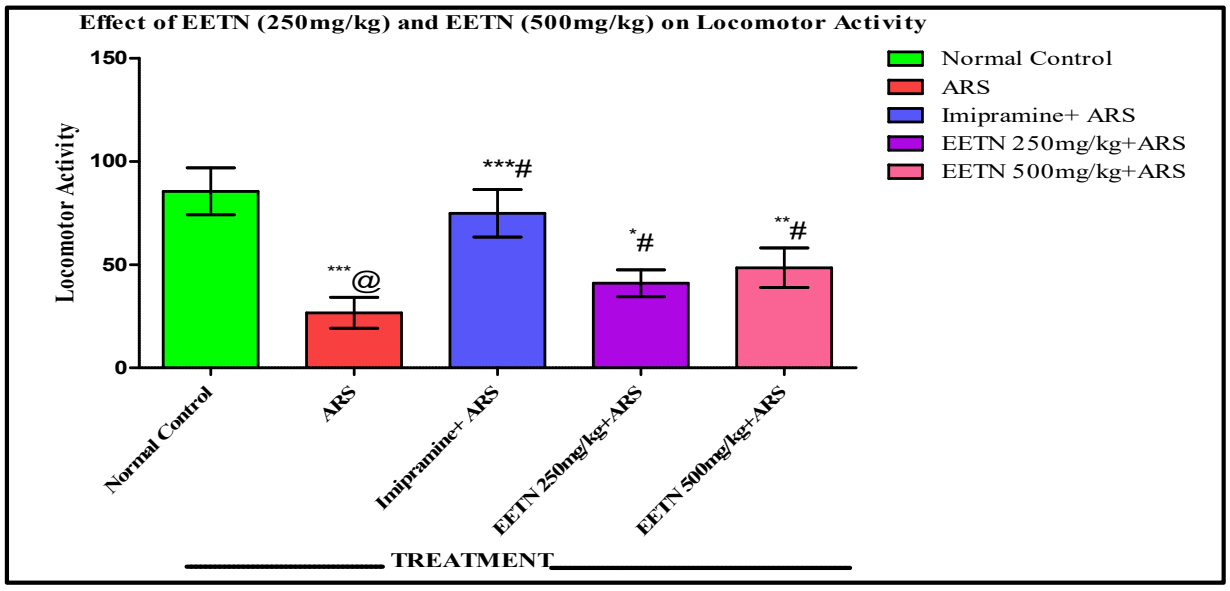
Result:



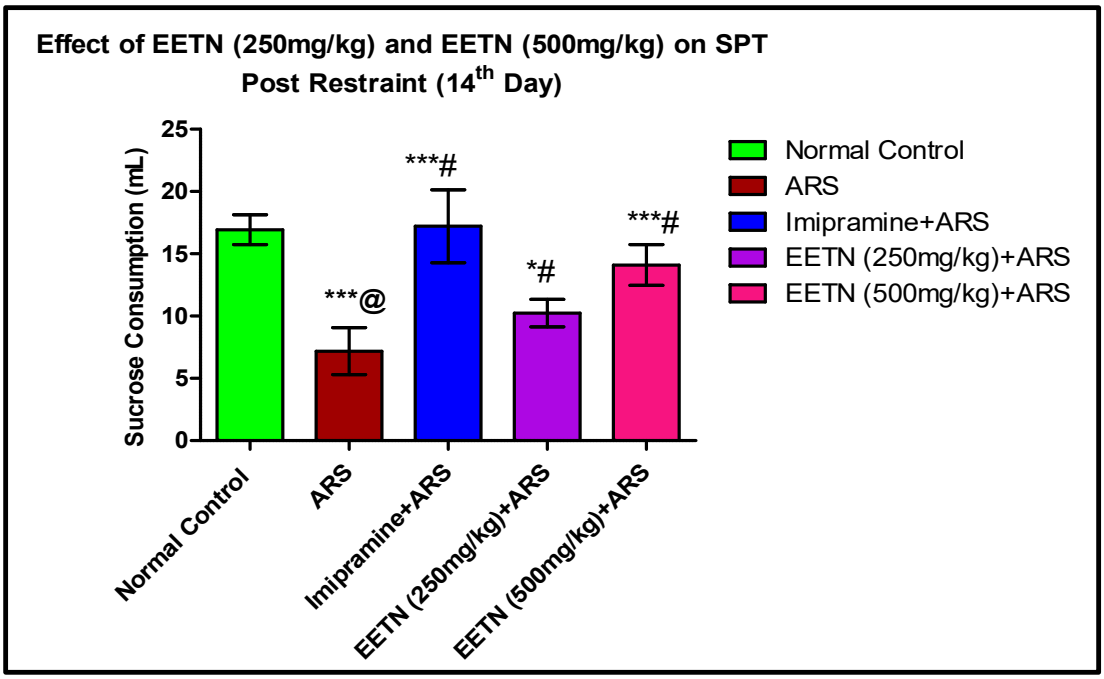
EETN (250 and 500 mg/kg, p.o., twice daily) significantly reduced immobility time in the Forced Swim Test in a dose-dependent manner. One-way ANOVA followed by Dunnett's test revealed that ARS markedly increased immobility (***$p<0.001$ vs. control), whereas Imipramine (***$p<0.001$) and EETN at 250 mg/kg (*$p<0.05$) and 500 mg/kg (*$p<0.001$) significantly attenuated this effect compared to the ARS group.



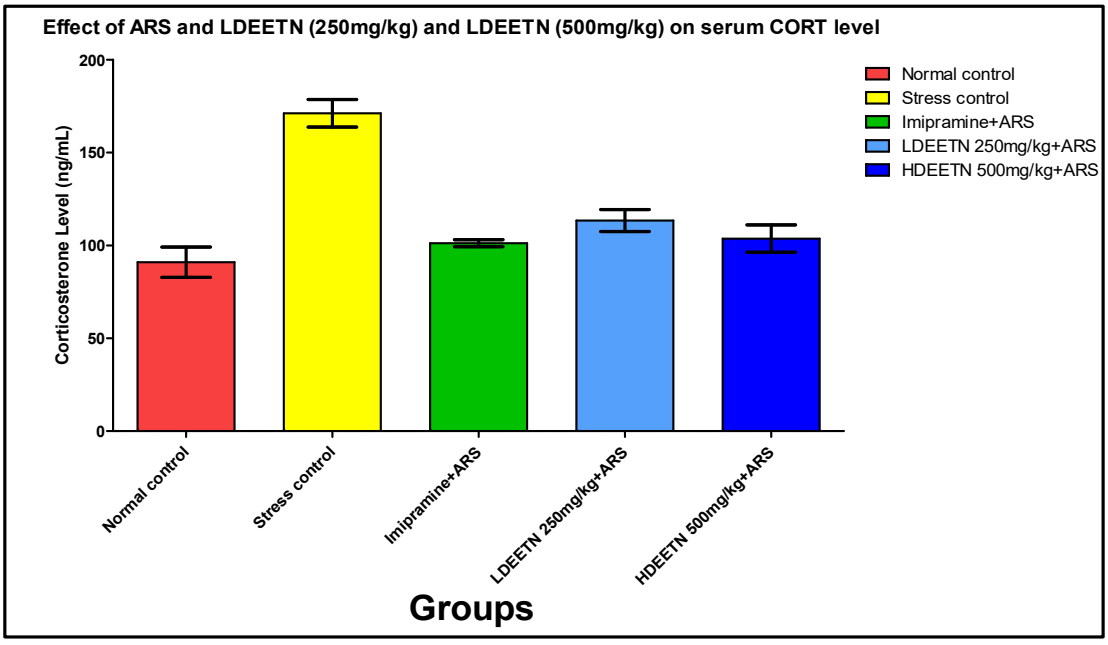
Effect of EETN (250 and 500 mg/kg; p.o., b.i.d.) on immobility time in the tail suspension test (TST) in Wistar rats. Data are expressed as mean ± SD (n=6). One-way ANOVA followed by Dunnett's t-test. ***p<0.001, **p<0.01 vs. normal control; #p<0.05, ***#p<0.001 vs. ARS group.



Effect of EETN (250 and 500 mg/kg, p.o., b.i.d.) on locomotor activity in Wistar rats. Data are expressed as mean ± SD (n=6) and analyzed by One-way ANOVA followed by Dunnett's t-test. Locomotor activity was significantly reduced in the ARS group (*@p<0.001 vs. normal control). Imipramine markedly restored activity (*#p<0.001 vs. ARS). Both EETN doses produced a dose-dependent improvement, with 250 mg/kg (*#p<0.05) and 500 mg/kg (#p<0.001) vs. ARS."**



ARS significantly reduced sucrose preference compared to the normal control (***p<0.001). Imipramine markedly reversed this effect. EETN at 250 mg/kg (*p<0.05) and 500 mg/kg (**p<0.001) produced a dose-dependent restoration of sucrose consumption versus the ARS group, indicating antidepressant-like activity.



One-way ANOVA followed by Bonferroni's test revealed a highly significant increase in corticosterone levels in the ARS group (***p<0.001) compared to normal and treatment

groups. EETN (250 mg/kg) showed no significant difference versus control, while EETN (500 mg/kg) significantly reduced corticosterone compared to the ARS group (*p<0.05).

Discussion:

Depression is a common mental disorder marked by persistent sadness, anhedonia, and cognitive impairment. Conventional antidepressants often have limited efficacy and adverse effects, driving interest in herbal alternatives. The present study evaluated the antidepressant potential of ethanolic fruit extract of *Trapa natans* var. *bispinosa* Roxb. (EETN) in Wistar rats using behavioral, biochemical, and histopathological parameters. Phytochemical analysis revealed the presence of flavonoids, saponins, tannins, glycosides, steroids, amino acids, proteins, and carbohydrates, with flavonoids and saponins reported to mediate antidepressant effects. Rats were divided into five groups: normal control, stress control, standard drug (imipramine, 15 mg/kg), and EETN-treated groups (250 and 500 mg/kg, p.o.). Depression was induced by acute restraint stress (ARS), and behavioral tests including Forced Swim Test (FST), Tail Suspension Test (TST), Sucrose Preference Test (SPT), and locomotor activity were conducted. Serum corticosterone (CORT) levels and hippocampal histopathology were also assessed. Results demonstrated that EETN significantly reduced immobility in FST and TST, improved locomotion, enhanced sucrose consumption, and lowered serum CORT levels in a dose-dependent manner (p<0.05–0.001). Histopathological examination showed marked neuronal protection and regenerative changes, particularly at the higher dose. The findings suggest that EETN exerts antidepressant-like effects, likely mediated through flavonoids and saponins that enhance monoaminergic neurotransmission and protect hippocampal integrity. Thus, EETN shows promise as a potential herbal antidepressant candidate.

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(Table-1) Botanical Description of *Trapa Natan var bispinosa roxb*:

Taxonomical Classification (Prafulla *et al.*, 2014)	**Vernacular Names (Prafulla *et al*., 2014)**
Kingdom: Plantae **Family:** Lythraceae **Genus:** *Trapa;* L.	**English:** Water chestnut **Sanskrit:** Smgtakah, Jalphala **Hindi**: Singhara, Singhara **Assamese:** Paniphal

(Table-2) Reported Pharmacological Activities of *Trapa Natan var Bispinosa* roxb:

S.NO	**Plant Name**	**Pharmacological activity**	**Reported by**
1.	*Trapa natan*	Hepatoprotective	(Majee *et al.,* 2022)
2.	*Trapa natan*	Anti-inflammatory	(Kim *et al.,* 2014)
3.	*Trapa natan*	Anthelmintic	(Verma *et al.,* 2013)
4.	*Trapa natan*	Antifungal	(Mandal *et al.,* 2011)
5.	*Trapa natan*	Antidiabetic	(Das *et al.,* 2011)
6.	*Trapa natan*	Antibacterial	(Razvy *et al.,* 2011)
7.	*Trapa natan*	Antiulcer	(Kar *et al.,* 2010)

(Table-3) Statistical analysis the data was expressed as Mean ± SD. The statistical significance between groups were analyzed by ANOVA.

S.no	Parameter/ Groups	Normal control	Stress control	Imipramine +ARS	EETN (250mg/kg) +ARS	EETN (500mg/kg) +ARS
1.	**FST**	50±5.9	107±11.4	61.1±5.12	93±9.3	73.8±10.9
2.	**TST**	65.3±13.9	115.3±6.8	73.1±4.3	92.4±7.33	80.4±8.8
3.	**Locomotor activity**	85.5±10.3	26.7±6.8	74.8±10.4	41±5.94	48.5±8.73
4.	**SPT**	16.2±2.52	17.2±2.13	17±1.87	18.8±196	18.9±1.52
5.	**CORT**	90.68±8.1	147.5±3.6	87.9±4.7***	117.9±7.10	106.5±5.1

(Fig 1). Showing the step 1 for extraction
(Shade dried of fruit *Trapa natan var bispinosa* roxb)

Fig 1.2 Showing the step 2 for extraction(Fruit crushed into a coarse powder)

Fig 1.3 Showing the step 3 for extraction(Soxhlet assembly)

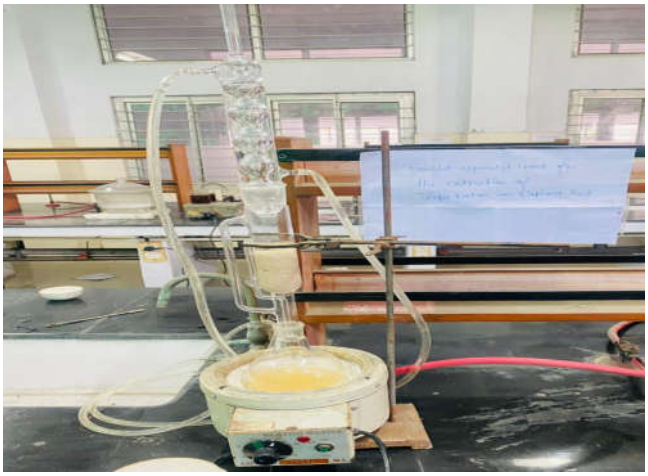

Fig 1.4 Showing the step 4 for(Liquid extract)

Fig 1.5 Showing the step 5 for (Liquid extract dried in dessicator)

Fig 1.6 Showing the step 6 for extraction (Extract ready for test)

Histological Study:

On 14th days after the completion of employed parameters, each rat was killed via cervical dislocation, and the brains and the two rats of each group was removed and dissected. Brain tissue was fixed in 10% neutral buffered formalin for histopathology. The histopathological analysis was out source from Vet Path Lab, Chhatarpur Extension, New Delhi 110074, India

illustrates the hippocampal histoarchitecture across experimental groups. Normal architecture was observed in Group I (A&B), while Group II (C&D, Stress Control) showed neuronal necrosis, degeneration, and vacuolization. Group III (E&F, Imipramine) exhibited near-normal architecture with mild regenerative and degenerative changes. Group IV (G, EETN 250 mg/kg) showed regenerative activity with minor degeneration, whereas Group V (H, EETN 500 mg/kg) revealed largely preserved hippocampal architecture with minimal degenerative alterations.