

Antidepressant Activity of Different Fractions of *Cassine albens* in an Olfactory Bulbectomized Mouse Model

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ABSTRACT

Background: Tribal peoples in north Maharashtra used *Cassine albens* for a range of illnesses, including some psychosomatic conditions. For treatment, these indigenous people employed a very raw form of this plant. The goal of this study is to test the traditional claims of antidepressant effect in an olfactory bulbectomized mice using a chloroform and ethyl acetate fraction of Methanolic extract of *C. albens*. **Materials and Methods:** For 28 days, olfactory bulbectomized mice were administered Ethyl acetate and Chloroform fractions of Methanolic extract of leaf of *Cassine albens* (200mg/kg and 400mg/kg). The forced swim test, open field test, and splash test were used to assess antidepressant efficacy. Throughout the study, imipramine (10 mg/kg) was used as a reference standard. **Results:** After a 28-day treatment, chloroform and ethyl acetate diminish hyperexcitation in an open field apparatus by reducing the number of lines crossing. When compared to the control group, the Chloroform fraction 400 mg/kg and 200 mg/kg demonstrated a significant ($p < 0.0001$ and $p < 0.05$) decrease in hyperactivity. In the FST, Chloroform fractions 200 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.01$) significantly reduced immobility time when compared to the control group, and in the Splash test, Chloroform fraction 400 mg/kg significantly increased grooming time ($p < 0.001$) when compared to the control group. **Conclusion:** *Cassine albens* chloroform fractions (200 mg/kg and 400 mg/kg) have considerable antidepressant effect.

Keywords: Olfactory bulbectomy, Splash test, Immobility period, Open Field, Forced swim test, number of line crossing.

INTRODUCTION

Natural goods that work on the brain and brain diseases have a long and well-documented history, and these naturally active chemicals are used to treat cognitive, psychotropic, mood, anxiety, and personality problems. *Cassine albens* (R.) Kosterm, locally known as Bhutekes or Bhutyapala, is a plant used in traditional medicine by tribal communities in north Maharashtra, particularly in the Nandurbar district.¹ Local tribal people have historically employed this plant for sternulatory and fumingatory purposes in hysteria² headache, common cold, and psychosomatic disorders.³ Watery exudation from the trunk is utilised to cure red eyes caused by conjunctivitis.⁴ The leaf paste is used to treat eruptions, wounds, and cuts on the skin, It has the potential to exhibit antimicrobial and wound-healing properties.⁵ Previously, Patil *et al.* 2011

investigated the effects of a hydro alcoholic extract of stem bark of *C. albens* in an animal model of depression and anxiety.⁶ In this study the chloroform and ethyl acetate fractions of Methanolic extract of leaves were investigated in an animal model of depression caused by olfactory bulbectomy. In open field tests, olfactory bulbectomized (OBX) mice show increased exploratory and locomotor behaviour, increased immobility time in the despair test (FST), and grooming behaviour in the splash test, all of which are depression-like behavioural abnormalities. In addition to behavioural changes, OBX altered neurochemical, neuroendocrinological, and neuroimmunological balance, including changes in monoaminergic, GABAergic, and glutamatergic neurotransmissions in the brain, hyperactivity of the HPA-axis, increased inflammatory cytokines,

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and decreased endogenous antioxidant enzyme levels. As a depression model, OBX's subsequent symptoms suggest that it has considerable face and construct validity.^{7,8}

METHODS AND MATERIALS

Animals

Swiss albino mice of either sex (20-30 g) were procured from the R.C. Patel Institute of Pharmaceutical Education and Research's Central animal house facility in Shirpur. 651/PO/ReBi/S/02/CPCSEA is the CPCSEA registration number. All of the animals were kept in normal polypropylene cages (at a temperature of 25 ± 0 °C, with a 12:12 hr Light/Dark cycle). The rodents were offered a conventional rat pellet diet (Nutrivet Lifesciences, Pune) as well as free access to water. IAEC accepted all of the study's experimental techniques and protocols (Protocol Approval Number: IAEC/RCPIPER/2017-18/10).

Chemicals

Sigma Aldrich Ltd provided the imipramine hydrochloride. Loba chemical ltd provided Methanol, Chloroform, and Ethyl acetate, while practising veterinarians provided Xylazine (Briliant BioPharma) and Ketamine (Themis medicare).

Phytochemical Screening and Extraction Procedure

In December 2017-January 2018, leaves of *Cassine albens* were collected in Leghapani village (Taluka- Dhadgaon; Dist- Nandurbar, Maharashtra) and the plant specimen was authenticated at SSVP'S college, Dept. of Botany, in Dhule. The leaves was washed with water, air dried for about one week, and was ground into a coarse powder. The powdered substance was extracted twice, first with Petroleum Ether (60:80) and subsequently with Methanol using soxlet continuous extractor. For fractionation, 10 gm of dried methanolic extract was triturated with water and kept in a separating funnel with Chloroform and subsequently Ethyl acetate. During fractionation, the separating funnel was shaken every now and then. After 72 hr, the chloroform and ethyl acetate fractions were separated and evaporated separately to produce a dried solid fraction.

Antidepressant activity: 2.5

Olfactory Bulbectomy in Mice

The surgery for bilateral olfactory bulbectomy was carried out according to the protocols previously described by.^{8,9} In a nutshell, mice were sedated with xylazine (6 mg/kg i.p.) and ketamine (80 mg/kg i.p.) diluted in saline (0.9 percent NaCl), and bregma was identified by making a midline longitudinal incision with a sterile scalpel blade, set the coordinates to AP= 4mm, ML= 1mm, and DV= 2mm. drill the selected ML- coordinates (using drill pointer- 1mm) and seal the burr hole with dental cement. Remove and place the mouse in a clean, warm cage until the anaesthetic wears off.¹⁰⁻¹⁴

Open Field Test

The arena was made up of nine 12 × 7 cm squares with black and white markings. The cage measured 37 cm in length, 25 cm in breadth, and 15 cm in height. Individual animals were placed in the Open field apparatus's central position and free to explore. During a 5-min test, the number of line crossings was recorded. In the OFT apparatus, a video tracking system based on ANYMAZE software (6.1.) was used to capture and evaluate mouse movement.¹⁵

Forced Swim Test

Individual mice were made to swim in a cylinder (20 cm height and 14 cm diameter) filled with fresh water at a height of 15 cm and kept at a temperature of 25°C. One day before the study, a 15-min pre-test session was held. The mice were subjected to the identical conditions again twenty-four hours later. Each animal adopts an inert posture after the first two minutes of vigorous locomotion. Mice was consider immobile when it just floats on the water surface without struggling, with only the smallest motions of its limbs required to maintain its head above water. During the 6-min test session, the immobility period was recorded using the ANYmaze Video Tracking system.¹⁶⁻¹⁷

Splash Test

The splash test was performed as described by Isingrini *et al.*¹⁸ after 24 hr following the last administered dose, with slight adjustments as previously performed.¹⁹⁻²⁰ On the dorsal fur coat of each mouse a 10% sucrose solution was sprayed and each mouse was kept individually in clear plexiglass cylinders. For a period of 5 min, the onset time of grooming and total grooming time were recorded. When the viscosity of the sucrose solution dirties the mouse hair, the animals begin grooming, which is used as a self-cleanness indicator. Mice's unusual grooming habit is thought to be an indicator of self-care and motivating behaviour. Grooming behaviour was reduced in animals suffering from moderate forms of depression.²¹

Statistical Analysis

Statistically analysis was done by using a one-way ANOVA with Dunnett's multiple comparison post-hoc tests and compared to the negative controls using GraphPad Prism 7 (USA) software. $P < 0.05$ was consider to be significant.

Result: 2.6

Phytochemical Preliminary Test

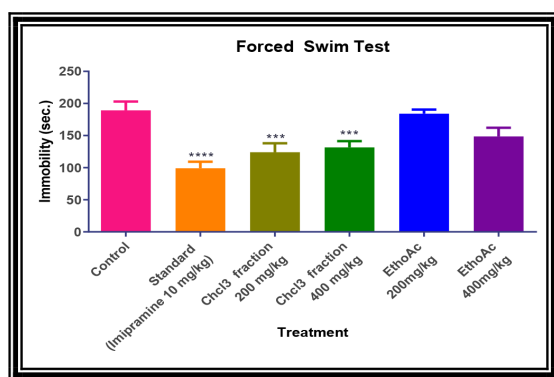
The chloroform and ethyl acetate fractions were found to be high in alkaloids, tannins, Triterpenoids, and steroids in a preliminary phytochemical screening.

Sl. no.	Plant constituents	Chloroform Fractions of CA	Ethyl acetate fraction of CA
1	Terpenoids	++	++

2	Steroids	+	+
3	tannins	-	+
4	Alkaloids	++	+

Effect of Chloroform and Ethyl Acetate Fractions of *C. albens* in Forced swim test

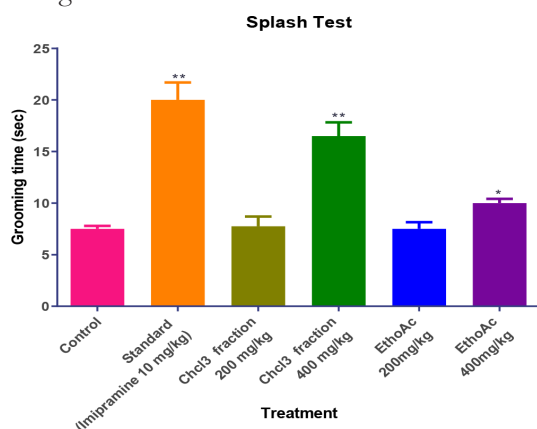
The immobility period was significantly reduced in Chloroform fractions 200 mg/kg ($P < 0.01$) and 400 mg/kg ($P < 0.001$) as compared to the control group. Imipramine hydrochloride (10 mg/kg p.o.) was employed as the reference standard drug in our study ($P < 0.0001$)



The data was analysed using a one-way ANOVA followed by a Dunnett post-hoc test and expressed as mean \pm S.E.M. In comparison to the control group, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ were significant.

Effect of Chloroform and Ethyl Acetate Fractions of *C. albens* in Splash Test

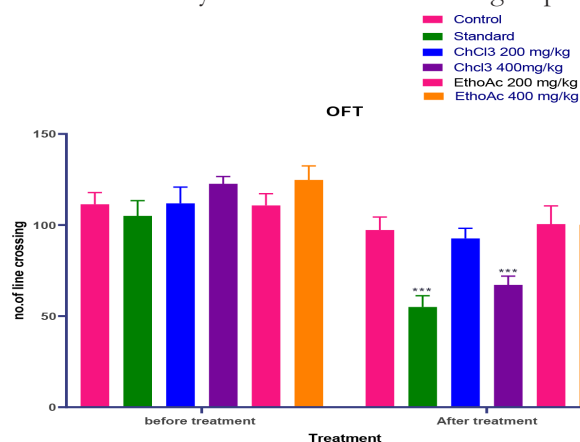
Grooming behavior in splash test was considered as self care and positive motivation. In this study Chloroform fraction (400mg/kg) was significantly increases ($P < 0.001$) grooming time as compared to control group. Ethyl acetate 400 mg/kg also showed slight increase in grooming time. Imipramine (10mg/kg) was most significantly ($P < 0.001$) showed increase in grooming time.



The data were analysed using a one-way ANOVA followed by a Dunnett post-hoc test and expressed as mean S.E.M. In comparison to the control group, * $P < 0.05$, ** $P < 0.01$ were found significant.

Effect of Chloroform and Ethyl Acetate Fractions of *C. albens* in Open Field Test

The open field test was used to investigate the effect of fractionated compounds on hyperactivity before and after treatment with chloroform and ethyl acetate fractions. In an open field test on the 15th day after surgery, hyperactivity in OBX animals was considerably higher than in the control group. When compared to the Control group, there was a substantial ($P < 0.0001$ and $P < 0.05$) decrease in hyperactivity and locomotion after 28 days of treatment with imipramine hydrochloride (10 mg/kg p.o.), Chloroform fraction 400 mg/kg and 200 mg/kg. No significant decrease in hyperactivity was observed in ethyl acetate fraction treated group.



The data were analysed using a Two-way ANOVA followed with Bonferroni's multiple comparison post-hoc test and expressed as mean S.E.M. *** $P < 0.001$, as compared to control group.

DISCUSSION

Depression is a symptomatic complicated disease with a wide range of clinical symptoms. Some symptom changes, such as psychomotor activity, sleep, and appetite changes, may even go in the opposite direction.²²⁻²³ The goal of this study was to evaluate the antidepressant activity of the chloroform and ethyl acetate fractions of *Cassine albens* Linn in an olfactory bulbectomized mouse model. This study revealed that both fractions (Chloroform and Ethyl acetate) of *Cassine albens* have antidepressant activity potential.

The FST was regarded as a classic animal model for assessing antidepressant activity. When an animal was forced to swim in a restricted space from which it could not escape, its immobility time was measured. When the animal is entirely helpless, it adopts a normal body posture and floats on the water's surface with very little hindlimb movement. This position is similar to the Depressive state in people when the animal remains absolutely immobilised. After an initial period of vigorous activity, mice forced to swim in a restricted space with no way out assume a typical immobile posture, moving only when necessary to maintain their heads above water. The animals' immobility was described as indicating that they had realised that escape was not possible and had taken an

immobile stance to conserve energy, anthropomorphically as though they had given up hope of escaping this awful situation. In the open field paradigm, changes in neurotransmitter levels in the brain of OBX-mice showed significant hyperactivity and increased immobility time in the forced swim test. In this work, OBX-mice had a substantially longer immobility time in the forced swim test than the control group. In a forced swim test, chloroform and ethyl acetate fractions (200 mg/kg) and (100 mg/kg); as well as imipramine significantly reduced immobility time. The obtained results are consistent with those of the prior study.²⁴

Hyperactivity is the most meaningful behavioural measure for bulbectomized animals in the open field test (OBX). The number of crossings (the number of squares crossed by the animal with its four paws) was used to assess locomotor activity in an open field apparatus.²⁵⁻²⁶ The open field test is a criterion for detecting the level of neuronal excitability and analysing the influence of drug therapy on general behaviour. Normally, animals freely explore the entire open field area, but the observations of the open field apparatus in the case of olfactory bulbectomized rodents are completely different; they resulted in hyper-aggressive behaviour after undergoing OBX surgery in the open field, and they resulted in an increase in the number of line crossings before treatment and a decrease in the number of line crossings after treatment with the drug. After the chloroform and ethyl acetate fractions had been treated for the entire study duration, there was a substantial reduction in the number of line crossings. It means that the *Cassine albens* fractions may have possesses antidepressant properties.

When compared to the OBX group, OBX-mice treated with chloroform and ethyl acetate (200 and 100 mg/kg p.o. demonstrated a significant decrease in hyperactivity or movement on the 28th day after chronic drug treatment. The chloroform fraction of 100mg/kg, on the other hand, had no effect.

Mice's unusual grooming habit is thought to be an indicator of self-care and motivating behaviour. Grooming behaviour was reduced in animals suffering from a mild form of sadness. The results demonstrated that the increase in delay to grooming caused by OB was significantly reversed by chloroform and ethyl acetate fraction (200 and 100 mg/kg, p.o.) treatment, indicating a improvement in self-care and motivated behaviour.

CONCLUSION

Phytochemical study of leaves of *C. albens* reveals that the presence of alkaloid, Terpenoids and steroids etc, these may be a compound of interest experimental data shows that significant antidepressant effect of chloroform fractions of *Cassine albens* in OBX mice model. This may be used in future

reference for development of better alternative molecule for treatment of depression.

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CONFLICT OF INTEREST

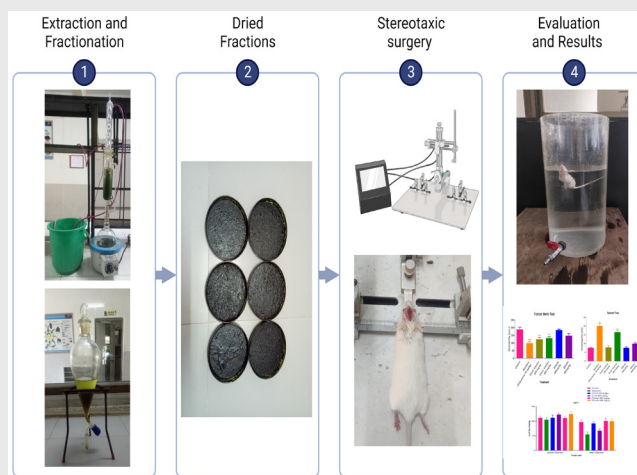
The authors declare that there is no conflict of interest.

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PICTORIAL ABSTRACT



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