BIOCHEMICAL ESTIMATION FOR ANTIOXIDANT AND ANTI-DEPRESSANT POTENTIAL OF *BOMBAX CEIBA*

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Abstract

Depression is a prevalent mental illness. According to World Health Organization, 5% adults worldwide experience depression. The Bombax ceiba is a huge, beautiful, and thrive in the hot climate of India, even reaching altitudes of 1,500 meters. The current research emphasizes on the biochemical estimation for antioxidant and anti-depressant potential of hydroethanolic flowers extract of *Bombax ceiba.* Fresh flowers of *B. ceiba* were collected from the UP east region. Diethyl ether was obtained from the local market. Imipramine was obtained as gift sample from the Lupin Pharma Pvt Ltd. Evaluation kits for antioxidant were purchased from Thermofisher Scientific. The fresh flowers were harvested from the Uttarakhand region of India. The plant was authenticated by Botanist at NBRI Lucknow with specimen no. NBRI/PA/2022/10/0035. Wistar rats of either sex of weight b/w 40-60g were procured from the animal house at TRC Mahavidyalaya, Department of Pharmacy, Satrikh, Barabanki, India. The rats were divided in 4 groups and treated for 21 days i.e., control: administered normal saline, standard: administered Imipramine (10mg/kg, *i. p.*), test 1: administered *Bombax ceiba* flower extract (200mg/kg, *p. o.*), and test 2: administered *Bombax ceiba* flower extract (400mg/kg, *p. o.*). The chronic unpredictable mild stress (CUMS) was used for induction of depression. The evaluation parameters including body weight determinations, light-dark arena, sucrose preference test (SPT), biochemical evaluation. In results, *Bombax ceiba* facilitated the no. of entries and time spent in open arm in light/dark arena model and increased the sucrose preference percentage. Thus, it exhibited anti-depressant effect in behavioral and biochemical parameters i.e., SOD, CAT, TBARS and GSH. It can be concluded that *B. ceiba* hydroethanolic flowers extract dominates the inhibitory action of central nervous system. Its mode of action is required to be confirmed by screening in clinical trials to confirm for safety and efficacy.

Keywords: *Bombax ceiba,* hydroethanolic, depression, biochemical, antioxidant.

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INTRODUCTION

Depression is a prevalent mental illness. According to World Health Organization, 5% adults worldwide experience depression. The largest cause of disability in the world today is depression, which also significantly contributes to the overall burden of sickness on the planet. Depression affects more women than males. Suicide can result from depression [1]. It is the main danger factor for self-destruction, a main source of death around the world, particularly in teenagers, young adults, and the older people [2]. Similar to this, having a first-degree family who suffers from depression increases the risk of developing major depression by 2.5-3 times but experiencing a terrifying life event in the months following the incident boosts the risk by 5-16 times [3]. The neurotransmitter serotonin influences a variety of physiological processes, including emotional thoughts, sleep, sexual behaviour, circadian rhythm, appetite, aggression, and pain sensitivity [4]. Studies have shown that depressed patients have greater cortisol levels and abnormal cortisol conc. than non-depressed people [5].

There are many reasons to use herbal plants over conventional pharmaceuticals, including the potential absence of adverse effects even after prolonged use [6]. The Bombax ceiba is a huge, beautiful, and endemic Northern Australian deciduous tree. Its range includes much of temperate and tropical Asia, as well as much of Africa. Plants thrive in the hot climate of India, even reaching altitudes of 1,500 metres. There are thick, stiff prickles all over the plant's juvenile stems and the wide, spreading branches that it produces at a height of 25 to 30 metres. The bark ranges in tint from silver grey to ash and is 1.8-2.5 cm thick [7].

a. Flower

b. Stem

Fig 1. Parts of *Bombax ceiba* plant

Taxonomy

Division	- Magnioliophyta
Class	- Magniolipsid
Order	- Malvales
Family	- Bombacaceae
Genus	- *Bombax*
Species	- *ceiba*

The leaves *B. ceiba* have been proved for different phytoconstituents as quercetin, aesculetin, aesculin, gentisic acid, protocatechuic acid, glucosyringic acid, luteolin-4-glucoside, scoparone, limettin, scopoletin, taraxerol, taraxerone, taraxeryl acetate, squalene, beta-sitosterol palmitate, 4-methyl stigmast-7-en-3-ol, 1H-indole-3-carboxylic acid, 6-O-palmitoylsitosteryl-D-glucoside, 12beta-hydroxyl-pregnane-4, 16-diene-3, 20-dione, loliolide and 5-(hydroxymethyl) furfural. [8].

The current research emphasizes on the biochemical estimation for antioxidant and anti-depressant potential of hydroethanolic flowers extract of *Bombax ceiba.*

MATERIALS AND METHODS

Experimental requirements

Fresh flowers of *B. ceiba* were collected from the UP east region. Diethyl ether was obtained from the local market. Imipramine was obtained as gift sample from the Lupin Pharma Pvt Ltd. Evaluation kits for antioxidant were purchased from Thermofisher Scientific.

Sucrose, ethanol, distilled water, rotatory evaporator, desiccator, and Wistar albino rats.

Collection and authentication of plants

The fresh flowers were harvested from the Uttarakhand region of India. The plant was authenticated by Botanist at NBRI Lucknow with specimen no. NBRI/PA/2022/10/0035. The flowers

were shade dried, crushed, and weighed for equal amounts of 15g. It was mixed and soaked into equal volume of ethanol and distilled water solution (1:1). After a week, all the macerates were filtered off to get the hydroethanolic flowers extract [9].

Preparation of animals

Wistar rats of either sex of weight b/w 40-60g were procured from the animal house at TRC Mahavidyalaya, Department of Pharmacy, Satrikh, Barabanki, India. The animals were housed separately in cages for acclimatization at a temp. of 25±2ºC and 50±15% relative humidity with a 12hours light/dark cycle. Animals were kept on standard pallet diet with drinking water *ad libitum* throughout the study. The study period was approved by institutional animal ethics committee (IAEC), TRC Mahavidyalaya, Department of Pharmacy, Satrikh, Barabanki. At the end of experiment rats were sacrificed with high dose of anaesthesia- diethyl ether [10].

Groups design

For the study, the rats were separated into 4 groups, each with 6 animals [11].

Control: administered normal saline once daily for 21 days.

Standard: administered Imipramine (10mg/kg, *i. p.*) once daily for 21 days.

Test 1- administered *Bombax ceiba* flower extract (200mg/kg, *p. o.*) once daily for 21 days.

Test 2- administered *Bombax ceiba* flower extract (400mg/kg, *p. o.*) once daily for 21 days.

Induction of depression

Chronic Unpredictable Mild Stress (CUMS) was employed for the induction of depression. In order to prevent adaptation, two stressors were applied every day and were chosen at random, with no stressor being delivered twice in a row for three days. The same daily stressors with the same type, onset, and duration were applied to all CUMS animals. Every day, the stressor application time was varied, and the interval between each stressor was between 3 and 8 hours [12].

Stressors include as below:

- 24 hours of food deprivation
- 1 hour in a 4 °C room
- 24 hours of cage tilting at a 45° angle
- overnight illumination
- 24 hours of exposure to a wet cage
- 5 minutes of swimming in 6-8 °C water
- 5 minutes of tail clamping
- 24 hours of water deprived
- Unpredictable shocks lasting 5 minutes
- 15 minutes of swimming
- 4 hours of limited movement
- 4 hours of cage disruption.

The stressors for CUMS were applied each day for consecutive 28 days.

EVALUATION PROTOCOLS

Body weight determinations

All the rats from each group were observed for their body weights. They were compared as before and after treatment weights.

Light-dark Arena Model

In light/dark arena test, a 100Watt bulb is being kept 30cm above to base of box. Rats are kept in middle- light arena (box) and must expose for 5 min. Number of entries, time spent in light arena segment are recorded for 5 min. It is cleansed prior placing a rat [13].

Sucrose preference test (SPT)

After the deprivation of water for 12 h, each mouse was simultaneously presented with two premeasured bottles complemented with water or 1% sucrose solution (w/v) for 6 h. Then the fluid intake will be recorded and the bottles will be exchanged their place for another 6 h [14]. The sucrose preference will be defined as follows:

Sucrose preference = sucrose consumption/ (sucrose consumption + water consumption) × 100%.

Biochemical evaluation

Animals were starved for 16 hours after 28 days, and blood samples from the recto orbital plexus were taken. The serum was then separated to examine the antioxidants levels i.e., SOD, CAT, TBARS & GSH. It was also evaluated for TG, HDL, LDL and VLDL [15].

RESULTS AND DISCUSSION

Body weights determination

Normal saline fed group showed a significant increase in body weight after treatment which was 85.23±0.52*g as before and 93.41±0.13*g as after. Imipramine fed group showed a remarkable increase in body weight as 85.62±0.18**g which was 74.30±0.61*g before the treatment. *B. ceiba* hydroethanolic flowers extract exhibited marked decrease in body weight & demonstrated body weight as 86.27±0.27**g and 88.43± 0.49*g after the treatment, at the dose of 200mg/kg and 400mg/kg respectively that was significant decrease in body weight. Thus, this maintained body weights of rats.

Following table depicted the body weight determination-

Table 1. Body weight determination of *Bombax ceiba* hydroethanolic flowers extract (BCFE) in treated animals

Treatment	Body weight (g) (Mean± SEM) before	after
Normal Saline	85.23±0.52*	93.41±0.13*
Imipramine (10mg/kg)	74.30±0.61*	85.62±0.18**
B. ceiba hydroethanolic flowers extract (200mg/kg)	78.62±0.57**	86.27±0.27**
B. ceiba hydroethanolic flowers extract (400mg/kg)	80.17±0.64**	88.43± 0.49*

Significance level (P<0.05) was denoted by *
Values were expressed as Mean± SEM & n=6.

Light/dark arena test

In light/dark arena test, no. of entries, time spent and % of time spent in light arena were recorded for 5 min. In Imipramine treated rats, no. of entries in light arena was recorded as 8.56±0.41** and time spent 152.40± 0.62 sec and thus % of time spent as 49.80±0.25** which was highest among all. The *Bombax ceiba* hydroethanolic flowers extract (BCFE) also showed increased no. of entries and time spent in light arena as 7.81±0.54** and 123.36±0.43 respectively at the dose of 400mg/kg. Whereas, BCFE exhibited 6.20±0.24* (no. of entries) and 94.29±0.57 (time spent) in light arena at the dose of 200mg/kg.

Table 2. Light/dark arena test of *Bombax ceiba* hydroethanolic flowers extract (BCFE) in treated animals

Treatment	In light arena (sec) Entries no.	time spent	(time spent %)
Normal Saline	5.47±0.35**	66.23± 0.71	22.07±0.32*
Imipramine (10mg/kg)	8.56±0.41**	152.40± 0.62	49.80±0.25**
B. ceiba hydroethanolic flowers extract (200mg/kg)	6.20±0.24*	94.29±0.57	32.43±0.19**
B. ceiba hydroethanolic flowers extract (400mg/kg)	7.81±0.54**	123.36±0.43	43.12±0.43**

Significance level (P<0.05) was denoted by *
Values were expressed as Mean± SEM & n=6.

Sucrose Preference Test (SPT)

In SPT model, *Bombax ceiba* hydroethanolic flowers extract showed increasing effect in treated rats as 81.70±0.57*% and 94.19±0.61**% at the dose of 200mg/kg and 400mg/kg, respectively. Imipramine fed rats demonstrated SPT as 105.36±0.638* % while normal saline treated rats exhibited lowest SPT as 78.21±0.34**%.

Table 3. SPT of *Bombax ceiba* hydroethanolic flowers extract (BCFE) in treated animals

Treatment	PST % (Mean± SEM)
Normal Saline	78.21±0.34**
Imipramine (10mg/kg)	105.36±0.638*
B. ceiba hydroethanolic flowers extract (200mg/kg)	81.70±0.57*
B. ceiba hydroethanolic flowers extract (400mg/kg)	94.19±0.61**

Significance level (P<0.05) was denoted by *
Values were expressed as Mean± SEM & n=6.

Estimation of anti-oxidant parameters

Anti-oxidant activity of rats was estimated in terms of SOD, CAT, TBARS and GSH. *B. ceiba* hydroethanolic flowers extract (200mg/kg) & *Bombax ceiba* hydroethanolic flowers extract (400mg/kg) showed SOD level as 0.24±0.03***U/µg and 0.19±0.01***U/µg; CAT level as 10.24±1.72***nM/µg and 12.92±0.88***nM/µg; TBARS as 0.85±0.02***nM of MDA/mg and 0.62±0.01***nM of MDA/mg and GSH as 4.53±1.62**µM/µg of protein & 5.87±1.98***µM/µg of protein. When these parameters were compared with control, they showed a remarkable change/ modulation behaviour on the anti-oxidant parameters.

Table 4. Estimation of anti-oxidative levels of *Bombax ceiba* hydroethanolic flowers extract (BCFE) in treated animals

Treatment	SOD (U/µg of protein)	CAT (nM of H_2O_2/min/µg of protein)	TBARS (nM of MDA/mg of protein)	GSH (µM/µg of protein)
Normal Saline	0.29±0.07	14.15±1.08	0.51±0.02	7.82±1.82
Imipramine (10mg/kg)	0.12±0.02	7.13±0.87	1.55 ±0.02	3.02±1.16
B. ceiba hydroethanolic flowers extract (200mg/kg)	0.24±0.03***	10.24±1.72***	0.85±0.02***	4.53±1.62**
B. ceiba hydroethanolic flowers extract (400mg/kg)	0.19±0.01***	12.92±0.88***	0.62±0.01***	5.87±1.98***

Significance level (P<0.05) was denoted by *
Values were expressed as Mean± SEM & n=6.

It is fairly normal for people to experience depression, and for many, it is a persistent problem that interferes with work & family obligations. It depletes the drive, vigor, and enjoyment needed to support and maintain social, parental, and marital connections. It can start at any age, can be chronic or waxing and waning, and it frequently coexists with a wide range of other problems, including anxiety disorders, substance abuse and behavioral disorders. It is frequently held responsible for or a contributing factor in medical disorders. There is a lot of information on the prevalence and symptoms of depression in the general population, but less information is available on depression among parents and other caretakers.

The effects on the central nervous system that have been shown in our in vivo tests may be related to this antioxidant activity in vitro. The central effects of the B. perennis ethanolic extract were assessed. The open-field test, which gives a strong indicator of the animal's emotional state [16] was used to analyze ethanol extract first. According to the findings, ethanolic extract was able to considerably reduce both the number of crossings and rearing, which is a symptom of a potential sedative action and muscle relaxant. Our findings from an open-field experiment point to a potential sedative effect of the B. perennis ethanolic extract. However, the mechanism by which this chemical produces its sedative effect is still unknown. Since the voltage-gated K+ channels from the Kv family are implicated in neuronal signaling, this effect may have been caused by an impact on neuronal excitability by ionic channel regulation [17].

It exhibits antidepressant action probably by facilitating the release of neurotransmitters i. e., serotonin, dopamine. It also increases the release of GABA (Gamma Amino Butyric Acid) and chloride ions influx that leads to hyperpolarization. The effect was determined in dose-dependent manner.

In results, *Bombax ceiba* facilitated the no. of entries and time spent in open arm in light/dark arena model and increased the sucrose preference percentage. Thus, it exhibited anti-depressant effect in behavioral and biochemical parameters i.e., SOD, CAT, TBARS and GSH.

CONCLUSION

People have been employing a variety of plants as a source of medicine in various forms since ancient times. We can conclude from the foregoing preclinical study that *B. ceiba* hydroethanolic flowers extract have antidepressant activity in behavioral and biochemical models of depression. It also showed marked antioxidant potential. It can be effectively used in the treatment of depression, mental agitation and other neurological disorders after successfully evaluating mechanism of action against the same.

It can be concluded that *B. ceiba* hydroethanolic flowers extract dominates the inhibitory action of central nervous system. Its mode of action is required to be confirmed by screening in clinical trials to confirm for safety and efficacy.

FUNDING STATUS

None.

CONFLICT OF INTEREST

'None' conflict of interest was declared by the authors.

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