

## Evaluation of antidepressant action of various fractions of methanolic extract of *Terminalia bellirica* roxb in rats

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### ABSTRACT

**Objective:** The present study was aimed to investigate the effect of various fractions of methanolic extract of *Terminalia bellirica* on depression in rat using forced swim test (FST).

**Materials and Methods:** Antidepressant activity was evaluated by Forced swim test. Gallic acid (10, 30, 50, 100mg/kg), Fluoxetine, and Imipramine were administered in separate group of male Sprague rats one hour before the test. Gallic acid equivalent doses of various fractions of methanolic extract of *Terminalia bellirica* has been administered to a particular group of animals. The interference of antidepressant activity with gross motor activity of rats was determined by Open field test.

**Results:** Gallic acid shows a significant decrease in immobility time in dose dependent manner in FST. The efficacy of Gallic acid (50mg/kg) was found to be similar to that of Imipramine (10.5 mg/kg, p.o.) and Fluoxetine (14.5 mg/kg, p.o.). Gallic acid equivalent dose of various fractions (200mg/kg) of methanolic extract of *Terminalia bellirica* showed a significant reduction in immobility time when compared to normal control and was not interfered with gross motor activity.

**Conclusion:** Antidepressant property of various fractions of methanolic extract of *Terminalia bellirica* is due to the presence of Gallic acid. The antidepressant effect of Gallic acid may be through maintaining the level of nor adrenaline, 5-HT, and dopamine because TB extracts reversed reserpine induced depression suggested that antidepressant like effect of TB extracts may be through the restoration of brain monoamines, like nor epinephrine, 5-hydroxy typtamine (5-HT) and dopamine levels.

**Key words:** *Terminalia bellirica* forced swimming test, fluoxetine, imipramine, gallic acid.

### INTRODUCTION

Traditional medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries. India, having a very old and rich tradition of folk medicine for centuries, has provided very simple but effective remedies to various ailments using plants and plant derived compounds<sup>1</sup>. *Terminalia bellirica* fruit is the main constituent of the ayurvedic formulation triphala. *Terminalia bellirica* belonging to the family Combretaceae. Its common name in Sanskrit is *Bibhitakahi*<sup>2</sup>. Studies has been proved that *Terminalia bellirica* fruit has antidepressant, anti bacterial, anti malarial, anti HIV, anti mutagenic, hypolipidemic, immunomodulator, anti diabetic and anti oxidant, and used in kidney and urinary

disorder<sup>3</sup>. Chief constituent of *Terminalia bellirica* fruit is poly phenol. Fruits of *Terminalia bellirica* contains about 17% Tannin, beta sitosterol, Gallic acid, ellagic acid, ethylgallate, chebulagic acid<sup>4</sup>.

In light of above information the present studies have been under taken i) to evaluate the action of various fractions of methanolic extract of *Terminalia bellirica* on depression in rats using forced swim test and ii) and to explore the possible underlying mechanism of antidepressant like effect of the fractions of T.B. Standard antidepressant drugs like Fluoxetine a selective serotonin reuptake inhibitors, Imipramine a tricyclic antidepressant and gallic acid were employed to standardize the animal model of depression and to compare the

antidepressant efficacy of the fractions.

## MATERIALS AND METHODS

### Animals

The study was approved and was carried out as per the guidelines of Institutional Animal Ethical Committee. Male Sprague Dawley rats weighing 250-300 gm were selected for the study. Animals were housed at  $25^{\circ} \pm 5^{\circ}\text{C}$  in a well-ventilated animal house under 12:12 h light dark cycle with a free access to food and water in an animal house approved by CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) (Ref:KCP/IAEC-31/2008-2009).

### Drugs and Chemicals

Methanolic extract of *Terminalia bellirica* roxb fruit (Patch no- PC/PT/0707035) was collected from Natural remedies Pvt Ltd, Bangalore. Fluoxetine and Imipramine (gifted samples from Torrent pharmaceuticals, Ahmadabad), Gallic acid (Merck Specialties Private Limited, Mumbai, India) were used in the present study.

### FRACTIONISATION OF METHANOLIC EXTRACT OF *TERMINALIA BELLIRICA*

The dried methanol extract (100 g) was suspended in 200 ml of distilled water and was extracted successively and exhaustively with solvents in increasing order of polarity<sup>5</sup>. The suspension mixture was extracted with ethyl acetate (200 ml X 3) and ethyl acetate fraction was collected. After extraction with ethyl acetate, the mother liquor was extracted with n-butanol (200 ml X 3) and separated the n-butanol fraction. The remaining mother liquor was the water fraction. Each fraction was freed of solvent by distillation under reduced pressure. The yield of each fraction was recorded viz., ethyl acetate fraction (g), n-butanol fraction (g) and water fraction (g).

### PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF VARIOUS FRACTIONS

Various fractions of methanolic extract of *Terminalia bellirica* was subjected to qualitative analysis to investigate

the presence of various phytochemical constituents like polyphenol, triterpenoides, glycosides, phytosterols, saponins, tannins and flavonoids<sup>6,7</sup>.

### THIN LAYER CHROMATOGRAPHY OF GALLIC ACID<sup>8</sup>

Stationary phase: Precoated silica gel G plate.

Mobile phase : Ethyl Acetate:Toluene: Formic acid (2.2:1.1:1.1)

Visualization : UV at 254 nm, after spraying with  $\text{FeCl}_3$  reagent<sup>8</sup>.

Retention factor ( $R_f$ ) =

$$\frac{\text{Distance traveled by the fractions}}{\text{Distance traveled by the solvent}}$$

Distance traveled by the solvent

### TEST FOR EVALUATING ANTIDEPRESSANT ACTIVITY

#### i) Forced swimming test (FST)

Forced swimming test is a behavioral test used as a screening test for antidepressant drugs. This test composed of two sections: initially pre test for 15 mins followed by a 5 min test section after 24 hours. In the first session (pretest) animals were individually placed in a glass tank (46 cm height and 20 cm in diameter) of 210 to 220°C water filled to a depth of 30 cm. The water depth 30 cm allowed the rat to swim or float to the surface without touching the hind limb to the bottom of the tank. Upon removal animals were towel dried before returning to the cage for maintaining body temperature. In the next session 24 hrs after pretest, animals were administered the drugs. 90 min after drug administration, rats were again placed in to the water tank and the time of immobility was determined. Immobility was defined as the time a rat remained floating in the water making only those movements necessary to keep its head above the water or floating motionless. After testing of each animals water of glass tank was replaced with the fresh water<sup>9</sup>.

#### ii) Gross motor activity

Gross motor activity was assessed in a chamber having a floor measuring 107×107cm (40×40 in) with wall 50 cm in height. The wall of the chamber was

Table 1. Drug protocol

Group No:	Treatment
I	Normal control (Administered only distilled water)
II, III, IV, V	Gallic acid (10 mg, 30 mg, 50 mg, 100 mg) respectively were administered orally for successive 10 days. At 90 mnts after the administration on 10 days, immobility period was recorded.
VI, VII	Fluoxetine((14.5 mg/kg, p.o.), Imipramine ((10.5 mg/kg, p.o.) ) respectively were administered orally for successive 10 days. At 90 mnts after the administration on 10 days, immobility period was recorded
VIII, IX, X,	n- Butanol fractions of methanolic extract of <i>T. bellirica</i> ( 50mg/kg,100mg/kg, 200mg/kg) respectively were administered orally for successive 10 days. At 90
XI, XII, XIII	mnts after the administration on 10 days, immobility period was recorded Ethyl acetate fractions of methanolic extract of <i>T. bellirica</i> ( 50mg/kg,100mg/kg, 200mg/kg) respectively were administered orally for successive 10 days. At 90
XIV,XV, XVI	mnts after the administration on 10 days, immobility period was recorded Water fractions of ofmethanolic extract of <i>T. bellirica</i> (50mg/kg, 100mg/kg, and 200mg/kg) respectively were administered orally for successive 10 days. At 90 mnts after the administration on 10 days, immobility period was recorded

painted black and floor white. Black line divided the white floor in to 25 square of equal size. This chamber was housed in a quiet, darkened testing room and was illuminated by a 25 watt bulb positioned above approximately 90 cm above the corner of the floor. The animals were brought to the testing room and placed the animal exactly at the centre of the square facing away from the observer who sat on a stool positioned above one corner of the field so as to see the entire movement of the animal. An animal was considered to have entered a square when it moved all four limbs across the boundary in to that square. Smaller movements of turning, spinning, or probing with the head or forelimbs were not counted as entering the square. The open-field test lasted 10 min. At the end of the test, the animal was removed and the observer noted whether it had urinated and counted the number of fecal boli deposited. The floor of the open field was then cleaned thoroughly and washed with a dilute acetic acid solution in preparation for the next animal<sup>10</sup>.

#### Drug protocol

Animals were divided in to 13 groups

and each group comprised a minimum 8 animal each.

#### Statistical Analysis

All the data were expressed as mean±SEM. Significance of difference between two groups was evaluated using Student's t-test. For multiple comparisons, one-way analysis of variance (ANOVA) was used. When ANOVA showed significant differences, post hoc analysis was performed with Tukey's test

#### RESULT

##### FRACTIONISATION OF METHANOLIC EXTRACT OF *TERMINALIA BELLIRICA*

Three fractions of methanolic extract of *Terminalia bellirica* have fractionized out ie. n-butanol fraction, Ethyl acetate fraction and finally water fraction.

##### PRELIMINARY PHYTOCHEMICAL INVESTIGATION

Phytochemical test revealed the presence of polyphenol, triterpenoides, glycosides, phytosterols, saponins, tannins and flavonoids in various fractions.

**THIN LAYER CHROMATOGRAPHY (TLC) OF GALLIC ACID**

Thin layer chromatography was done for each fraction and detected the presence of gallic acid in each fraction.

**QUANTITATIVE ANALYSIS BY HPLC**

By HPLC method particular quantity of gallic acid in each fraction has to be calculated.

Table 2. Quantitative analysis of gallic acid in different fractions by HPLC

S.no	Fractions	Result(% of gallic acid)
	Ethyl acetate fraction	14.0%
	n-butanol fraction	24.9%

**ANTIDEPRESSANT ACTIVITY**

**Effect of various doses of gallic acid in forced swimming test (In normal rat):**In forced swim test various doses of gallic acid (10mg/kg, 30mg/kg, 50mg/kg, 100mg/kg) has showed significant decrease in immobility time in dose dependent manner when compared to normal. The immobility time produced by gallic acid 10mg/kg, 30mg/kg, 50mg/kg, and 100mg/kg were found to be 33.04, 21.44, 12.49, and 24.04 sec respectively. The dose 50mg/kg was found to be more significant ( $p < 0.01$ ) when compared to normal control and its efficacy was found to be similar to Fluoxetine, and Imipramine (54.33sec Figure 1)

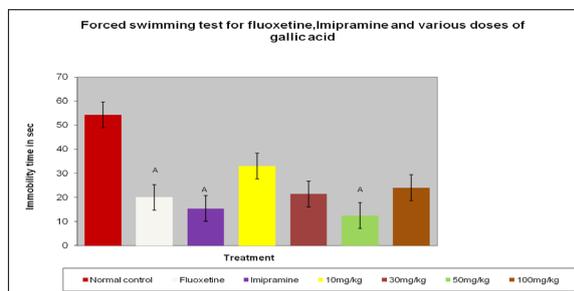


Figure 1. Forced swimming test for fluoxetine, Imipramine and various doses of gallic acid

**Effect of various doses of fractions of methanolic extract of Terminalia bellirica (n-butanol, ethyl acetate, and water fraction) in normal rats:** All three fraction of methanolic

extract of *Terminalia bellirica* have shown dose dependent decrease in immobility time when compared to normal. 200mg/kg of each fraction showed extremely significant antidepressant activity when compared to normal control (Figure 2).

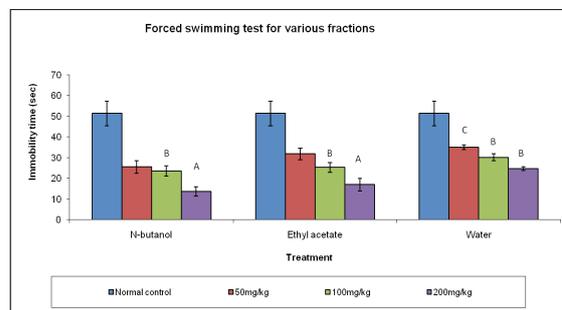


Figure 2. Forced swimming test for various fractions

**GROSS MOTOR ACTIVITY**

Gallic acid (50mg/kg) and 200mg/kg of n-butanol, ethyl acetate, and water fraction treated animals were did not significantly interfere with gross motor activity (Figure 3).

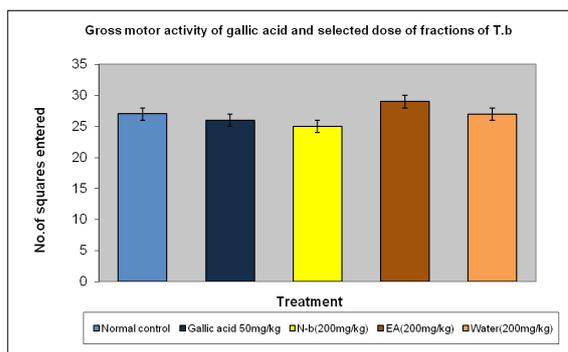


Figure 3. Gross motor activity of gallic acid and selected dose of fractions of T.b.

All values are mean  $\pm$  SEM,  $n=8$ ,  $P < 0.001A$  when compared to normal control group (Fig.1). All values are mean  $\pm$  SEM,  $n=8$ ,  $P < 0.05C$   $P < 0.01B$ ,  $P < 0.001A$  when compared to normal control group. (Fig.2. All values are mean  $\pm$  SEM,  $n=8$ ,  $P < 0.05A$ ,  $P < 0.01B$ ,  $P < 0.001D$  when compared to normal control group (Figure 3).

**DISCUSSION**

In the present study standard Gallic acid (50mg/kg) produce significant antidepressant activity when compared to normal rats and the efficacy was found to be similar to Fluoxetine and Imipramine.

Among the various doses of fractions of methanolic extract of *Terminalia bellirica* dose 200mg/kg shows significant antidepressant activity. In FST rats are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression.

The selected dose of Gallic acid and various fractions of methanolic extract of *Terminalia bellirica* did not show significant change in locomotor functions of rats as compared to control so they did not produce any locomotor effect. It confirms the assumption that the antidepressant like effect of the fractions is specific. The precise mechanism by which fractions of TB extract produced antidepressant like effect are not completely understood.

*Terminalia bellirica* roxb fruit (TB) extracts may produce anti-depressant like effect by increasing the levels of norepinephrine, dopamine and serotonin levels in the brain of mice. p-CPA (serotonin synthesis inhibitor) significantly reversed the antidepressant effect of fluoxetine (specific serotonin reuptake inhibitor) in TST, suggesting that fluoxetine has antidepressant effect through serotonergic system. Reserpine, an antihypertensive drug that depleted neuronal storage granules of norepinephrine, serotonin and dopamine, produced clinically significant depression in 15% or more of patients<sup>11</sup>. Reserpine produced significant increase in immobility period 4 and 24 hrs of treatment when tested in TST. TB extracts reversed reserpine induced depression, as indicated by decrease in extension of immobility period in TST, suggests that antidepressant like effect of TB extracts may be through the restoration of brain monoamines, like norepinephrine, 5-hydroxy typtamine (5-T) and dopamine levels. The extracts of TB fruit have antioxidant activity due to the presence of phenolic compounds, particularly gallic acid. There is evidence of dearrangement of oxidant and antioxidant system in depression. Thus antidepressant like

activity of TB extracts might be due to its antioxidant property<sup>8</sup>.

## CONCLUSION

Preliminary studies have proved that extract of *Terminalia bellirica* fruit have antidepressant activity and hence N-butanol, ethyl acetate, and water fractions of methanolic extract of *Terminalia bellirica* have shown significant antidepressant activity when compared with standard drugs like gallic acid, fluoxetine and Imipramine. There is evidence of dearrangement of oxidant and antioxidant system in depression. Antidepressant like activity of TB extracts might be due to its antioxidant property. TB extracts reversed reserpine induced depression, as indicated by decrease in extension of immobility period in TST, and suggests that antidepressant like effect of TB extracts may be through the restoration of brain monoamines, like norepinephrine, 5-hydroxy typtamine (5-HT) and dopamine level and that depends upon the amount of Gallic acid present.

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