# Original Article:
# Neurochemical Modulating Effect of *Boswellia serrata* Roxb. ex Colebr: A Preclinical Research

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## ABSTRACT

**Background:** *Boswellia serrata* has been known for many decades and mentioned in the ancient Ayurvedic texts. Many previous studies have demonstrated its role in depression and anxiety in animal models.

**Objectives:** The present study is carried out to evaluate the effect of *Boswellia serrata* on neurotransmitter levels of Swiss albino mice by spectrophotometer.

**Methods:** Eighteen (n=18) Swiss albino male mice were procured for this study. All mice were divided into three groups of six mice in each. The first group of mice (control) received normal saline (10 mg/kg); the second group (standard) received imipramine (10 mg/kg), and the third group (test) received *Boswellia serrata* (100 mg/kg) orally for 21 days. On the 22nd day, all mice were sacrificed as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. The mice brains were dissected, and their brain tissue was collected and stored in a preservative. The mice brain tissue was centrifuged, and samples were used for the estimation of serotonin (5-HT), Acetylcholinesterase (AChE), dopamine, Gamma-Aminobutyric Acid (GABA), and glutamate levels by spectrophotometry.

**Results:** The levels of neurotransmitters are expressed in Mean±SE. Analysis of results was done by 1-way ANOVA and Tukey Kramer tests. The statistical tests revealed that imipramine-treated mice have significantly increased the levels of AChE, GABA, and glutamate when compared to control ($P<0.05$). However, imipramine treated group showed statistically significant lower levels of 5-HT and dopamine levels when compared to the control ($P<0.05$). Similarly, the test drug *Boswellia serrata*-treated group had significantly higher levels of 5-HT, AChE, GABA, and glutamate when compared to the control group ($P<0.05$) and lower levels of dopamine when compared to the control ($P<0.05$).

**Conclusion:** The present study establishes the role of *Boswellia serrata* in various psychiatric disorders like depression and anxiety in animal models by modulating multiple neurotransmitters in the brain.

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## Introduction

The nervous system is the key system to control most body functions. The human brain is the main integral part of the nervous system, which not only receives sensory inputs from the internal organs but also from the external environment. Once the interpretation of sensory signals is made, precise responses are produced based on these signals. These nervous system functions are mainly carried out by endogenous chemical mediators called neurotransmitters. The neurotransmitters are helpful in the transmission of nerve impulses from the site of origin to the destination site. Neurotransmitters are broadly classified as excitatory and inhibitory neurotransmitters based on their action on neurons [1].

Glutamate is the main excitatory neurotransmitter in the central nervous system involved in cognitive functions, such as learning and memory. However, continuous excitation of a neuron can be dangerous, causing neurotoxicity, which may end up in stroke, epilepsy, amyotrophic lateral sclerosis, Huntington's disease, Parkinsonism, and Alzheimer disease [2].

Gamma-Aminobutyric Acid (GABA) is the major inhibitory neurotransmitter in the central nervous system with a significant role in anxiety disorders, and many sedative-hypnotics act by facilitating GABA action [3]. Many other neurotransmitters have excitatory and inhibitory actions, such as acetylcholine, serotonin, and dopamine. Acetylcholine (ACh) exerts its activity in both the central and peripheral nervous system. It is mainly associated with memory and learning. ACh is quickly metabolized by the Acetylcholinesterase (AChE) enzyme, which has ubiquitous distribution. Several studies have demonstrated reduced AChE activity in brain specimens of patients suffering from Alzheimer disease [4].

Serotonin is required to regulate mood, sleep, appetite, anxiety, and sexual behavior. Drugs like tricyclic antidepressants and selective serotonin inhibitors increase serotonin levels in the brain and are effective in treating depression, anxiety, and panic disorders [5].

Dopamine plays an important role in reward function, motivation, and body movements. Many illicit drugs increase the dopamine level in the brain, causing addiction and sometimes psychosis. Parkinsonism, on the other hand, occurs due to the degeneration of dopaminergic neurons in the basal ganglia [6, 7].

The therapeutic role of dried resinous gum (Guggulu) from *Boswellia serrata* has been known for many decades and mentioned in the ancient Ayurvedic texts-Sushruta Samhita and Charaka Samhita. Many previous studies have demonstrated its role in depression and anxiety in animal models [8]. However, the actual mechanism of *Boswellia serrata* is still not known. So this study was carried out to establish its role in neurotransmitter levels in the brain.

## Materials and Methods

### Study animals

The study was started after the approval of the Institutional Animal Ethical Committee (IAEC) clearance from Yenepoya University, Mangalore, Karnataka, India. A total of 18 (n=18) healthy male Swiss albino mice (3-4 months old, weighing 25-35 g) were procured for the study. The mice were inbred in the central animal house of our institution under suitable conditions of housing, temperature, ventilation, and nutrition. The study was conducted as per standard CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

### Drugs

The pure form of the test drug, *Boswellia serrata*, was purchased from Natural Remedies, Bangalore. The dose of *Boswellia serrata* used in the present experiment was based on previous studies [8]. The standard drug, imipramine (pure form), was obtained from Torrent Pharmaceutical Company, Ahmadabad. The test and standard drugs were dissolved and diluted in normal saline to get desired concentration. Normal Saline (NS) was purchased from our institutional pharmacy.

### Experimental design

Eighteen Swiss albino male mice were procured and divided into 3 groups of six each. Mice were weighed, and an appropriate drug dose was given to the different groups orally. The mice in the control group received normal saline (10 mg/kg), the standard group received imipramine (10 mg/kg), and the test group received *Boswellia serrata* (100 mg/kg) orally for 21 days. On the 22$^{nd}$ day, all the mice were sacrificed as per CPCSEA guidelines. The mice brain was dissected, and brain tissue was collected and stored in preservative and used for the estimation of Acetylcholinesterase (AChE), serotonin (5-HT), dopamine, Gamma-Aminobutyric Acid (GABA), and glutamate levels by spectrophotometry.

## Estimation of Acetylcholinesterase (AChE) enzyme

AChE enzyme activity was estimated as per the method described by Ellman et al. [9]. After the euthanasia, mice brains were removed and stored in ice-cold saline. The frontal cortex, hippocampus, and septum are dissected on a Petri dish and chilled on crushed ice. The tissue samples were weighed and homogenized in 0.1 M phosphate buffer (pH 8). Then, 0.4 mL aliquot of the homogenate was added to a cuvette containing 2.6 mL phosphate buffer (0.1 M, pH 8) and 100 µL of DTNB (5,5'-dithio-bis-[2-nitrobenzoic acid]). The contents in the cuvette were mixed thoroughly by bubbling air, and absorbance was measured at 412 nm in the spectrophotometer. When absorbance reaches a stable value, it is noted as the basal reading. Afterward, 20 µL of acetylcholine is added, and the change in absorbance per minute is determined.

Calculations: The AChE enzyme activity is calculated using the following formula:

$$Acetylcholinesterase\ activity\ (M/mL) = \frac{A/min \times Vt}{\varepsilon \times b \times Vs}$$

, where A/min is the change in absorbance/minute, ε refers to $1.361 \times 10^4\ M^{-1}cm^{-1}$, b denotes path length (1 cm), Vt is the total volume (3.1 mL), and Vs is the sample volume (0.4 mL). The final reading of enzyme activity is expressed as µmoles/min/mg.

## Estimation of serotonin (5-HT)

The method followed for the estimation of serotonin in the mouse brain was as described by Schlumpf [10]. First, 0.2 mL of mouse brain extract was taken, and 0.25 mL of O-phthaldialdehyde (20 mg in 100 mL conc. HCL) was added. The fluorophore was developed by heating to 100°C for ten minutes. When the samples reached equilibrium with the ambient temperature, readings were taken at 360-470 nm using a spectrophotometer. For serotonin tissue blank, 0.25 ml cont. HCL without O-phthaldialdehyde was added. The internal standard-serotonin (500 µg/ml) was synthesized in distilled water: HCL-butanol in a 1:2 ratio.

## Estimation of glutamate and GABA

About 1.0 mL of the brain supernatant homogenate was evaporated to dryness at 70°C in an oven, and the residue was reconstituted in 100 mL of distilled water. The standard solutions of glutamate (2.942 mg in 10 mL distilled water) and GABA at the concentration of 2 mM along with the sample are spotted on Whatman No.1 chromatography paper using a micropipette. The paper was placed in the chamber containing butanol:acetic acid:water (12:3:5 v/v) as solvent. When the solvent front reached the top of the paper, it was removed and dried. A second run was performed again. Then, the papers were dried and sprayed with 0.25% ninhydrin reagent and placed in the oven at 100°C for four minutes. The portions which contain glutamate corresponding with the standard are cut and eluted with 0.005% $CuSO_4$ in 75% ethanol. Their absorbance is read against blank at 515 nm in the spectrophotometer.

Calculation:The glutamate and GABA levels are calculated by using the following formula:

$$A = \frac{Unknown\ OD \times Standard\ in\ mg\ (3\ \mu g) \times 1000}{Standard\ OD \times Volume\ spotted\ (10\ \mu L/) \times W}$$

, where A refers to amino acid values in µmoles per gram wet weight tissue, 1000= conversion factor, and W is the weight of the tissue in grams.

## Estimation of dopamine

The mice brain tissue was weighed and homogenized in HCl-butanol for about one minute (in 1:10 ratio). The sample was centrifuged for 10 minutes at 3000 rpm. An aliquot supernatant phase (1 mL) was removed and transferred to a centrifuge tube containing 2.5 mL hexane and 0.3 mL of 0.1 M HCl. The aqueous phase (0.2 mL) was then taken to estimate dopamine. All steps were carried out at 0°C (on ice). To 0.2 mL of aqueous phase, 0.05 mL 0.4 M HCl and 0.1 mL of sodium acetate buffer (pH 6.

**Table 1.** Levels of neurotransmitters in mice brain tissues in the treatment groups

| Groups | 5-HT levels [U/g tissue×$10^3$] | AChE [mol/mL×$10^{-5}$] | Dopamine [U/g tissue×$10^3$] | GABA [µmole] | Glutamate [µmole] |
|---|---|---|---|---|---|
| I. Control (Normal saline, 10 mL/kg) | 10.335±0.06 | 2.492±0.03 | 12.445±0.03 | 221.686±0.56 | 155.17±0.35 |
| II. Standard (Imipramine, 10 mg/kg) | 7.513±0.04 [a] | 3.44±0.05 [a,c] | 2.133±0.04 [a] | 331.61±0.36 [a] | 262.98±0.33 [a] |
| III. Test (*Boswellia serrata*, 100 mg/kg) | 17.30±0.09 [b] | 3.363±0.04 [a] | 6.57±0.064 [b] | 278.72±0.47 [b] | 177.22±0.12 [b] |

Values are expressed as Mean±SE; n=6, One way ANOVA followed by Tukey Kramer test.

[a]P<0.001 comparing II with I and III groups; [b]P<0.001 comparing III with I and II groups; [c]P<0.05 comparing II with III groups.

9) were added followed by 0.1 mL iodine solution (0.1 M in ethanol) for the oxidation. The reaction was stopped after two minutes by adding 0.1 mL sodium sulfite solution. Then, 0.1 mL acetic acid is added after 1.5 minutes. The entire solution was heated to 100°C for six minutes. When the sample reached room temperature, excitation and emission spectra were read from the spectrophotometer at 330-375 nm. Tissue blanks for dopamine estimation were prepared by adding the reagents of the oxidation step in reversed order (sodium sulfite before iodine)

Calculation: The dopamine level is calculated using the following formula:

*X dopamine=(Sample O.D – Blank O.D) X [Conc. of Standard (500 µg/mL) O.D – Blank O.D]*

It gives the dopamine level present in 1 mL of the sample. The final reading of dopamine level is expressed as µmoles/g tissue.

### Statistical analysis

The neurotransmitter levels are expressed in Mean±SE. Results are analyzed by 1-way ANOVA followed by the Tukey Kramer test. For all tests, a $P<0.05$ is considered significant.

## Results

Table 1 depicts the levels of neurotransmitters in mice brain tissues of various treatment groups. The mice group treated with normal saline (control) recorded 10.335±0.06 U/g of 5-HT, 2.492±0.03 Mol/mL of AChE, 12.445±0.03 U/g of dopamine, 221.686±0.56 µmoles of GABA, and 155.17±0.35 µmoles of glutamate levels. The imipramine treated group recorded values of 7.513±0.04 U/g for 5-HT, 3.44±0.05 Mol/mL for AChE, 2.133±0.04 U/g for dopamine, 331.61±0.36 µmoles for GABA, and 262.98±0.33 µmoles for glutamate. Similarly, *Boswellia serrata* 100 mg/kg treated group showed 17.30±0.09 U/g of 5-HT, 3.363±0.04 Mol/mL for AChE, 6.57±0.064 U/g for dopamine, 278.72±0.47 µmoles of GABA, and 177.22±0.12 µmoles for glutamate levels. Statistical tests revealed that imipramine-treated mice have significantly increased AChE, GABA, and glutamate levels compared to the control ($P<0.05$). However, imipramine treated group showed statistically significant lower levels of 5-HT and dopamine levels when compared to the control ($P<0.05$). Similarly, the test drug *Boswellia serrata*-treated group (100 mg/kg) has significantly higher levels of 5-HT, AChE, GABA, and glutamate but lower dopamine level when compared to the control ($P<0.05$).

## Discussion

Most neuropsychiatric and even degenerative disorders of the brain result from imbalances in the neurotransmitters. More than 200 neurotransmitters have been identified in the human brain, and research is still ongoing. However, ACh, serotonin, dopamine, GABA, noradrenaline, and glutamate are the key neurotransmitters that are involved in many disorders of the brain. So in the present study, we attempted to measure levels of various neurotransmitters in mice brains.

*Boswellia serrata* is no more a neglected drug. Numerous clinical trials are going on to elucidate the safety and effectiveness of *Boswellia serrata* in disease conditions like osteoarthritis, bronchial asthma, irritable bowel syndrome, cerebral edema, Crohn's disease, and even in psoriasis [11, 12].

Only a few studies were carried out on the effect of *Boswellia serrata* on neurotransmitter levels, especially on AChE. We did not find any previous studies on the effect of *Boswellia serrata* on various neurotransmitter levels like 5-HT, dopamine, GABA, and glutamate.

Rajib Paul and Anupom Borah found reduced levels of AChE activity in all regions of hypercholesterolemic mice brain with cognitive impairment [4]. The reduced brain AChE activity is evident in many neurological conditions, including neurodegenerative diseases, mainly in Alzheimer and Parkinson diseases [13, 14].

Nemat AZ Yassin et al. concluded that *Boswellia serrata* increases ACh level and reduces AChE activity in rat brain tissue. Our study findings are contradictory to the results of this study. We have found an increase in the levels of AChE activity in *Boswellia serrata* treated mice [15].

## Conclusion

The present study establishes the role of *Boswellia serrata* in various psychiatric disorders like depression and anxiety in animal models by modulating multiple neurotransmitters in the brain. Possibly, *Boswellia serrata* can be an alternative herbal drug to conventional ones for the above conditions if subjected to clinical trials in the near future.

## Ethical Considerations

### Compliance with ethical guidelines

The study protocol was approved (YU/IAEC/2-3/2013) by the Institutional Animal Ethics Committee of Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore.

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### Authors' contributions

All authors contributed equally to preparing, conducting, and analyzing this study.

### Conflict of interest

The authors declared no conflict of interest.

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