

EVALUATION OF ANTIDEPRESSANT ACTIVITY OF LEAF EXTRACTS OF HOLOPTELEA INTEGRI FOLIA (ROXB) PLANCH IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: In traditional system of medicine *Holoptelea Integrifolia* is useful in treating various ailments. The main objective of the work was to evaluate the antidepressant activity of petroleum ether and methanolic extract of *Holoptelea Integrifolia* leaves using forced swim test (FST) and tail suspension test (TST) in mice.

Methods: The petroleum ether extract (100 and 300 mg/kg) and methanolic extract (100 and 300 mg/kg) were administered to mice for 14 days for evaluating antidepressant activity using forced swim test (FST) and tail suspension test (TST) in mice.

Results: In case of Forced swim test the petroleum ether extract was more significant than methanol extract and showed dose dependent effect on immobility and climbing behaviour however it didn't show any effect on swimming suggesting role of noradrenergic transmission in its antidepressant activity while in case of Tail Suspension Test also the petroleum ether extract was more significant than the methanol extract and showed dose dependent effect on immobility suggesting antidepressant action. Although the methanolic extract of 100 mg/kg didn't show significant action but the reading are too close to methanolic extract of 300 mg/kg and petroleum ether extract of 100 mg/kg which indicates its antidepressant potential.

Conclusion: The results indicate that petroleum ether and methanol extracts of *holoptelea integrifolia* leaves contained flavonoids and phytosterols which might be active in case of Forced swim test (FST) and Tail Suspension Test (TST) to show antidepressant activity. which support the ethnomedicinal application of the plant as an antidepressant agent.

Keywords: Forced swim test, Tail suspension test, *Holoptelea integrifolia* leaves, Depression, *Fluoxetine*.

INTRODUCTION

According to the World Health report, approximately 450 million people suffer from mental or behavioral disorders, yet only a small minority of them receives even the most basic treatment [1]. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020 [2]. According to WHO anxiety and Depression are the two most common disorders. Mental health problems are currently making about 8% of the worldwide cases of disease and 15% of adults in developing countries are found to suffer from mental diseases. It is estimated that more than 20% of the adult population suffers from these conditions at least some time in their life time.

The World Health Organisation predicts that depression will become the second leading cause of premature death or disability all over the world by the year 2020. Medicinal herbs are becoming popular day by day. Both developed and developing countries have a great demand for medicinal plants due to increased identification of natural products and it is sometimes the primary source of health care available to the poor. Since old time herbal sources are useful in the treatment of many diseases. Many plants have traditional claim in the treatment of several harmful diseases still they are not scientifically confirmed or not properly used. For this reason such plant drugs require detailed studies as a need of modern medicine. In traditional system of medicine, bark and leaves of *Holoptelea Integrifolia* used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [3,4]. The plant *Holoptelea integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea [5].

But, its antidepressant activity is not yet validated scientifically as on date. The literature and ethnobotanical survey and the use of this herb for various ailments shows its importance from ancient time.

Due to these reasons the current dissertation for antidepressant activity of Petroleum ether and Methanol extract of leaf of *Holoptelea Integrifolia* in experimental animals was under taken.

MATERIALS AND METHODS

Plant Introduction & Collection

Holoptelea Integrifolia belongs to the family Ulmaceae commonly called as Indian Elm and commonly used in India by the tribal people for its medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings [6].

Leaves of *Holoptelea Integrifolia* were collected in the Month of August from the agricultural fields of Tirunelveli district, Tamilnadu, India. The plant was identified and leaves of *Holoptelea Integrifolia* were authenticated and confirmed from Dr.V.Chelladurai,

Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by comparing morphological features (leaf and stem arrangement, flower /inflorescence arrangement, fruit and seed morphology etc.). The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Instruments and Chemicals used

The solvents used for extraction were Petroleum ether and Methanol. Other reagents used were of laboratory grade and obtained from various other commercial sources. All the reagents used were of laboratory and analytical grade. Solvents were obtained from SD Fine Chem Ltd. (Mumbai). Standard drug *fluoxetine* was obtained from Crescent Therapeutics limited, Himachal Pradesh. The extracts were suspended in CMC (0.5 %). A gastric catheter was used for oral drug administration. The extracts did not show any sign of toxicity till the oral dose of 2000 mg/kg hence the extracts were used in the range of 100–300 mg/kg orally assuming that LD₅₀ dose is 2000 mg/kg.

Preparation of Different Extracts

Successive extraction of leaves of *Holoptelea Integrifolia* was prepared on the basis of solvent polarity

Preparation of petroleum ether and methanol extract

The powder of *Holoptelea Integrifolia* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using petroleum ether. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50° C to get petroleum ether extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10°C. After obtaining the petroleum ether extract the marc was pressed and it is air dried and again it was extracted using methanol. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50° C to get semi solid mass of methanol extract. The extract was stored in an airtight container in a refrigerator below 10°C. The two extracts namely Petroleum ether and Methanol were examined for their color and consistency. Their percentage yield was calculated with reference to air dried sample.

The Petroleum ether and Methanol extracts of *H.Integrifolia* leaves were subjected to the following investigations,

1. Preliminary phytochemical screening.
2. Pharmacological activities
 - a. Determination of acute toxicity (LD₅₀)
 - b. Antidepressant activity

Preliminary phytochemical testing of extracts

The extracts were subjected to following chemical tests to detect the chemical constituents present in them. 0.5 gm of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents [7,8].

Animals

Albino wistar mice of either sex weighing between 20-25g were procured from Central Animal House, Rajah Muthiah Medical College & Hospital, Faculty of Medicine, Annamalai University, Annamalai Nagar- 608002, Tamilnadu, India for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet. Water was allowed ad libitum under hygienic conditions. All animal studies were performed in accordance to guideline of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Central Animal House, Rajah Muthiah Medical College & Hospital, Annamalai University, Tamilnadu, India (CPCSEA registration number- 160/1999/IAEC/ CPCSEA).

Determination of LD₅₀ of Leaf Extract of *Holoptelea Integrifolia* and standard drug Preparation

The acute toxicity of leaf extracts of *H. Integrifolia* was determined by using albino mice of either sex weight between (20-25 g), maintained under standard conditions. The animals were fasted for 3 hr prior to the experiments. Animals were administered with single dose of either Petroleum ether or Methanol leaf extract of *H. Integrifolia* and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. Since no mortality was observed upto dose 2000 mg/kg From the LD₅₀ dose, 100 mg/kg and 300 mg/kg doses were selected and considered as low and high doses respectively. *Fluoxetine* was used as the reference standard drug for evaluating antidepressant activity. *Fluoxetine* suspension was prepared using saline.

Experimental Design

Treatment Schedule

The antidepressant activity of the test drug was evaluated using the following experimental models of depression such as forced swim test (FST) and Tail Suspension Test (TST): Thirty six albino wistar mice with body weights between 20-25g were divided in six groups each consisting of 06 mice. All these mice were subjected to daily treatment for the period of 14 days as follows. One group received vehicle and served as the control group, two groups received petroleum ether extract (PEHI) in a dose of 100 and 300 mg/kg, two groups received methanol extract (MHI) in a dose of 100 and 300 mg/kg and the sixth group received the reference standard fluoxetine as follows:

- Gr 1- Vehicle (10ml/kg)-po
- Gr 2- PEHI- 100 mg/kg-po
- Gr 3- PEHI- 300 mg/kg-po
- Gr 4- MHI- 100 mg/kg-po
- Gr 5- MHI- 300 mg/kg-po
- Gr 6- *Fluoxetine* -10 mg/kg -po

Forced Swim Test (FST)

On the 14th day immediately after administration of last dose, each mouse individually was allowed to swim freely in a transparent glass vessel (25 cm high, 10 cm diameter) filled with 10 cm of water at room temperature for a period of 05 minutes as a pre test session without any recording for any parameters. 24 hours later forced swim test was conducted in the same cylinder for 05 minutes wherein

- Swimming (Active movements of extremities and circling in the cylinder)
- Climbing/Trashing (Active upward directed movements of forelimbs on the container wall)
- Immobility (floating in water without swimming i.e. mice made no further attempts to escape except the movements necessary to keep its head above the water)

Were noted down (using stop watch) as an indices of depression. The water was changed after each test. The increase in active responses, such as climbing/trashing as well as swimming and reduction in immobility were considered as behavioral profiles that indicated an antidepressant like action [9-14].

Tail Suspension Test (TST)

On the 14th day immediately after administration of last dose, each mouse individually was suspended on the edge of the table 50 cm above the floor by adhesive tape placed 01 cm from the tip of the tail for the period of 05 minutes using stop watch and Immobility duration was recorded. Mice are considered immobile when they hanged passively and completely motionless. The principle of this test is that suspending mice suspended upside down leads to characteristic behaviour of immobility which resembles to human depression. The decrease in immobility duration is considered as behavioral profiles that indicates an antidepressant like action [15,16].

Statistical Analysis

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dunnetts test to calculate the significance difference if any among the groups. p<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical Examination of Extracts

Preliminary phytochemical analysis of petroleum ether extract of *Holoptelea integrifolia* Leaves showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and

carbohydrates. Methanolic extract of *Holoptelea integrifolia* leaves showed the presence of steroids, alkaloids, flavonoids, proteins and carbohydrates.

Acute toxicity study

Both the petroleum ether and methanol extracts of *Holoptelea integrifolia* leaves did not produced any sign of toxicity till the oral dose of 2000 mg/kg hence the extracts were used in the range of 100–300 mg/kg orally assuming that LD₅₀ dose is 2000 mg/kg.

Evaluation of Antidepressant activity

Assessment of Antidepressant Activity of *H. Integrifolia* Leaves

Forced swim Test (FST)

It was observed that the PEHI was more significant than MHI and showed dose dependent effect on immobility and climbing behaviour however it didn't show any effect on swimming suggesting role of noradrenergic transmission in its antidepressant activity. The observations are given in table no.1

Table 1: Effect of petroleum ether (PEHI) and methanol (MHI) extracts of *holoptelea integrifolia* leaves on duration of immobility climbing and swimming time in Forced Swim Test (FST) In Mice

Treatment (mg/kg)	Immobility (second)	Climbing (second)	Swimming (second)
Vehicle Control	106.77 ± 4.08	92.52± 2.49	99.03±6.5
PEHI-100	93.20 ± 3.38*	101.30±1.82*	105.48±3.47
PEHI -300	82.91 ± 2.25**	104.41±2.14**	112.66±2.60
MHI-100	104.58 ± 2.87	90.39±2.77	103.35±4.23
MHI-300	92.38 ± 2.81*	99.89±1.66*	107.72±2.70
Fluoxetine -10	78.75± 2.75**	105.20±1.28**	116.03±2.95

Statistical Analysis- one way ANOVA followed by Dunnetts 't' test, *P<0.05, **P<0.01, ***P<0.001

Table 2: Effect of petroleum ether (PEHI) and methanol (MHI) extracts of *holoptelea integrifolia* leaves on duration of immobility in Tail Suspension Test (TST) In Mice.

Treatment (mg/kg)	Immobility (second)
Vehicle Control (10 ml/100 gm)	83.73 ± 2.63
PEHI -100	76.50 ± 1.73*
PEHI -300	70.78 ± 1.70**
MHI-100	77.71 ± 1.26
MHI-300	76.49 ± 1.61*
Fluoxetine -10	71.29 ± 1.53**

Statistical Analysis- one way ANOVA followed by Dunnetts 't' test *P<0.05, **P<0.01, ***P<0.001

Tail Suspension Test (TST)

PEHI was more significant than MHI and showed dose dependent effect on immobility suggesting antidepressant action Although the MHI 100 mg/kg didn't show significant action but the reading are too close to MHI 300 mg/kg and PEHI 100 mg/kg which indicates its antidepressant potential. The observations are given in table no. 2

DISCUSSION

Depression is a common, debilitating, life threatening illness with a high incidence. Numerous antidepressant compounds are now available, which presumably act via different mechanisms involving the serotonergic, noradrenergic and/or dopaminergic systems. Heterogeneity of clinical response to antidepressant and mood-stabilizing drugs and susceptibility to adverse effects are major clinical problems [17]. Therefore, new drugs are still needed for the control of depression-related disorders. The present study has shown that oral administration of the petroleum ether and methanol extracts of *holoptelea integrifolia* leaves were effective in producing significant antidepressant effects in the forced swimming test and tail suspension test in mice, as is evident from the reduction in the immobility time in mice [15]. In FST, mice were forced to swim in a restricted space from which there was no escape, and will, after periods of agitation, cease attempts to escape and become immobile. It is accepted that immobility is seen in rodents during swimming reflects behaviour despair as seen in human depression and that the antidepressant drugs are able to reduce the immobility time in mice [13].

The chronic treatment of the extracts significantly reduced the immobility time however it didn't show any effect on swimming suggesting role of noradrenergic transmission in its antidepressant activity. This shows that the petroleum ether and methanolic extract of the *holoptelea integrifolia* possesses antidepressant activity and its specificity towards particular behaviour may depend on the dose of the extracts. There are reports

to indicate that immobility, swimming and climbing behaviours are enhanced by different groups of antidepressant drugs [18]. The NE-selective uptake inhibitors like desipramine (DMI) and maprotiline (MAP) enhances the climbing behaviour where as the serotonin specific reuptake inhibitors (SSRIs) like fluoxetine (FLX), sertraline (SRT) and paroxetine (PRX) enhance swimming but not climbing behavior. However, both the types of antidepressants reduce immobility behaviour. The tail suspension test has been described by Steru et al [16]. As a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by tail. Also recent studies have shown that the dopaminergic activation is also involved in struggling (climbing) behaviour [18, 19, 20].

CONCLUSION

The present results shows that petroleum ether and methanolic extracts of *holoptelea integrifolia* leaves produced antidepressant like effect as it decreases the immobility time during depression in animal model (FST & TST).

It was found to be similar to that of *fluoxetine*. The petroleum ether extract of *holoptelea integrifolia* was found to be more potent when compared to methanol extract. The antidepressant like effect of the petroleum ether and methanol extracts seems mostly likely to be mediated through an interaction with adrenergic, dopaminergic and serotonergic system.

Thus petroleum ether extract of *holoptelea integrifolia* may have potential therapeutic value for the management of depressive disorders. The phytochemical test results indicate that petroleum ether and methanol extracts of *holoptelea integrifolia* leaves contained flavonoids and phytosterols which might be active in case of Forced swim test (FST) and Tail Suspension Test (TST).

Since beta sitosterol is present in the plant [21]. but this phytochemical is not quantified till so far and this beta-sitosterol has shown central inhibitory and neuromodulatory effects which may also be responsible for showing the observed antidepressant. [22]. activity. Further studies are going on to isolate and quantify the beta sitosteol alongwith finding a new phytosterol for the observed anti-depressant effect.

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