Antidepressant-like effects of seeds of *Citrullus lanatus* in the reserpine-induced depressed mouse model

Efeitos antidepressivos das sementes de *Citrullus lanatus* no modelo de rato deprimido induzido pela reserpina

Efectos antidepresivos de las semillas de *Citrullus lanatus* en el modelo de ratón deprimido inducido por reserpina

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**ABSTRACT**
Depression is a mental health disorder that affects millions of people worldwide. Plants and their products are widely used in the treatments of various diseases including depression for their therapeutic effectiveness with least or no side effects. The present study has therefore been conducted to evaluate the antidepressant like effects of *Citrullus lanatus* seed extract (CLSE) in the reserpine-induced depressed mouse model by assessing the phytoconstituents of CLSE, body weight, behaviour, histopathology of the adrenal gland and level of plasma corticosterone. Twenty-four adult male mice of Swiss strain were distributed into four groups of six each (n=6). Group I served as control while groups II, III and IV received reserpine (RES: 0.75mg/kg/BW/day) for 14 days, CLSE (300mg/kg/BW/day) only, for 28 days, and RES+CLSE [RES treatment for 14 days
followed by CLSE treatment for 28 days), respectively. Behavioural alterations were analysed by food consumption, sucrose preference, eight-arm radial maze, and forced swim tests during the treatment. RES-induced depression caused marked alterations in the body weight, all behavioural parameters, histopathology of the adrenal cortex, and in the level of plasma corticosterone, compared with the control. Administration of CLSE only, did not cause significant alterations in all the studied parameters, compared with the control. However, CLSE administration in RES-treated mice resulted in significant restoration in all the studied parameters, similar to the control. The findings therefore suggest the antidepressive activity of the seeds of *C. lanatus*, that may emerge as one of the potential sources among the natural therapies of depression disorder.

**Keywords:** behavioral parameters, depression, *Citrullus lanatus* seeds, corticosterone.

**RESUMO**
A depressão é uma perturbação da saúde mental que afecta milhões de pessoas em todo o mundo. As plantas e os seus produtos são amplamente utilizados no tratamento de várias doenças, incluindo a depressão, devido à sua eficácia terapêutica com poucos ou nenhuns efeitos secundários. Por conseguinte, o presente estudo foi realizado para avaliar os efeitos antidepressivos do extrato de sementes de Citrullus lanatus (CLSE) no modelo de ratinho deprimido induzido pela reserpinia, avaliando os fitoconstituintes do CLSE, o peso corporal, o comportamento, a histopatologia da glândula suprarrenal e o nível de corticosterona plasmática. Vinte e quatro ratos adultos machos de estirpe suíça foram distribuídos em quatro grupos de seis cada (n=6). O grupo I serviu de controlo, enquanto os grupos II, III e IV receberam reserpinia (RES: 0,75mg/kg/BW/dia) durante 14 dias, CLSE (300mg/kg/BW/dia) apenas, durante 28 dias, e RES+CLSE [tratamento com RES durante 14 dias seguido de tratamento com CLSE durante 28 dias], respetivamente. As alterações comportamentais foram analisadas através do consumo de alimentos, preferência por sacarose, labirinto radial de oito braços e testes de natação forçada durante o tratamento. A depressão induzida por RES causou alterações acentuadas no peso corporal, em todos os parâmetros comportamentais, na histopatologia do córtex adrenal e no nível de corticosterona plasmática, em comparação com o controlo. A administração de CLSE apenas, não causou alterações significativas em todos os parâmetros estudados, em comparação com o controlo. No entanto, a administração de CLSE em ratos tratados com RES resultou numa restauração significativa de todos os parâmetros estudados, semelhante ao controlo. Os resultados sugerem, portanto, a atividade antidepressiva das sementes de *C. lanatus*, que pode emergir como uma das fontes potenciais entre as terapias naturais do distúrbio depressivo.

**Palavras-chave:** parâmetros comportamentais, depressão, sementes de Citrullus lanatus, corticosterona.

**RESUMEN**
La depresión es un trastorno mental que afecta a millones de personas en todo el mundo. Las plantas y sus productos se utilizan ampliamente en el tratamiento de diversas enfermedades, incluida la depresión, por su eficacia terapéutica con efectos secundarios mínimos o nulos. Por lo tanto, el presente estudio se ha llevado a cabo para evaluar los efectos antidepressivos del extracto de semillas de Citrullus lanatus (CLSE) en el modelo de ratón deprimido inducido por reserpinia mediante la evaluación de los fitoconstituyentes del CLSE, el peso corporal, el comportamiento, la histopatología de la glándula suprarrenal y el nivel de corticosterona plasmática. Se distribuyeron 24 ratones.
machos adultos de cepa suiza en cuatro grupos de seis cada uno (n=6). El grupo I sirvió de control, mientras que los grupos II, III y IV recibieron reserpina (RES: 0,75mg/kg/PC/día) durante 14 días, CLSE (300mg/kg/PC/día) sólo, durante 28 días, y RES+CLSE [tratamiento con RES durante 14 días seguido de tratamiento con CLSE durante 28 días], respectivamente. Durante el tratamiento se analizaron las alteraciones del comportamiento mediante el consumo de alimentos, la preferencia por la sacarosa, el laberinto radial de ocho brazos y las pruebas de natación forzada. La depresión inducida por RES causó marcadas alteraciones en el peso corporal, en todos los parámetros conductuales, en la histopatología de la corteza suprarrenal y en el nivel de corticosterona plasmática, en comparación con el control. La administración de CLSE únicamente, no causó alteraciones significativas en todos los parámetros estudiados, en comparación con el control. Sin embargo, la administración de CLSE en ratones tratados con RES produjo un restablecimiento significativo de todos los parámetros estudiados, similar al del control. Los hallazgos sugieren, por tanto, la actividad antidepresiva de las semillas de C. lanatus, que puede emerger como una de las fuentes potenciales entre las terapias naturales del trastorno depresivo.

Palabras clave: parámetros conductuales, depresión, semillas de Citrullus lanatus, corticosterona.

1 INTRODUCTION

Depression is a complex and pervasive mental health disorder that affects approximately 280 million of people worldwide (Institute of Health Metrics and Evaluation, 2023). It is characterized by persistent feelings of sadness, irritability, insomnia or excessive sleeping, changes in eating habits, hopelessness, and a lack of interest or pleasure in daily activities. The impact of depression extends beyond the emotional realm, often interfering with one's ability to function in various aspects of life, such as work, relationships, and physical health. Depression results in several health issues related to gastrointestinal problems, cancer, diabetes, cardiovascular, and respiratory diseases (Qiao et al., 2021; Goodwin, 2022; Frank et al., 2023). Several neurochemicals, such as tetrabenazine (Carratella-Ros et al., 2023), parachloramphentamine (Pawlowski, 1988), 6-hydroxydopamine (Beppe et al., 2015), and reserpine (Dudau et al., 2023), are therapeutic drugs used in treatment of chorea (one of the common symptoms in Huntington’s disease), symptoms of allergic reaction, Parkinson’s disease, and hypertension through different relative monoamine-depleting specificity. However, such drugs are known to exert side effects on the physiological system of the body, causing mental illness (Cryan; Leonardo, 2000).
Reserpine is a rauwolfia indole alkaloid that acts as a sympatholytic and sedative agent and used in the treatment of hypertension (Guo et al., 2015). However, evidences from basic research and clinical trials have reported that reserpine causes severe depression (López-Muñoz et al., 2004; Veselinović et al., 2011; Chen et al., 2018; Strawbridge et al., 2023). Reserpine-mediated depression is reported to be caused by depletion of the monoamines in the brain, such as catecholamines including epinephrine, dopamine, and nor-epinephrine (Nagakura et al., 2009; Gao et al., 2013; Zhu et al., 2019; Van Onselen; Downing, 2021). This has been referred as the monoamine theory of depression, supported by numerous reports regarding reserpine-induced depression (Hanff et al., 2010; Govindarajulu et al., 2021).

Herbal remedies are widely used across the world for their effectiveness with least or no side effects which in turn have accelerated the scientific research regarding the antidepressant activity of several plants. Indian traditional system of medicine, claims a vast number of herbal constituents used in the treatment of several types of mental illness. Depression, a type of mental disorder, is reported to be improved in the experimental animals by use of some medicinal plants such as Bacopa monnieri (Hazra et al., 2012; Girish et al., 2016), Ocimum sanctum (Pemminati et al., 2010; Chatterjee et al., 2011), Piper longum (Lee et al., 2005; Kilari et al., 2015), Tinospora cardifolia (Dhingra; Goyal, 2008; Mutilak and Mutilak, 2011), Zingiber officinale (Olanrewaju et al., 2020; Gopal; Sudha, 2023), and Citrullus lanatus (Adnaik et al., 2014; Sandhya et al., 2020).

Citrullus lanatus (common name: watermelon), belonging to vine-like family Cucurbitaceae, is used in the treatments of various diseases. Leaves, pulp, rind, and seeds of C. lanatus possess antioxidant, anti-inflammatory, antifungal, antimicrobial, antiulcer, hepatoprotective, and analgesic activities (Erhirhie; Ekene, 2013; Alka et al., 2018; Gautam et al., 2023). It also possesses an antidepressant activity (Rahman et al., 2013; Adnaik et al., 2014; Sandhya et al., 2020). The ethanolic extract of C. lanatus seeds has shown similar effects with that of several synthetic antidepressant drugs like fluoxetine and imipramine at different doses (Adnaik et al., 2014; Sandhya et al., 2020). These authors have studied the antidepressant activity of C. lanatus seed extracts and synthetic antidepressants in the normal rats and mice. However, no study has been carried out so far regarding the efficacy of C. lanatus seed extract in the treatment of depression. The present study therefore focusses the evaluation of the antidepressive effects of the seeds of C. lanatus in the reserpine-induced depressed mice.
2 MATERIALS AND METHODS

2.1 ANIMALS

Adult male mice (8-10 weeks old) of Swiss strain, weighing 22-24 g were purchased from Central Animal House, Institute of Medical Science, Banaras Hindu University, Varanasi for the experimental studies. Mice were maintained in polypropylene cages, with rice husk as the bedding material under 12 h light and 12 h dark cycle at controlled temperature (25± 2 °C), and fed on pelleted food and water *ad libitum*. The experimental protocol was approved by the Animal Ethical Committee, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India (BHU/DOZ/IAEC/2021-2022/029, February 15, 2022) for the use of laboratory animals.

2.2 COMPOUNDS

Reserpine (C_{33}H_{40}N_{2}O_{9}) was purchased from Otto Chemie Pvt. Ltd. Chemika - Biochemika - Reagent Chemical Company, Mumbai, India. Seeds of *C. lanatus* were collected from local market of Varanasi, Uttar Pradesh, India and authenticated by Prof. N. K. Dubey, Department of Botany, Banaras Hindu University, (Voucher specimen no. Cucurbita. 2019/01) Varanasi, India. The extract of seeds of *C. lanatus* was prepared by adopting the method of Adnaik *et al.* (2014). Standardization of *C. lanatus* dose was determined by its ameliorative efficacy on reserpine-induced alterations in the behaviour of the mice.

2.3 EXTRACTION OF PLANT MATERIALS

The air-dried, coarsely-powdered *C. lanatus* seeds were subjected to cold maceration extraction with 70% alcohol for 72 hrs. The liquid extract was filtered and transferred to the rotatory evaporator for complete evaporation of the solvent. After evaporation, the remaining *C. lanatus* seed extract was kept in the refrigerator at 4°C for conducting the experiments.
2.4 HIGH-RESOLUTION AUTOMATED MASS SPECTROSCOPY ANALYSIS OF SEEDS OF *C. LANATUS*

The characterization of bioactive compounds analysis of *C. lanatus* seed extract was performed by using High-Resolution Accurate Mass Spectrometry (HRAMS) system equipped with quaternary pump, autosampler, and diode array detector (Thermo Fisher Scientific) at Sophisticated Analytical and Technical Help Institute (SATHI), BHU. Pump parameter settings included flow rate: 3.0 µl/min, volume: 250 µl, run time: 30 minutes. Solvent composition for small molecules was solvent A: 100% water + 0.1% formic acid, solvent B: 100% acetonitrile+ 0.1% formic acid, solvent C: 100% methanol + 0.1% formic acid. During the whole process, column temperature was maintained at 40 °C. The mass spectrum analysis was carried out using positive mode within the scan range of 100-2000 m/z.

2.5 EXPERIMENTAL DESIGN AND DOSAGE

Twenty-four adult male mice of Swiss strain were distributed into four groups of six each (n=6). Group I served as control while groups II, III and IV received reserpine (RES: 0.75mg/kg/BW/day) for 14 days, CLSE (300mg/kg/BW/day) only, for 28 days, and RES+CLSE [RES treatment for 14 days followed by CLSE treatment for 28 days], respectively. All the treatments were given through the oral route. The control group received corn oil only, by the same route.

2.6 ANIMAL SACRIFICE AND COLLECTION OF THE BLOOD

Behavioral analyses, including food consumption test, sucrose preference test, and eight-arm radial maze test were conducted daily in all the groups. However, forced swimming test was done weekly. After recording the final body weights, mice were sacrificed by cervical dislocation. Adrenal gland and blood were collected for the histopathological analysis and measurement of the level of plasma corticosterone, respectively.
2.7 BODY WEIGHT MEASUREMENT

The body weights were measured during the treatment period and calculated by using the following formula:

\[
\text{Body weight} = \frac{\text{Average of body weight}}{\text{Total number of days}}
\]

3 BEHAVIOURAL ANALYSES

3.1 FOOD CONSUMPTION TEST

This test was used to measure the appetite behaviour of the mice and was previously reported by the method of Várkonyi et al. (2022) with minor modifications. Briefly, mice were subjected to 1 week of acclimatization before testing of food consumption. During the test, three mice/empty cage were placed without bedding followed by ad libitum access to standard weighed chow (feed) for 2 hours. The mice were weighed before the test. After completion of the 2 hours of the test, the mice were kept back in their home cages. The consumed chow was weighed after a mouse was placed back in their home cage, and calculated by the difference in weight of feed kept in the cage and the left-over feed at the end of 2 hours.

3.2 SUCROSE PREFERENCE TEST

The sucrose preference test (SPT) is used to assess the level of depression (Teegarden, 2012) and is measured by the previously reported method of Cline et al. (2015) with minor modifications. Briefly, mice were given 2 hours of free choice between two bottles of either 1% sucrose solution or normal drinking water (equal volumes) to analyse the preference rate. Sucrose intake was correctly calculated as the amount of consumed sucrose in mg/g BW, while sucrose preference was calculated as a percentage of the volume of sucrose solution intake over the total volume of fluid (volume of sucrose solution+ volume of water) using following formula:
Sucrose preference = \( \frac{\text{Volume of sucrose solution intake}}{\text{Total volume of fluid}} \times 100 \)

3.3 EIGHT-ARM RADIAL MAZE TEST

Behavioral test using the eight-arm radial maze was conducted to know the spatial learning and behaviour of the mice as previously reported by Pu et al. (2004) with minor modifications. Briefly, the maze consisted of a central circular platform of 24 cm in diameter, with 8 arms extending radially. The end of each arm was provided with a cup for keeping the feed. Each mouse was allowed to visit in all the arms for consuming the feed kept in all food cups, for 5 minutes. Before starting the experiment, each test mouse was trained day 1 to 7 to memorize the apparatus. After training, the feed (pellets) was put in alternate food cups. Then, each mouse was allowed to choose arms until both either food cup was chosen or 5 minutes were elapsed. The performance of the test animals in each trial was assessed using time record.

3.4 FORCED SWIMMING TEST

This test was performed to measure the behavioural despair of the mice by adopting the method of Porsolt et al. (1978) with minor modifications. Briefly, on test day, each mouse of control and treated groups was placed in plexiglass cylinder (height: 30 cm, diameter: 15 cm), containing water (25 ± 2 °C; depth of 17 cm) in such a way that mice could neither escape nor touch the bottom. Mice were allowed to swim for 5 minutes and then taken out immediately, dried with a towel and kept back to their home cages. This test was performed weekly in each mouse of control and treated groups during the experiment to analyse the immobility. Immobility is the summation of the time during which mice remain in floating state without movement of the limbs.

3.5 HISTOPATHOLOGICAL STUDIES

The adrenal gland was dehydrated in a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were obtained by using an ultramicrotome, dehydrated in graded series of alcohol, stained with Eosin, and counterstained with Ehrlich's Haematoxylin.
3.6 ESTIMATION OF CORTICOSTERONE

The level of plasma corticosterone was estimated spectrophotometrically by adopting the method of Bartos and Pesez (1979).

3.7 STATISTICAL ANALYSES

All the data were analyzed statistically by Student’s T-test and one-way ANOVA followed by Post hoc Dunnett’s test. Analyses were performed by SPSS software version 17 (IBM, USA). Values were considered significant at p<0.05. The values were represented as mean ± SEM of the six animals.

4 RESULTS

4.1 HRAMS ANALYSIS OF SEEDS OF *C. LANATUS*

The HRAMS analysis of the seeds of *C. lanatus* has shown the existence of numerous bioactive phytoconstituents, among which few like choline, diosmetin, vitexin, luteolin, apigenin, linoleic acid, trigonelline, piperine, ferulic acid, pyridoxine, etc. are responsible for the treatment of depression (Figure 1 and Table 1).

![Figure 1. The chromatogram of HRAMS analysis of seeds extract of *C. lanatus*](image-url)

Source: from authors original raw data
Table 1. HRAMS analysis of seed extract of *C. lanatus* showing presence of phytochemicals

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<th>S.N.</th>
<th>Name of compounds</th>
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<th>Retention time [min]</th>
<th>Area [Max.]</th>
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<td>0.842</td>
<td>812184629</td>
</tr>
</tbody>
</table>

Source: from authors original raw data

4.2 BODY WEIGHT

Body weight was significantly decreased in the RES-treated mice, compared with the control (Figure 2). Only CLSE treatment in the normal mice did not cause any significant alteration in the body weight, compared with the control. CLSE administration in RES-treated mice caused a significant increase in the body weight, as compared with the RES-treated mice, thus similar to the control (Figure 2).
Figure 2. Effects of RES, CLSE and RES+CLSE on the body weight of the mice. Values represent the mean± SEM of six animals. * indicates significant difference from control group at p < 0.05. ** indicate significant difference from RES-treated group at p < 0.05

Source: from authors original raw data

4.3 FOOD CONSUMPTION TEST

Food consumption was significantly decreased in RES-treated mice, compared with the control (Figure 3). No significant alteration was observed in the food consumption of only CLSE treatment in normal mice, compared with the control. Reduced food consumption in RES-treated mice was restored significantly following administration of CLSE, as compared with the RES-treated mice, thus similar to the control (Figure 3).

Figure 3. Effects of RES, CLSE and RES+CLSE on the food intake of the mice in the FST. Values represent the mean± SEM of six animals. * indicates significant difference from control group at p < 0.05. ** indicate significant difference from RES-treated group at p < 0.05

Source: from authors original raw data
4.4 SUCROSE PREFERENCE TEST

RES administration led to pronounced significant decrease in sucrose preference and consumption of the mice, compared with the control (Figure 4). No significant alteration was observed in the immobility time of the mice treated only with CLSE in the normal mice, compared with the control. CLSE administration in RES-treated mice marked recoupment in sucrose preference and consumption, as compared with the RES-treated mice, thus similar to that of control (Figure 4).

Figure 4. Effects of RES, CLSE and RES+CLSE on the sucrose preference and intake of the mice in the SPT. Values represent the mean± SEM of six animals. * indicates significant difference from control group at p < 0.05. ** indicate significant difference from RES-treated group at p < 0.05.

Source: from authors original raw data

4.5 EIGHT ARM-RADIAL MAZE TESTS

Significant increase was noted in the impairment of spatial learning and memory of the RES-treated mice, compared with the control (Figure 5). Only CLSE treatment in the normal mice did not cause any significant alteration in their spatial learning and memory, compared with the control. CLSE administration in RES-treated mice restored the impaired spatial learning and memory significantly, as compared with the RES-treated mice, thus similar to the control (Figure 5).
4.6 FORCED SWIMMING TEST

RES treatment caused a significant reduction in the immobility time of the mice, compared with the control (Figure 6). No significant alteration was observed in the immobility time of the mice treated only with CLSE in the normal mice, compared with the control. CLSE administration in RES-treated mice was significantly restored the immobility time of the mice, as compared with the RES-treated mice, thus similar to the control (Figure 6).
4.7 HISTOPATHOLOGY OF ADRENAL GLAND

RES administration resulted in marked histopathological alterations in the adrenal cortex. The cells of all the three layers of adrenal cortex showed disorganized architecture, especially the cells of zona fasciculata, a corticosterone secreting layer which appeared hypertrophied, filled with lipid droplets, large cytoplasmic vacuoles, condensed and vacuolated nuclei, compared with the control (Figure 7). No histopathological changes were observed in the adrenal cortex of the mice treated only with CLSE in normal mice, compared with the control. CLSE administration in RES-treated mice resulted in marked recovery in the zona fasciculata layer of adrenal cortex, as compared with the RES-treated mice, thus similar to the control (Figure 7).

Figure 7. A-D: 40X T.S. of the adrenal cortex of control showing normal histopathology A, RES administered mouse showing the disorganized and hypertrophied zona fasciculata cells filled with lipid droplets, large cytoplasmic vacuoles (red arrows), condensed and vacuolated nuclei (black arrows) B, Administration of CLSE only showing normal histopathology C, CLSE administration in RES-treated mice showing complete recovery in the zona fasciculata layer of adrenal cortex D

Source: from authors original raw data

4.8 PLASMA CORTICOSTERONE LEVEL

The level of plasma corticosterone showed a significant increase in RES-treated mice, compared with the control (Figure 8). Only CLSE treatment in the normal mice did not alter the level of plasma corticosterone significantly, compared with the control. CLSE administration in RES-treated mice resulted in a significant restoration in the level of plasma corticosterone, as compared with the RES-treated mice, thus similar to the control (Figure 8).
5 DISCUSSION

In contrast to the synthetic antidepressant drugs, very few studies have reported the antidepressive effect of *C. lanatus* in the normal/untreated rats and mice (Rahman *et al.*, 2013; Adnaik *et al.*, 2014; Sandhya *et al.*, 2020). However, its antidepressant effects in the drug-induced depressed mice have not been studied so far. Therefore, the present study is focused to evaluate the effects of seeds of *C. lanatus* in the reserpine-induced depressed mouse model by assessing the analysis of phytoconstituents of seeds of *C. lanatus*, body weight, behaviour, histopathology of the adrenal gland, and the level of plasma corticosterone.

Significantly reduced body weight of RES-treated depressed mice is consistent with the previous findings reported in the mice (Park *et al.*, 2020; Tang *et al.*, 2022). Reduction in food intake during depression is a contributing factor in decreased body weight (Tang *et al.*, 2022). Unaltered body weight of only CLSE-treated mice has also been reported in the rat (Oyenihi *et al.*, 2016). Remarkable restoration in the body weight of CLSE-treated depressed mice is due to increased food intake as measured by food consumption test. Similar finding has been reported in diabetic rats treated with fruit’s pulp of *C. lanatus* (Oseni *et al.*, 2015).

The preference and consumption of palatable sweet solutions (sucrose or saccharin) are the most frequently used test to measure sensitivity to reward ‘anhedonia’ in rodents (Hoffman, 2016). Depressed mice, analysed for SPT, had a significant
reduction in sucrose preference and consumption indicating anhedonic behaviour. Such behaviour has also been reported previously during chronic mild stress or chronic social defeat exposure in the mice (Slattery et al., 2007; Krishnan and Nestler, 2011). This behavior could be related to dysfunction of the dopaminergic and noradrenergic pathways of the limbic reward system (Kaminska et al., 2017). Unaltered sucrose preference and consumption observed in only CLSE-treated mice in the present study, has also been reported previously by Ge et al. (2023) in the mice by the treatment of cucurbitacin B. Significant improvement in sucrose preference and consumption noticed in the CLSE-treated depressed mice has also been reported in the unpredictable chronic mild stressed rat treated with Cannabis sativa essential oil (Kabdy et al., 2024). According to these authors the Cannabis sativa essential oil improves the impaired dopaminergic and noradrenergic pathways in the brain.

Spatial learning and memory are neurological functions that allow to retain important informations associated with the environment. Lesions, pharmacological inhibition, the act of learning itself, and genetic mutations of signalling molecules or receptors within the hippocampus region of the brain result in impaired spatial learning and memory (Burgess et al., 2002; Whitlock et al., 2006; Penner; Mizumori, 2012; Buzsaki and Moser, 2013; Suh et al., 2013). The hippocampus and medial entorhinal cortex of the autonomic-hypothalamic-limbic system of the brain is implicated as an essential region for memory, learning, exploration, anxiety and fear. This form of neurobehavioral cognition in the animals is assessed by several maze test including eight-arm radial maze. Reserpine-induced impaired spatial learning and memory found in our study is consistent with the finding reported in the rats (Naidu et al., 2004; Tseng et al., 2015). Significant increase in the spatial learning and memory noticed in the depressed mice may be possibly due to inhibition of hippocampus neurogenesis. Culig et al. (2017) have reported the inhibition of hippocampus neurogenesis in unpredictable chronic mild stress-induced depressed mice. Unaltered spatial learning and memory in only CLSE-treated mice is consistent with the finding of Wahid et al. (2020) in the same animal. CLSE-induced improvement in learning and memory of RES-treated depressed mice has also been reported previously by Maifitrianti and Suryadi (2021) in the mice induced the state of dementia treated with steamed C. lanatus juice. These authors have reported the C. lanatus juice improved the damaged hippocampus neurogenesis of the brain.

In forced swimming test (FST), the animals are forced to swim in a restricted and inescapable space showing characteristic of depressive-like behavior. The immobility
signifies behavior resembling a state of depression. Drugs that enhance the activity of the central dopaminergic and α-adrenergic systems reduce the immobility time in rodents (Koneru et al., 2010). Monoamines are transported into presynaptic vesicles via the vesicular monoamine transporter (VMAT), which is blocked by reserpine (De Freitas et al., 2016). Reserpine-induced depression causes monoamine depletion resulting in anxiety- and depressive-like behaviours via reduction in immobility time by conducting the FST and tail suspension test (Samad et al., 2021). Such alterations in the levels of monoamines induce depression. The present study showed the RES-induced depression-like behaviours in mice, as indicated by significant decrease in immobility time while performed FST. Our findings demonstrated that such decrease in the immobility time was due to reductions in spontaneous motionless activity. Unaltered immobility time in only CLSE-treated mice has also been reported in the rat treated with Curcuma aromatica (Jaseela et al., 2016). Significant restoration in the immobility time in CLSE-treated depressed mice is consistent with the findings reported in the rats treated with Ocimum sactum and Camellia sinensis (Tabassum et al., 2010). Restoration in the immobility time may be possibly due to protective effects of CLSE on the monoaminergic receptor mediated neurotransmission.

The main mechanism behind depression is depletion of monoamine neurotransmitters such as dopamine, nor-epinephrine and serotonin. Treatment with reserpine (RES) significantly reduces monoamine levels thus resulting in increased metabolites of monoamines such as 5-HTAA, DOPAC and HVA (Gao et al., 2016). RES inhibits pre-synaptic catecholamine reuptake and storage, depleting monoamines and accelerating neurotransmitter oxidative catabolism with monoamine oxidase (Nagakura et al., 2009) which successfully prove the depressive action of this drug.

Disorganized and hypertrophied zona fasciculata cells filled with lipid droplets, noted in the adrenal cortex of the RES-treated depressed mice is similar to that of rats during chronic mild stress-induced depression (Wang et al., 2018) and stress (Zaki et al., 2018). Unaltered histopathology of the fasciculata cells in the adrenal cortex of only CLSE-treated mice have also been reported previously by Savici et al. (2017) in the rat, treated only with leaf extract of Hypericum perforatum, that also acts as an antidepressant (Lazzara et al., 2015). Restoration in the impaired histopathology of the zona fasciculata layer of the adrenal cortex of the depressed mice, treated with CLSE is consistent with the finding of Sadek et al. (2021) in the mice treated with Licorice extract during chronic unpredictable mild stress. It is generally known that stress affects the hypothalamic-
pituitary-adrenal (HPA) axis, an important element of the neuroendocrine system, which may be measured by the levels of adrenocorticotropic hormone and corticosterone. The HPA axis offers negative feedback to maintain normal circulatory glucocorticoid levels. Long-term stress may promote HPA axis hyperactivity, which may elevate adrenocorticotropic hormone and corticosterone/cortisol levels, linked to depressive illness in mice and humans (Jozuka et al., 2003). Corticosterone is a main glucocorticoid, involved in regulation of energy, immune reactions, and stress responses. Significant increase in the level of plasma corticosterone may be one of the causes of depression. Similar to reserpine (Wang et al., 2023), nicotine also causes depression by inducing significant increase in the level of corticosterone in the rat (Zhang et al., 2019). Unaltered level of plasma corticosterone noted in the CLSE-treated mice in the present study is consistent with that of *Papaver rhoeas*-treated mice (Shahyad et al., 2023). Restoration in the level of plasma corticosterone in the CLSE-treated depressed mice is consistent with stress-induced depressed mice, treated with natural antidepressants, such as *Fraxinus rhynchophylla* and *Butea superba* (Kim et al., 2018). According to these authors, these natural antidepressants modify the downregulation of hyperactive HPA axis, resulting in restoration in the level of corticosterone.

The antidepressant effects of the seeds of *C. lanatus* depends on the existence of several bioactive phytochemical constituents. The HRAMS of seeds of *C. lanatus*, conducted in the present study, has revealed the presence of several active phytoconstituents among which choline (Glenn et al., 2012), diosmetin (Saghaei et al., 2020), vitexin (Abbasi et al., 2013; Lima et al., 2018), luteolin (Yoo et al., 2013), apigenin (Qi et al., 2020; Kim et al., 2021), linoleic acid (Nagata et al., 2005), trigonelline (Chowdhury et al., 2018a; 2018b), piperine (Mao et al., 2014), ferulic acid (Fleck et al., 2010), pyridoxine (Kuypers; Hoane, 2010) are reported to improve the mental disorder in the experimental animals. In our study, it is speculated that more than one phytoactive compounds might have involved in restoring the depression disorder. Therefore, further study is required in this direction and for elucidation of the precise mechanism underlying the antidepressant effects of seeds of *C. lanatus*. 
6 CONCLUSION

The findings of the present study prove the antidepressant activities of seeds of *C. lanatus* as a recommended natural medicine and provide a clue in emergence of natural antidepressant from well-known folk remedies.

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ABBREVIATIONS

CLSE: *Citrullus lanatus* seed extract;
EARMT: eight-arm radial maze test;
FCT: food consumption test;
FST: forced swimming test;
HPA: hypothalamus-pituitary-adrenal;
HRAMS: High-resolution automated mass spectroscopy;
RES: reserpine;
SPT: sucrose preference test;
VMAT: vesicular monoamines transporter.