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Ameliorative effect of Betulinic Acid in CUMS induced depression in Swiss-albino mice

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Abstract
Depression is a widespread mental health disorder characterized by persistent feelings of sadness, hopelessness, and a lack of interest in daily activities. Chronic unpredictable mild stress (CUMS) is an established method to induce depressive-like behavior in animal models, closely mimicking the human condition of depression. Betulinic acid, a naturally occurring triterpenoid found in the bark of *Betula utilis*, has shown promise in various pharmacological activities, including anti-inflammatory, anti-tumor, and neuroprotective effects. This study investigates the ameliorative effects of Betulinic acid on CUMS-induced depression in Swiss albino mice. Methods involved dividing mice into different groups: control, CUMS, CUMS + standard antidepressant (Imipramine), and CUMS + Betulinic acid. Mice in the CUMS groups were subjected to a variety of mild stressors over several weeks. Behavioral assessments, such as the sucrose preference test, forced swim test, and tail suspension test, were conducted to evaluate depressive-like behaviors. Additionally, biochemical analyses were performed to measure oxidative stress markers and neuroinflammatory cytokines in the brain tissue. Results demonstrated that Betulinic acid significantly alleviated depressive-like behaviors in CUMS- induced mice, as evidenced by improved performance in behavioral tests compared to the untreated CUMS group. Biochemical analysis revealed a reduction in oxidative stress and neuroinflammation markers in the brain tissue of Betulinic acid -treated mice. These findings suggest that Betulinic acid exerts its antidepressant effects through the modulation of oxidative stress and neuroinflammation pathways. In conclusion, Betulinic acid shows potential as a natural antidepressant agent, offering a novel therapeutic approach for the treatment of depression. Further studies are warranted to explore its mechanisms of action and potential clinical applications.

Keywords: Betulinic acid, *Betula utilis*, Chronic Unpredictable Mild Stress (CUMS), depression, Swiss albino mice, oxidative stress, neuroinflammation, antidepressant, behavioral assessment

1. Introduction
Depression stands as a widespread mental health problem hurting millions of adults globally. According to the World Health Organisation (WHO), depression is the primary cause of disability worldwide and accounts for a substantial portion of the total disease burden. According to recent estimates, depression affects 3.8% of the population, with rates among adults at 5% (4% for males and 6% for women) and among those over 60 at 5.7%. Interestingly, women are around 50% more likely than males to have this disease. Furthermore, depression affects more than 10% of expectant mothers and those who have just given birth. The burden of depression is enormous, impacting more than 280 million people worldwide. With over 700, 000 suicide fatalities per year, depression is also a significant risk factor for suicidality and the fourth largest cause of death for those between the ages of 15 and 29. According to the WHO World Mental Health (WMH) Surveys, more than 75% of people in low- and middle-income countries do not receive treatment for mental disorders, even though there are proven and efficient treatments for them. Inadequate financing for mental health services, a lack of qualified medical personnel for all residents, and the persistent social stigma attached to mental illnesses are all barriers to receiving quality care [1].
Chronic unexpected mild stress (CUMS) is commonly used as a model to induce conduct similar to depression. Previous investigations have shown that antidepressant medications considerably attenuate several of the negative effects of CUMS [2, 3]. Rats given CUMS show signs of depression, including a reduction in their desire for sucrose [4], which is

known as anhedonia, a key sign of depression [5]. It has long been recognised that depression causes an increase in plasma corticosterone levels due to the hyperactivity of the hypothalamic-pituitary-adrenal cortical axis (HPA axis) [6]. Certain antidepressants reduce the blood levels of corticosterone that depression produces, according to Jin *et al*. [7]. This implies that one of the drugs' possible antidepressant effects could be neuroendocrine modulation. As a result, the model has strong construct validity (because CUMS reduces sensitivity in the brain reward system), predictive validity (since antidepressant drugs reverse behavioural changes), and face validity (because CUMS encourages the behavioural changes seen in sad people) [2].

Evidence from the literature suggests that the serotonergic system is involved in the pathogenesis of depression [8]. Various antidepressants have been found in numerous investigations to limit the absorption of serotonin (5-HT), hence reducing the behavioural abnormalities caused by CUMS [9]. Prolonged activation of the HPA axis has been proposed as a potential cause of mitochondrial damage. It then causes an increase in 5-HT flow caused by monoamine oxidase (MAO), which kills serotonergic neurones in the hippocampus (HIP) and prefrontal cortex (PFC), among other parts of the brain [10, 11].

The BDNF, which is linked to neuronal survival and neurogenesis and is somewhat connected to the expression of proinflammatory mediators, is essential in animal models of depression. While long-term antidepressant treatment, particularly imipramine, alters BDNF levels during the depressive episode, CUMS and other stress management techniques reduce BDNF concentration in the brain [12-14]. In the brain, BDNF expression is lower in the more proinflammatory mediators. Furthermore, by lessening the impact of proinflammatory mediators, a range of therapeutic strategies have been demonstrated to offer antidepressant efficacy through BDNF-mediated activities [15-17]. These findings demonstrate a strong relationship between proinflammatory, serotonergic, and mitochondrial mediators during depression.

2. Materials and Methods

2.1 Chemicals and Reagents

Sigma-Aldrich supplied Betulinic acid & imipramine, while Merck Pvt. Ltd. in New Delhi and HiMedia Laboratories Pvt. Ltd. in Mumbai, India, supplied all other HPLC and analytical grade chemicals and reagents.

2.2 Experimental animal and Their Housing

Mice weighing between 20 and 25 grams were used in this experiment. They were from Rajiv Academy for Pharmacy's Animal House in Mathura, India. The mice were housed in groups within husk-coated polyacrylic cages. The temperature was 25±2 °C with a relative humidity of 45-55%, and there were twelve hours of light and twelve hours of darkness each day. Water and their regular grain diet were available to the animals at any time. The animals were allowed to drink as much water as they want prior to the experiments, but they were not fed for sixteen to eighteen hours.

2.3 Experimental Design

Six groups of mice were created, with six mice in each group. With the exception of the control group, mice were depressed using the CUMS model. The first day, or day 1, was when the mice's behaviour was examined. Following CUMS administration, mice exhibited depression-like behaviours on day 28, while rats displayed similar behaviours. For seven days following the onset of depression, three groups received BA (100, 200, and 400 mg/kg, respectively) [18], one group received imipramine (10 mg/kg) [19], and one group remained as normal control and one group remained as CUMS (disease control). On day 35, all mice received the last dose of BA (100, 200, and 400 mg/kg) an hour later. They were then tested for antidepressant activity using the OFT, TST, FST, and sucrose feeding test. The mice were sacrificed by cervical dislocation, and the brain was dissected in order to examine the antidepressant effectiveness of BA for the following conditions: altered serotonergic system, proinflammatory cytokine expressions, impaired HPA axis high activity, and impaired mitochondrial function and integrity in the rats' HIP and PFC.

2.4 CUMS (Chronic Unpredictable Mild Stress) Protocol

The CUMS standard technique [2] was used to create depression-like behaviour, with some modifications [20]. Two hours of coupled caging, three hours of tilted cage at a 45 ° angle, three hours of wet caging, eighteen hours of food deprivation followed by an hour of severely restricted access to food, two periods of water deprivation followed by an hour of exposure to an empty water bottle, one period of wet cage with 200 mL water in 100 grams of sawdust bedding, and one period of continuous light exposure were all included in the CUMS cycle, which lasted for four weeks. Therefore, during both their active (dark) and inactive (light) phases, the mice were subjected to stressors. The cages and living areas of the control mice were not altered.

2.5 Observation of Behavioral Parameter and Biochemical Estimation

2.5.1 Forced Swim Test (FST)

The test was carried out in compliance with the reference recommendations [21, 22], with a few minor adjustments. Swim drills were conducted in transparent glass cylinders filled with water that was 23-25 °C, 46 cm high, 20 cm in diameter, and 30 cm deep. Between 10:00 and 16:00, two swim evaluations were carried out, each consisting of a 15-minute pretest and a 5-minute post-test. The mice were taken out of the cylinders after both swimming sessions, dried with paper towels, and then placed in heated cages for approximately fifteen minutes before being returned to their original cages. Climbing behaviour is the upward-directed movements of the forepaws along the swim cylinder's side. Movement (mostly horizontal) across the swim cylinder's quadrants and throughout the entire swim cylinder is referred to as swimming activity. Immobility, which is defined as the lack of further movement beyond that necessary to maintain the mice's head above the water, was one of the behaviours chosen for observation in the modified FST. The sampling process counts the instances of each behaviour every five seconds during the test session.

2.5.2 Tail-Suspension Test (TST)

With its body pointing downward, the mice were suspended by its tail from a stable metal rod that was 50 cm above the ground. In order to avoid the stressful situation, animals would normally try to climb up the rod, but depressed animals would give up and stay still. Assessed was the

amount of time spent immobile during the test's five minutes, which suggested depressive-like behaviour [23, 24].

2.5.3 Open Field Test (OFT)

A common method for assessing mice's movement and emotional response is OFT [25]. The field was an open hardwood field that was 60 60 cm and had obstacles that were 30 cm high. For five minutes, the behaviour of each individual mouse—including the distance walked, the amount of time it spent immobile, the number of rearing, and the number of line crossings with its hind leg—was monitored in the centre of the open space. Under ideal lighting, the percentage of total transit distance and rearing as an exploratory behaviour were calculated from grid crossings through the hind legs [26, 27].

2.5.4 Sucrose preference Test

The technique for assessing the demand for sucrose as outlined by [28, 29] was applied. Throughout the trial, mice were placed in cages one at a time. The mice were not given any water for a full day before to use. The mice were housed in different cages, and each cage had a single bottle of 1% w/v sucrose solution that they could freely access. The second bottle was replaced with water for a full day. The early-morning sucrose preference test was finished. Following the test, the volume percentage of water and sucrose solution consumed was recorded.

2.5.5 Corticosterone Estimation

As described by Woodward and Emery [30], an HPLC with an ultraviolet (UV) detection system was used to assess the serum corticosterone concentration, with dexamethasone added as a primary reference [31]. Essentially, 5 mL of dichloromethane was used to extract 500 µL of plasma that contained a specific dosage of dexamethasone. After being completely dried by evaporation, the dichloromethane was diluted in 100 µL of mobile phase. For measurement, 20 microlitres of the extract were put into the HPLC analyser. A UV detector (model: LC-20AD, Shimadzu, Japan) was used to detect CORT at 250 nm in a mobile phase of methanol/water (70:30) at a flow rate of 1.2 mL/min. The chromatogram was recorded and analysed using Empower software.

2.5.6 Brain Dissection

Cervical dislocation was used for animal sacrifice. After being quickly extracted, the brain was washed right away in 0.9% saline solution [32]. Using a homogeniser, HIP and PFC tissues were mixed together in 1 millilitre of 0.1 M perchloric acid. After 15 minutes in polypropylene tubes, the homogenate was centrifuged at 4000 g for 15 minutes after adding 50 µL of 4 M potassium acetate to bring the pH down to 4.0 [33].

2.5.7 Estimation of Monoamines

Using HPLC fitted with an ultraviolet (UV) detection system (model: LC-20AD, Shimadzu, Japan), the levels of neurotransmitters and associated metabolites, including serotonin (5-HT) and 5-hydroxy indole acetic acid (5-HIAA), as well as their ratio, were assessed in two brain regions, the HIP and PFC [31, 34].

2.5.8 Estimation of Mitochondrial Function (MTT Assay)

- **Isolation Mitochondria from Rat Brain:** The normal protocol had been followed to extract mitochondria from the HIP and PFC tissues [35]. The usual technique was used to determine the quantity of mitochondrial protein [36]. Rat tissues were taken out and put in an ice-cold isolation buffer containing 225 mM mannitol, 5 mM HEPES, 75 mM sucrose, and 1 mg/mL fatty acid free BSA (isotonized with KOH; pH 7.4) after the rats were executed. In a Dounce-type glass homogenizer, the tissue was chopped and homogenized using a chelator of 1 mM EDTA and isolation buffer (1/10, w/v). The homogenate was centrifuged at 600 rpm for 10 minutes. The aggregates were suspended in 10 mL of buffer and centrifuged at 600 rpm for 10 minutes in order to recover the fast-sediment mitochondria from the supernatant, which was collected in a chilled receiver. The supernatants were collected and centrifuged for eight minutes at 12, 000 rpm. The pellets were reconstituted in buffer and then centrifuged for 10 minutes at 12, 000 rpm. The top layer of the pellet is whitish and fluffy and is made up of lipids, synaptosomes, and myelin. The other layers vary in stiffness and appearance. The brown mitochondrial pellets were collected after the synaptosomal surface was removed, reconstituted in buffer, and centrifuged for ten minutes at 12, 000 rpm. Once more, the final mitochondria pellets were resuspended in isolation buffer without chelators.
- **Mitochondrial function (MTT) assay estimation:** In an Eppendorf tube, the pellet was suspended in phosphate buffer saline. After adding 10 µL MTT to 300 µL of mitochondrial solution, let it sit for half an hour. After centrifuging the suspension for five minutes at 12, 000 rpm, the supernatant was properly disposed of. The LMSF-V320 Spectrophotometer, manufactured by Labman Scientific Instruments Pvt. Limited, was used to dissolve the sample in 200 µL of DMSO. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction was used to measure the formazan generated at 595 nm [37] in order to ascertain the mitochondrial function. mg protein/min/mg formazan generated was the format used to display the results.

2.5.9 Pro-Inflammatory Cytokines Expression Estimation in HIP &PFC

To assist homogenise the HIP and PFC tissues, proteinase blocker (100 mg tissue/mL) was added to phosphate-buffered solution. After that, the mixture was centrifuged for ten minutes at 12, 000 rpm. The concentrations of tumour necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-1 (IL-1) in the supernatant were determined spectrophotometrically at 450 nm using an enzyme-linked immunosorbent assay kit (Abcam Pvt. Ltd., India) in compliance with manufacturer guidelines (model: UV-1800, Shimadzu, Japan). The cytokine concentrations in homogenates were expressed as pg/100 mg tissue sample, standardising to total tissue weight [38].

2.6 Data Analysis

The data was displayed as a standard error mean using the mean and standard deviation first from the mean. GraphPad Prism 5 software was used to do the statistical analysis. To find the differences between the groups, the analysis of variance (ANOVA) test was employed. FST, TST, OFT, and sucrose preference tests were conducted using two-way ANOVA followed by the Bonferroni post hoc test, while the estimation of plasma corticosterone, serotonergic system,

and proinflammatory cytokine expression was conducted using one-way ANOVA followed by the Newman-Keuls post hoc test. The null hypothesis was always excluded at the ≥5% ($P < 0.05$) level of significance.

3. Results

3.1 Effect of BA (Betulinic Acid) (100 AND 200 MG/KG & 400 Mg/Kg) IN CUMS-Induced Mice

3.1.1 Effect of BA (100 and 200 mg/kg & 400 mg/kg) on CUM-induced changed behaviorism FST

In Figure 1, you can see how 100 mg/kg, 200 mg/kg, and 400 mg/kg of BA affected the Forced Swim Test (FST) times that mice spent not moving, swimming, and climbing. In Table 1, you can see the relevant facts. There were big changes between the FST times for not doing anything ([F (2, 75) = 79.15, $p<0.001$]), swimming ([F (2, 75) = 328.6, $p<0.001$]), and climbing ([F (2, 75) = 82.95, $p<0.001$]). There were also big differences in the times of climbing, swimming, and not doing anything ([F (4, 75) = 20.68, $p<0.001$], [F (4, 75) = 96.49, $p<0.001$], and [F (4, 75) = 43.99, $p<0.001$, respectively). It was also seen that groups talked to each other and that people in the FST did nothing during breaks. Each number is the average (within a certain range) of six mice that weigh between 20 and 25 grams. *P < 0.05, **P < 0.001, and ***P < 0.0001 show that the changes are very important compared to the vehicle control. Post-hoc analysis showed that there were no significant differences between the groups in the amount of time spent sitting, swimming, and climbing on the first day of the experiment. In the FST on day 28, chronic unexpected mild stress (CUMS) made people less likely to swim and climb and more likely to be inactive. After 29 to 35 days of treatment with BA (200 mg/kg) and IMP, the animals were much less inactive than before CUMS. They could also swim for shorter amounts of time.

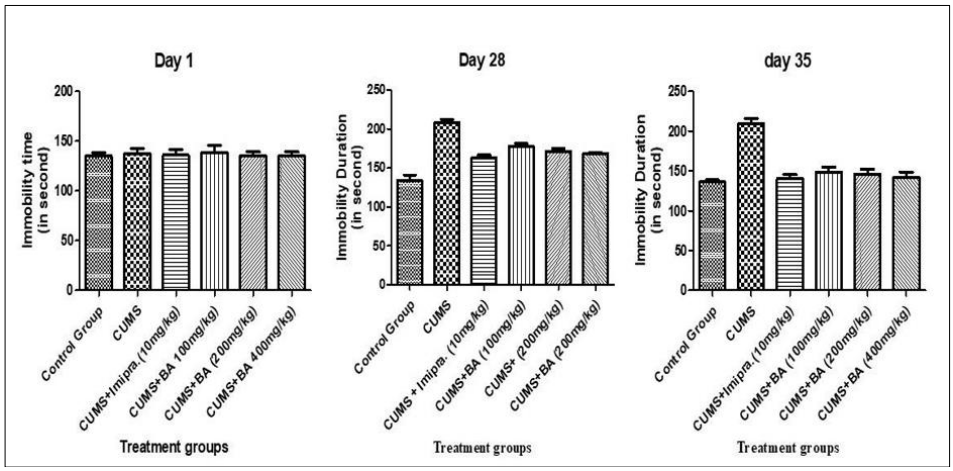

Fig1: Effect of Betulinic Acid (100 mg/kg and 200 mg/kg & 400 mg/kg) on CUMS-induced changed behaviors in FST

Table 1: Effect of Betulinic Acid (100 mg/kg and 200 mg/kg & 400 mg/kg) on CUMS-induced changed behaviour in FST

Forced Swim Test (in seconds)			
Groups	Immobility Period (in Second)		
	Day 1	Day 28	Day 35
Control	135.0±3.225	134.0±7.239	136.2±2.691
CUMS	137.8±4.565	208.4±4.202	209.4±6.809
CUMS + Imipramine (10 mg/kg)	136.0±5.486	163.2±3.569***	140.4±5.192***
CUMS + Betulinic Acid (100 mg/kg)	138.8±7.003	178.2±3.169***	148.4±6.501***
CUMS + Betulinic Acid (200 mg/kg)	135.8±3.470	171.6±3.669***	146.4±5.879***
CUMS + Betulinic Acid (400 mg/kg)	135.4±3.919	168.6±1.327***	142.2±6.398***

An ANOVA followed by Dunnett's test was conducted. The results are presented as the mean ± standard error for a sample size of 6 mice, each weighing between 20-25 grams. Statistical significance compared to the vehicle control group is indicated as follows: *P < 0.05, **P < 0.001, ***P < 0.0001.

3.1.2 Effect of BA (100 mg/kg and 200 mg/kg & 400 mg/kg) against CUMS-induced altered immobility in TST

Figure 2 depicts the impact of different dosages of BA (100 mg/kg, 200 mg/kg, and 400 mg/kg) on the alterations in immobility time generated by CUMS during the Tail Suspension Test (TST). The results pertaining to this matter are elaborated upon in Table 2 Each statistic indicates the average (±standard error) of six mice weighing between 20 and 25 grams. Significant deviations from the vehicle control are denoted by *P < 0.05, **P < 0.001, and ***P < 0.0001. On the first day, there were no notable disparities in the duration of immobility among the groups. However, on the 28th day, the Chronic Unpredictable Mild Stress (CUMS) had noticeably elevated the duration of immobility in all experimental groups. On the 35th day, the administration of BA-200 mg/kg effectively decreased the CUMS-induced rise in immobility duration.

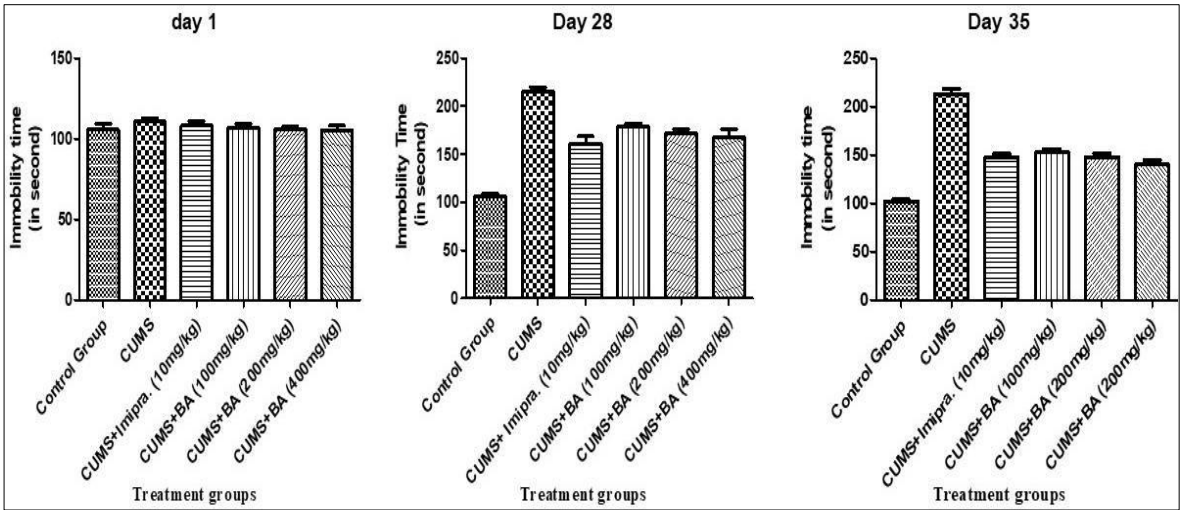

Fig 2: Effect of Betulinic Acid (100 mg/kg and 200 mg/kg & 400 mg/kg) on CUM-induced changed behaviours in TST

Table 2: Effect of Betulinic Acid (100 mg/kg and 200 mg/kg & 400 mg/kg) on CUM-induced changed behaviours in TST

Tail Suspension Test (TST)			
Groups	**Immobility Period (in Second)**		
	Day 1	**Day 28**	**Day 35**
Control	105.8±3.639	105.8±3.262	102.6 + 1.965
CUMS	110.8±2.245	214.8±5.113	213.4 + 5.006
CUMS + Imipramine (10 mg/kg)	108.6±2.694	161.0±7.694***	148.2 + 3.441***
CUMS + Betulinic Acid (100 mg/kg)	107.0±2.530	178.4±3.600***	153.0 + 3.633***
CUMS + Betulinic Acid (200 mg/kg)	106.2±1.828	171.8±4.24***	148.4+ 3.669***
CUMS + Betulinic Acid (400 mg/kg)	105.6±2.786	167.6±8.559***	140.8 + 4.443***

An ANOVA followed by Dunnett's test was conducted. The results are presented as the mean ± standard error for a sample size of 6 mice, each weighing between 20-25 grams. **P* < 0.05, ***P* < 0.001, and ****P* < 0.0001 show statistical significance compared to the car control group.

3.1.3 Effect of BA (100 and 200 mg/kg) on CUMS-induced altered behaviors in OFT

The effects of different dosages of BA (100 mg/kg, 200 mg/kg, and 400 mg/kg) on the changes generated by CUMS are shown by changes in (A) walking, (B) standing, (C) time spent in the centre, and (D) total distance travelled in the Open Field Test (OFT). The data is depicted in Figure 3 and displayed in Table 3. Subsequent analysis revealed that there were no statistically significant variations in any behavioral measures across the groups during the Open Field Test (OFT) on the initial day of the experiment. Nevertheless, the administration of CUMS resulted in a substantial decrease in all behavioral measures on day 28, and these reductions remained evident until day 35. On the 35th day, the treatment of BA (200 mg/kg) and IMP effectively reduced the decrease in all behavioral activities caused by CUMS.

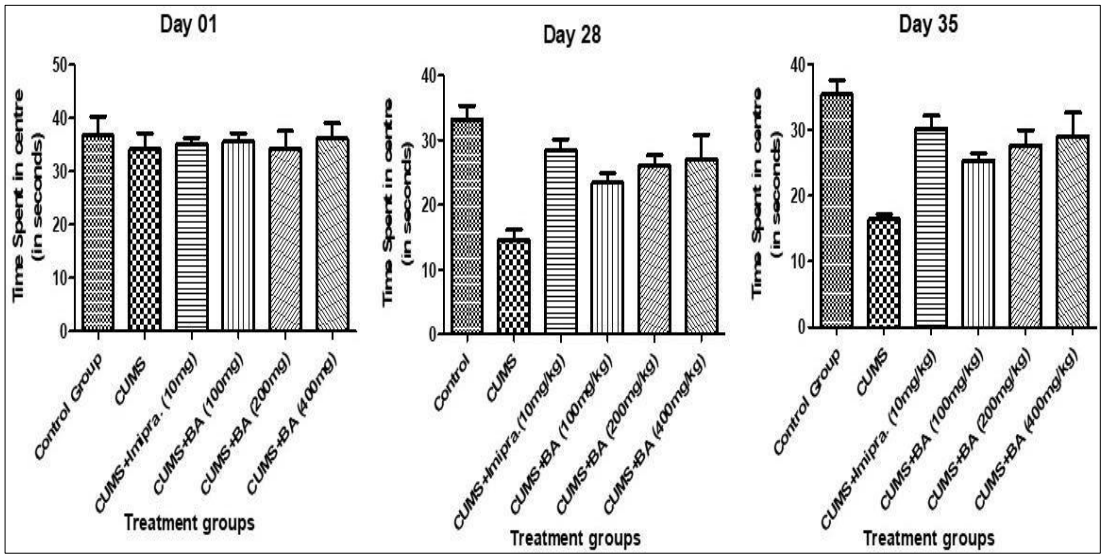

Fig 3: Effect of Betulinic Acid (100 mg/kg and 200 mg/kg &400 mg/kg) on CUM-induced changed behaviours in OFT

Table 3: Effect of Betulinic Acid (100 mg/kg and 200 mg/kg &400 mg/kg) on CUM-induced changed behaviours in OFT

Open Field Test (OFT) (in seconds)									
Groups	Ambulation			Rearing's			Time spent in Centre		
	Day 1	Day 28	Day 35	Day 1	Day 28	Day 35	Day 1	Day 28	Day 35
Control	67.60±3.776	65.00±2.236	65.60± 3.187	29.40±2.337	29.20±1.562	30.80±2.223	36.80±3.611	33.20±2.154	35.40±2.135
CUMS	64.40±5.810	31.40±2.993	32.00±3.688	32.40±2.112	11.20±1.828	12.80±3.262	34.20±3.072	14.60±1.536	16.40±0.7483
CUMS + Imipramine (10 mg/kg)	62.60±2.159	55.00±4.722***	56.20±4.420***	30.00±1.924	26.20±2.973***	28.20±2.973***	35.20±1.158	28.40±1.720***	30.20±1.934+***
CUMS + Betulinic Acid (100 mg/kg)	63.80±3.072	46.00±3.536*	47.00±2.510*	29.80±3.878	19.40±1.208*	23.00±1.732*	35.60±1.631	23.40±1.470*	25.40±1.030*
CUMS + Betulinic Acid (200 mg/kg)	63.20±3.813	47.80±2.764**	49.80±3.611**	31.00±2.828	21.40±2.293**	25.60±2.379**	34.40±3.187	26.00±1.673**	27.60±2.400**
CUMS + Betulinic Acid (400 mg/kg)	63.40±3.709	48.80±2.55**	52.40±4.069**	30.40±2.421	22.60±2.015**	26.40±2.542**	36.20±2.853	27.00±3.860**	29.00±3.619+**

An ANOVA followed by Dunnett's test was conducted. The results are presented as the mean ± standard error for a sample size of 6 mice, each weighing between 20-25 grams. Statistical significance compared to the vehicle control group is indicated as follows: *P < 0.05, **P < 0.001, ***P < 0.0001.

3.1.4 Effect of BA (100 and 200 mg/kg) on CUMS-induced altered anhedonia behavior

In Figure 4, you can see how different doses of BA (100 mg/kg, 200 mg/kg, and 400 mg/kg) affected the amount of sugar that CUMS-treated mice. Table 4 shows the full results. There were big changes in how much sucrose people are between groups [F (4, 75) = 61.56, p<0.001] and over time [F (2, 75) = 195.6, p<0.001]. Also, when it came to sucrose intake, there were important interactions between group and time [F (8, 75) = 21.01, p<0.001]. Post-hoc tests showed that there were no significant changes in how much sucrose the groups ate on the first day of the experiment. On the other hand, sucrose consumption dropped significantly on day 28 after CUMS treatment and stayed low until day 35. The effects of CUMS were greatly lessened by treatment with BA (200 mg/kg) and the common drug IMP. This caused a decrease in sugar consumption on day 35.

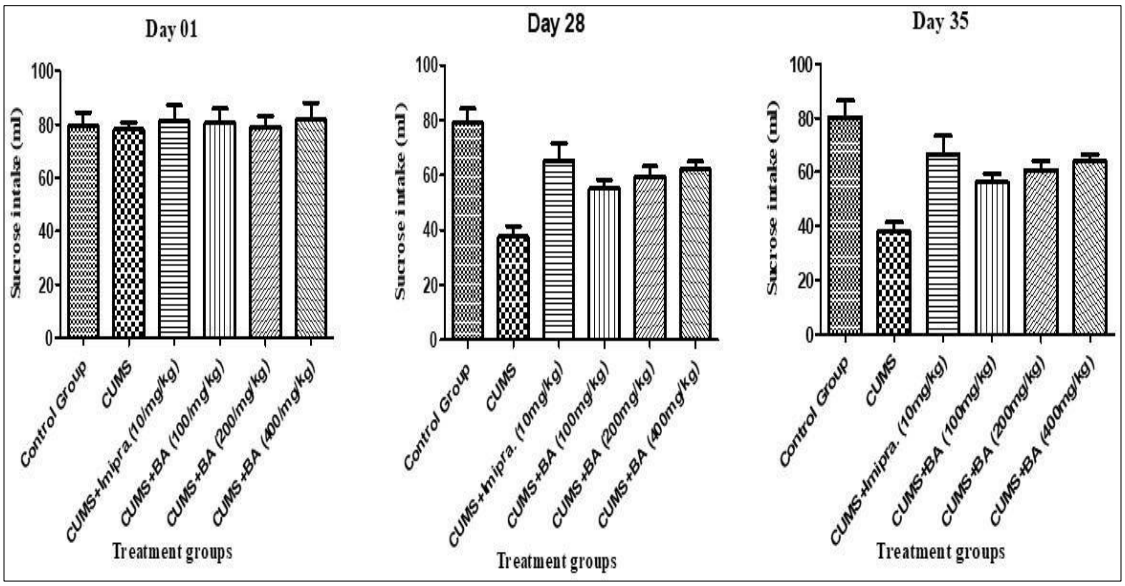

Fig 4: Effect of Betulinic Acid (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUM-induced altered anhedonia behaviour.

Table 4: Effect of Betulinic Acid (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUM-induced altered anhedonia behaviour.

Sucrose intake (ml)			
Groups	Day 1	Day 28	Day 35
Control	79.20±5.314	79.00±5.177	80.40±6.046
CUMS	78.00±2.683	37.60±3.982	38.00±3.564
CUMS + Imipramine (10 mg/kg)	81.20±5.886	65.40±6.210***	66.80±6.771***
CUMS Betulinic Acid (100 mg/kg)	80.40±5.381	55.20±3.023*	56.20±3.137*
CUMS + Betulinic Acid (200 mg/kg)	79.00±3.834	59.60±3.487**	60.80±3.200**
CUMS + Betulinic Acid (400 mg/kg)	81.60±6.524	62.20±2.691**	64.0±2.608**

An ANOVA followed by Dunnett's test was conducted. The results are presented as the mean ± standard error for a sample size of 6 mice, each weighing between 20-25 grams. Statistical significance compared to the vehicle control group is indicated as follows: *P < 0.05, **P < 0.001, ***P < 0.0001.

3.1.5 Effect of BA (100 and 200 mg/kg) against CUMS-induced changes in concentration of plasma corticosterone

Figure 5 illustrates the effect of various dosages of BA (100 mg/kg, 200 mg/kg, and 400 mg/kg) on alterations in plasma corticosterone levels in mice induced by chronic unpredictable mild stress (CUMS). The pertinent statistics can be found in Table 5. The statistical analysis revealed substantial disparities in plasma corticosterone levels among the groups [F (4, 25) = 95.68, p<0.001]. On the 35th day of the study, a further analysis showed that Chronic

Unpredictable Mild Stress (CUMS) caused a significant increase in plasma corticosterone levels. The administration of BA (200 mg/kg) and IMP successfully mitigated the rise in plasma corticosterone levels induced by the CUMS regimen in mice.

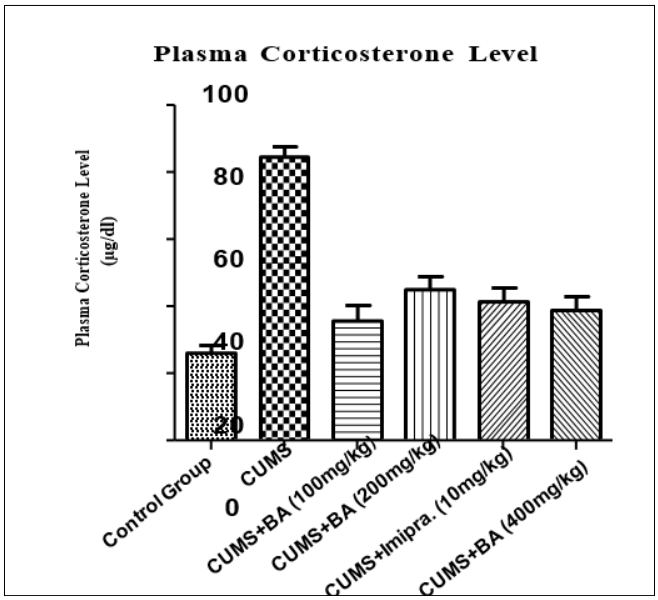

Fig 5: Effect of Betulinic Acid (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUMS-induced plasma corticosterone.

Table 5: Effect of Betulinic Acid (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUMS-induced in Plasma Corticosterone.

Groups	Plasma Corticosterone (µg/dl)
Control Group	26.00±2.366
CUMS	84.80±3.072
CUMS + Imipramine (10 mg/kg)	35.80±4.652***
CUMS + Betulinic Acid (100 mg/kg)	45.20±3.929***
CUMS + Betulinic Acid (200 mg/kg)	41.60±4.167***
CUMS + Betulinic Acid (400 mg/kg)	38.80±4.079***

An ANOVA followed by Dunnett's test was conducted. The results are presented as the mean ± standard error for a sample size of 6 mice, each weighing between 20-25 grams. Statistical significance compared to the vehicle control group is indicated as follows: *P < 0.05, **P < 0.001, ***P < 0.0001.

3.1.6 Effect of BA (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUMS-induced changes altered serotonergic activity in HIP and PFC

The effects of CUMS at dosages of BA (100 mg/kg, 200 mg/kg, and 400 mg/kg) on variations in 5-HTi and 5-HIAA levels as well as the ratio of 5-HIAA/5-HT are shown in Figure 6. You may find the appropriate findings in table 6. The 5-HT and 5-HIAA concentrations, together with their ratio (5-HIAA/5-HT), exhibited notable variations in the HIP and PFC areas. The levels of 5-HT and 5-HIAA, as well as their ratio 5-HIAA/5-HT (F (4, 25) = 262.3, p<0.001), were significantly different in the HIP region (F (4, 25) = 46.57, p<0.001 and F (4, 25) = 422.2, p<0.001, respectively). The levels of 5-HT and 5-HIAA, as well as their ratio 5-HIAA/5-HT (F (4, 25) = 294.5, p<0.001), were also significantly different in the PFC region (F (4, 25) = 76.54, p<0.001 and F (4, 25) = 407.2, p<0.001, respectively). A post-hoc test revealed that, in comparison to the control group, the injection of CUMS caused a substantial drop in i5-HT and an increase in 5-HIAA in the brain areas of HIP and PFC. Furthermore, chronic unexpected mild stress (CUMS) significantly increased the ratio of 5-HIAA/5-HT in all brain areas in mice as compared to control mice. In the mice brain areas, IMP and BA (200 mg/kg) both considerably reduced the 5-HT level decline brought on by CUMS. All brain regions showed a significant reduction in the CUMS-induced elevation in i5-HIAA levels when BA (200 mg/kg) was administered. On the other hand, IMP significantly exacerbated the rise in i5 -HIAA levels brought about by CUMS in both mouse brain areas. In all brain regions, BA (200 mg/kg) also lessened the rise in the i5-HIAA/5-HT ratio that was brought on by CUMS. The standard medicine IMP treatment markedly exacerbated the CUMS-induced rise in 5-HIAA/5-HT levels in the HIP and PFC.

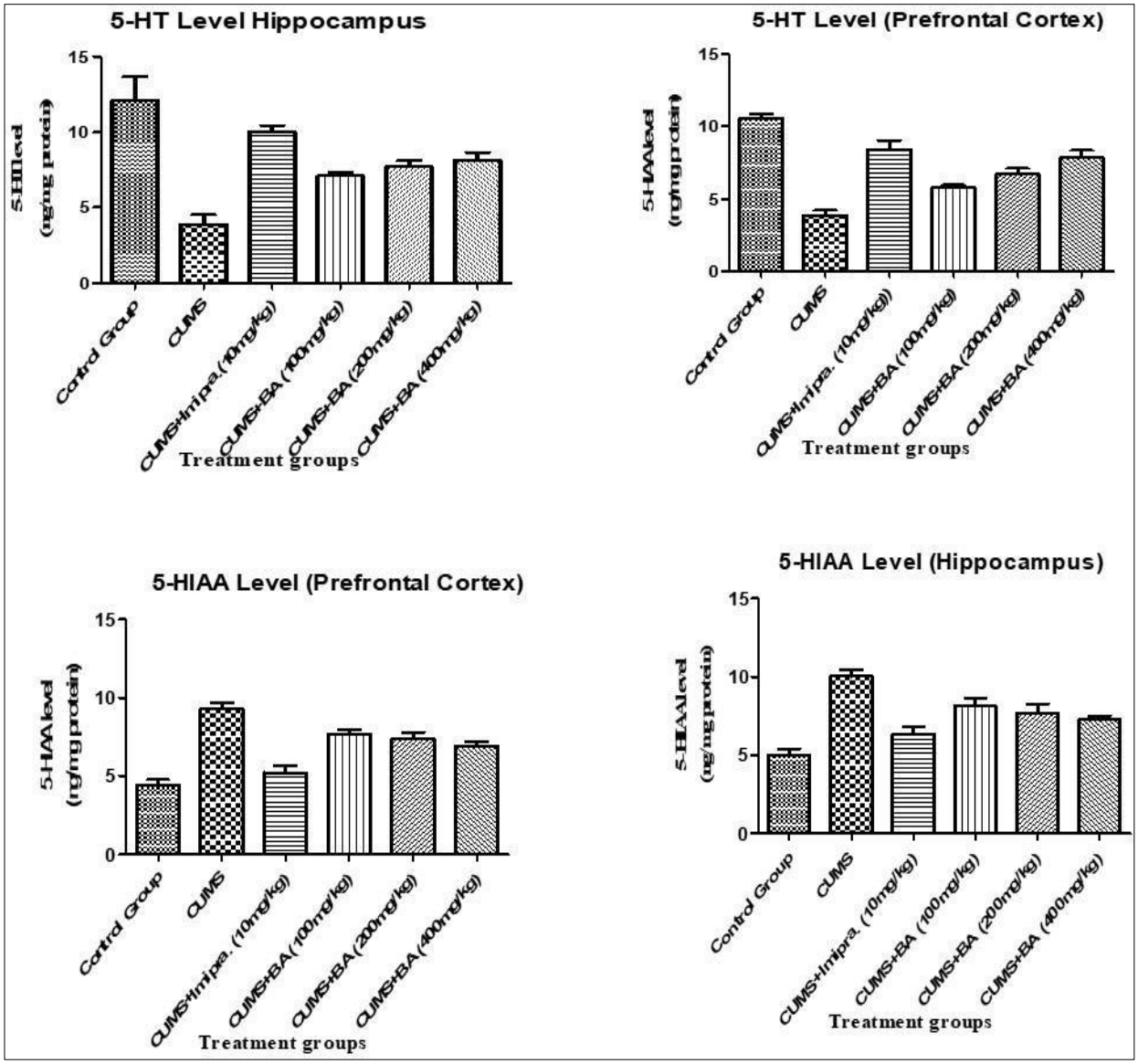

Fig 6: Effect *of* Betulinic Acid (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUMS-induced changes altered Serotoninergic activity in HIP and PFC.

Table 6: Effect of Betulinic Acid (100 mg/kg, 200 mg/kg and 400 mg/kg) on CUMS-induced changes altered Serotoninergic activity in HIP and PFC.

	HIP		PFC	
Groups	**5-HT**	**5-HIAA**	**5-HT**	**5-HIAA**
Control Group	12.14±**1**.534	5.003±0.408	10.54±0.355	4.400±0.364
CUMS	3.875±0.6477	10.03±0.426	3.860±0.346	9.280±0.386
CUMS + Imipramine (10 mg/kg)	10.05±0.4084***	6.360±0.458***	8.375±0.672***	5.235±0.443***
CUMS + Betulinic Acid (100 mg/kg)	**7**.105±0.2689*	8.115±0.532*	5.775±0.175*	7.675±0.275*
CUMS + Betulinic Acid (200 mg/kg)	7.710±0.4102**	7.640±0.644**	6.675±0.421**	7.380±0.425**
CUMS + Betulinic Acid (400 mg/kg)	8.150±0.5223**	7.250±0.251**	7.835±0.509***	6.940±0.274**

An ANOVA followed by Dunnett's test was conducted. The results are presented as the mean ± standard error for a sample size of 6 mice, each weighing between 20-25 grams. Statistical significance compared to the vehicle control group is indicated as follows: *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$.

Discussion

The purpose of this study was to determine whether BA could aid Swiss albino mice suffering from depression due to chronic unpredictable mild stress (CUMS). It emphasised both physiochemical and biochemical aspects. CUMS is a well-known method of eliciting depressive symptoms in rodents by mimicking the enduring and erratic stressors that causes depression in humans. Over the course of this investigation, Swiss albino mice were subjected to a variety of minor stressors. As a result, they exhibited behaviours that were obviously depressed. These behaviours were quantitatively measured using standard techniques such as the tail suspension test (TST) and the forced swim test (FST). These actions indicate behavioural despair. Betulinic acid was administered to the CUMS-induced animals, and the results were contrasted with those of a control group and a group that had received a standard antidepressant. The findings indicated that BA significantly reduced rest time in both the FST and the

TST, which raises the possibility that it has an antidepressant-like effect. The improved conduct of the treated mice indicated that their depression was improving. These behavioural results were further supported by biochemical studies. Long-term concern can disrupt the hypothalamic-pituitary-adrenal (HPA) axis, which raises stress chemicals like corticosterone. In this investigation, mice exposed to CUMS showed normal corticosterone levels after receiving BA. This indicates that the HPA axis is controlled by BA. In addition, reactive stress markers—which are frequently elevated in depressed individuals—were examined. Malondialdehyde (MDA), a marker of lipid peroxidation, significantly decreased after betulinic acid therapy, whereas antioxidant enzyme activity such as catalase (CAT) and superoxide dismutase (SOD) increased. These findings essentially demonstrate that BA aids in preventing oxidative stress, which is a component of the physiological mechanisms behind depression. Physiochemical parameters were also examined to gain a better understanding of BA's overall effects on the body. When changes in body weight and organ indices are brought on by long-term stress, they can reveal how healthy an organism is overall. Mice given CUMS in this study experienced significant weight loss and alterations in the weight of their organs, particularly the adrenal glands and spleen. The weight remained constant and the organ indicators reverted to normal when betulinic acid was used to treat these abnormalities. This demonstrates that BA not only helps those who are sad but also strengthens their bodies to better with stand chronic stress. Additional histological analyses of several brain regions, particularly the hippocampus, revealed that BA therapy reduced glial cell activation and neuronal damage, two conditions that are prevalent in sad individuals. An essential component of the brain that regulates mood and facilitates clear thinking is the hippocampus. Reducing the symptoms of depression requires protecting this area of the brain. The potential medical utility of BA is demonstrated by the fact that it protects neurones in this region. The study's findings indicate that betulinic acid considerably lessens the depression brought on by CUMS in Swiss albino mice. The antidepressant-like effects shown in behavioural tests are supported by biochemical and physiochemical data, such as the normalisation of corticosterone levels, the reduction of oxidative stress, and improvements in physiological parameters. Furthermore, the hippocampus's neuroprotective advantages demonstrate that BA may have multiple therapeutic applications. The study's findings pave the way for further investigation into how BA alleviates sadness and how it might be applied to treat depression in humans.

In conclusion, Swiss albino mice with CUMS-induced sadness exhibit encouraging antidepressant-like effects when exposed to betulinic acid extracted from the bark of *Betula utilis*. BA's potential as a treatment for depression is influenced by its diverse pharmacological actions, which include neurotransmitter modulation as well as anti-inflammatory, antioxidant, and neuroprotective properties. These results lay the groundwork for further investigation and advancement of BA as a cutting-edge antidepressant. However, more clinical research is required to verify its safety and effectiveness in people.

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