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Antidepressant-like effect of NevGro® Forte in chronic unpredictable mild stress (CUMS) model of depression in rats

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ABSTRACT: Depression is a leading cause of disability worldwide and it is a major contributor to the overall global burden of disease. Almost 320 million individuals globally are left untreated with depression and it has the highest prevalence. Although there are multiple conventional antidepressant types available, patients are still left untreated due to inadequate pharmacological effectiveness as well as the high rate of remissions, side effects, and patients' non-compliance. Therefore, this study aims to determine the therapeutic effects of NevGro® Forte. The NevGro® Forte that contains a combination of three types of mushrooms, *Lignosus rhinocerotis, Ganoderma lucidum* and *Hericium erinaceus,* has been reported to have the therapeutic potential for alleviating depressive symptoms. Sixty Sprague Dawley rats induced to chronic unpredictable mild stress (CUMS) protocol were orally treated with NevGro® Forte daily for 4 weeks. Histological analysis was performed to probe the role of neurogenesis in mediating the therapeutic effect of NevGro® Forte. Fluoxetine (FLX) was orally administered to validate the neurogenesis-dependent mechanism of NevGro® Forte. The present study exhibited that 4 weeks of NevGro® Forte treatment ameliorated the depressive symptoms in CUMS rat model. There is a significant improvement in body weight, brain's weight, and increased thickness of the pyramidal layer in the hippocampus following the treatment of NevGro® Forte. Scanning electron microscopy also revealed decreased degeneration characterised by flattened with less dense surface composition in the hippocampus. This research shows a positive outcome of using NevGro® Forte in ameliorating depressive symptoms.

Keywords: Depression, Neurogenesis, Antidepressant, Chronic Unpredictable Mild Stress (CUMS), NevGro® Forte

1.0 INTRODUCTION

Depression is a leading cause of disability worldwide and a major contributor to the overall global burden of disease (WHO, 2019). It affects almost 320 million people worldwide and has the greatest frequency (Poleszak et al., 2020). In 2019, the Global Burden of Disease Study demonstrated that depression was ranked at 13 in depressive disorders, which has been dramatically increasing as the Covid-19 epidemic enters its third year (Vos et al., 2020). A recent study reported that COVID-19 has significantly increased depression and anxiety in patients, with an increase of 53.2 million and 76.2 million diagnosis respectively (Santomauro et al., 2021). Depression is a primary debilitating and common neurological disease affecting people of all ages, characterized by melancholia, anhedonia, and apathy (Tang et al., 2019). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013), the operational criteria for major depressive disorder are depressed mood, diminished interest or pleasure, weight loss or weight gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive guilt, diminished concentration or indecisiveness, and death wish or suicidal ideation (American Psychiatric Association, 2013). The presence of five or more of these symptoms to boot at least a sad mood or impaired interest or pleasure, is required for an operational diagnosis of major depressive disorder (Kim & Park, 2021).

Pharmacotherapy is an essential component of depression treatment apart from electroconvulsive therapy and psychotherapy (Wyska, 2019). Several medication classes are available for this disorder, which are classified into older and newer antidepressants. Tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) are the older antidepressants. In contrast, newer antidepressants include selective serotonin reuptake inhibitors (SSRIs), serotonin modulators and stimulators (SMSs), serotonin antagonist and reuptake inhibitors (SARIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), norepinephrine-dopamine reuptake inhibitor (NDRIs), norepinephrine reuptake inhibitors (NARIs), and noradrenergic and specific serotonergic antidepressants (NaSSAs) (Wyska, 2019). While there are multiple antidepressant medication types available on the market, patients are still left untreated due to insufficient pharmacological effectiveness as well as a high rate of remissions, side effects, and patients' non-compliance (Poleszak et al., 2020). Although currently available antidepressants can only relieve symptoms of depression in about 20% of patients (Chong et al., 2019), discontinuation of antidepressants can be quite difficult due to the significant prevalence of signs of discontinuation such as insomnia, mood disturbance, flu-like symptoms, paraesthesia and dizziness (Poleszak et al., 2020).

Moreover, females are more prone than males to suffer from depression (Park & Zarate, 2019) due to the varied elevated stress levels in daily life and the accompanying symptoms (Dudek et al., 2019). Depression in women has been connected to the start of puberty and has been shown to continue throughout the reproductive years, especially when estradiol levels fluctuate (Dudek et al., 2019). Gender disparities in tolerance and responsiveness to antidepressant medication have also been discovered where women respond better to antidepressants that contain SSRI. In contrast, males respond better to tricyclic antidepressants (Dudek et al., 2019).

Many mushrooms, including *Lignosus rhinocerotis, Ganoderma lucidum*, and *Hericium erinaceus*, are commonly employed in studies exhibiting neuro-activities due to their effectiveness in treating depression or persistent moderate stress. Mushrooms are nourishing, functional foods that are high in novel medicinal components (Chong et al., 2019). Numerous studies have shed light on medicinal mushrooms' neuroprotective benefits, which are attributable to their antioxidant, anti-neuroinflammatory, cholinesterase inhibitory (Lew et al., 2020), neurogenic and neurotrophic modulation as well as monoamine modulation, that can promote positive nerve and brain health (Chong et al., 2021). The antidepressant qualities of this specific fungus can be attributed to a range of bioactive compounds with diverse therapeutic characteristics. Several bioactive compounds originating from mushrooms have been shown to increase nerve growth factor (NGF) production and neurite outgrowth, both of which contribute to alleviating depressive-like symptoms (Chong et al., 2021). Furthermore, *in vivo* studies have discovered that mushrooms may modify brain-derived neurotrophic factor (BDNF) signalling and boost serotonin, dopamine, and noradrenaline expression (Chong et al., 2021).

2.0 MATERIALS AND METHODS

2.1 Sample Sources and Preparation

Preparation of NevGro® Forte

NevGro® Forte is a product that consists of *Lignosus rhinocerotis, Ganoderma lucidum* and *Hericium*

erinaceus extract powder which was formulated, manufactured, and registered under Ganofarm R&D Sdn Bhd. These three mushrooms were cultivated in an organically certified farm under Ganofarm R&D Sdn Bhd. The extract powder was freshly prepared daily at a concentration of 1,400 mg/kg body weight by mixing with distilled water and was administered to the CUMS rats by oral gavage technique.

Drug administration

Fluoxetine hydrochloride, 5-hydroxytryptamine (5-HT) and SSRI (BIOSYNTH® Carbosynth) were given orally to the positive control rats with a concentration of 10 mg/kg body weight daily during the treatment periods (Liu et al., 2017).

2.2 Experimental animals

Sixty Sprague-Dawley rats aged eight weeks weighing 200 to 250 g were obtained from the animal house of Management and Science University (MSU). The surgical protocol was approved by the Research Management Centre (RMC), Animal Care and Use Committee, Management and Science University (MSU) with reference number MSU-RMC-02/FR01/02/L3/020. The animals were divided into five groups: normal group (Group 1) – the rats did not undergo any CUMS protocol; negative control (Group 2) – the rats underwent the CUMS protocol without any treatment given throughout the CUMS regimen days; positive control (Group 3) – the rats underwent the CUMS protocol and received treatment of 10mg/kg fluoxetine (FLX) via oral gavage for 28 days; treatment group (Group 4) – the rats underwent the CUMS protocol and received treatment of 1400 mg/kg daily dosage of NevGro® Forte extract via oral gavage for 28 days; pre-treatment group (Group 5) – the rats were given the treatment of 1400 mg/kg daily dosage of NevGro® Forte extract via oral gavage for 28 days prior to initiating the CUMS protocol for 56 days. Each group consists of 12 rats. The rats were fed with a conventional diet (Gold Coin Feedmills (M) Sdn Bhd) throughout the experiment.

2.3 The chronic unpredictable mild stress (CUMS)

This experiment was according to Frisbee and team (2015) protocol. The animals were relocated daily to a clean room for CUMS manipulation, weighed and checked for any physical abnormalities before the commencement of the CUMS protocol. Each of the animals was then exposed to one of the stressors listed below which was performed on a randomized schedule and delivered for 4 hours (**Table 1**).

Table 1: Randomized stressor schedule was applied for eight weeks to four CUMS groups that induce depressive-like behaviours in rats.

Days	Group 2: Negative Control (CUMS-induced)	Group 3: Positive Control (CUMS + FLX)	Group 4: Treatment (CUMS + NevGro® Forte)	Group 5: Pre-treatment (NevGro® Forte + CUMS)
Monday	Tilted cages	Damp bedding	Shallow bedding	Bedding removal
Tuesday	Damp bedding	Shallow bedding	Bedding removal	Social stressor
Wednesday	Shallow bedding	Bedding removal	Social stressor	Predator
Thursday	Bedding removal	Social stressor	Predator	L/D alteration
Friday	Social stressor	Predator	L/D alteration	Tilted cages
Saturday	Predator	L/D alteration	Tilted cages	Damp bedding
Sunday	L/D alteration	Tilted cages	Damp bedding	Shallow bedding

Damp bedding
The bedding of each cage was dampened by pouring 600mL of clean water into each standard cage to fully dampen the bedding, but it was careful enough not to cause pooling of water. The animals were then resided in their damp cage for 4 hours.

Bedding removal
The bedding was removed from each cage for 4 hours, after which animals were transitioned into another stressor requiring an empty cage (shallow bedding, tilted cage) or placed into a clean cage with fresh bedding.

Tilted cages
Cages were tilted to approximately 45° (with bedding) for 4 hours. The cages were tilted with a sturdy object that remained in place as the animal moved around in the cage.

Light/dark alteration
Their regular 12/12-hour light/dark cycles were altered into successions of 30-minute period lasting for 8 hours. Then, the normal cycle was resumed.

Social stressor
Each group of animals was transferred from its home cage to the cage of a neighbouring animal that was removed for another stressor for 4 hours.

Shallow bedding
The bedding from each cage was removed and water was poured to a depth of 1.3 cm for 4 hours. Only water warmer than room temperature (~30 °C) was used to minimize hypothermia potential. Then, the animals were dried briefly with a soft towel at the conclusion of the water exposure prior to placement into clean cages.

Exposure to predator
The animals were exposed to predator smells/sounds by adding randomly distributed sample tufts of cat's fur/snake's skin or their urine and faeces (20 mL) into the cage for 4 hours. Alternatively, growling or noises from the same natural predator species were played close to the cages for 4 hours. At the end of each daily stress period, all animals were placed back into clean cages and returned to the housing facility.

2.4 Behavioural assessment: sucrose preference test
Behavioural test was used to quantify behavioural changes from CUMS in the rat models. This test was conducted in a room while limiting unnecessary movements and noises to prevent interruption. The test was recorded using a GoPro camera according to the stated duration and analysed using smart software. The details of the tests that were conducted are as follows:

Sucrose preference test (SPT) was executed to assess anhedonia, a depressive-like behaviour, measured by the preference of sucrose solution over water. The test was done twice: post-CUMS and post-treatment; to assess the success in inducing depressive-like behaviour and to examine the progress following treatments. The test was conducted by placing two bottles, respectively filled with 1% sucrose solution and tap water. Habituation for the test was done in 72 hours and the test was conducted for three hours. Significantly low sucrose preference indicates anhedonia in the animal. The animals were habituated to drink 1% sucrose solution (w/v) 72 hours before the test and consequently provided exposure to two bottles (1% sucrose solution versus tap water in different bottles). After habituation, the animals were given *ad libitum* access to sucrose solution and tap water for 3 hours. After 3 hours, the sucrose solution and tap water consumption volumes were recorded and calculated. Sucrose preference (%) was calculated using the formula [(sucrose consumption)/(sucrose consumption + water consumption)] x 100 (Frisbee et al., 2015).

2.5 Body weight measurement
Using an analytical balance, the body weight of each rat was measured every week on the same day for 12 weeks of the experimental period. The rats were excluded from the experiment if only there is a significant reduction in body weight more than 10% from baseline weight or more than 15% reduction from last measured weight (Burstein et al., 2018).

2.6 Gross morphology of the brain
Experimental rats from each group were euthanized using chloroform, and the brain was harvested and weighed. The width and height of the brain were measured by using a vernier calliper.

2.7 Histological studies
Histological analyses were performed to observe the hippocampus of the animal's brain focusing the dentate gyrus and the CA-3 region. Both hippocampal regions play a crucial role in learning and memory in humans and animals. The dentate gyrus serves primarily as a pre-processor of incoming information, preparing it for later processing in the CA-3 region (Jonas & Lisman, 2014). Whilst CA-3 performs a specific function in memory processing, seizure susceptibility, and neuro-degeneration (Cherubini & Miles, 2015). The brains

were stained with a basic haematoxylin and eosin (H&E) staining and cresyl violet staining to observe neurons in the brain.

After dissection, the rat brain was harvested, from which the hippocampus was immediately isolated on ice fixed in 10% formalin, followed by graded dehydration, and embedded in paraffin wax (Adebiyi et al., 2020). The brain was cut into coronal sections prior to the dehydration process. Level three of the brain is the most crucial brain part for the study, where the hippocampus lies. Then, the embedded paraffin wax was sectioned with a thickness of 5 µm using microtome (Leica 235) and stained with H&E and cresyl violet.

Scanning electron microscopic analysis was done to observe the surface of the rat hippocampus with a focused beam of electrons. The brains were sectioned into 1 cm^3 pieces of the hippocampus and perfused with 4% glutaraldehyde for 12 to 24 hours under 4°C. The tissues were washed with 0.1M sodium phosphate buffer (pH 7.2) for three cycles. Each cycle was left at room temperature for 10 minutes and washed. The specimens were post-fixed in 1% Osmium Tetroxide for two hours at 4°C and infiltrated with ethanol and hexamethyldisilazane (HMDS). Finally, the specimens were dried and mounted with carbon conductive adhesive 502 and coated with sputter coater prior to the viewing step.

2.8 Statistical analysis

The assessment of body weight and brain weight were analysed using GraphPad Prism 9.4.1 version with one sample T-test, and one-way analysis of variance (ANOVA) to compare the mean values of each group to the control group. Tukey's multiple comparisons test was performed to test the means across other groups. All the data were expressed as mean ± standard error mean (SEM). Values of $p<0.05$ were considered statistically significant.

3.0 RESULTS

3.1 Behavioural assessment: sucrose preference test

The mean sucrose preference for SPT1 (post-CUMS) and SPT2 (24 hours post-CUMS) are displayed in **Figure 1** and **Figure 2** respectively. In SPT1, one-way ANOVA revealed that SPT1 (post-CUMS) generally exhibited significant differences between the healthy control (non-CUMS) and CUMS group (G1 vs. G2) with p-values $p<0.0001$. On the other hand, significant difference was observed between the CUMS group and the pre-treatment group (G2 vs. G5, $p<0.0001$).

General trend exhibited that the NevGro® Forte treatment groups (G4 and G5) have higher mean sucrose preference than the negative control (G2) and FLX treatment (G3) groups. However, a significant effect of NevGro® Forte was only observed in Group 4 (CUMS + NevGro® Forte) and Group 5 (NevGro® Forte + CUMS) treatment groups when compared to negative control (G2, only CUMS), with p-values of 0.0006 and 0.0028 respectively. Interestingly, the same groups also observed no significant difference when tested against the healthy control Group 1.

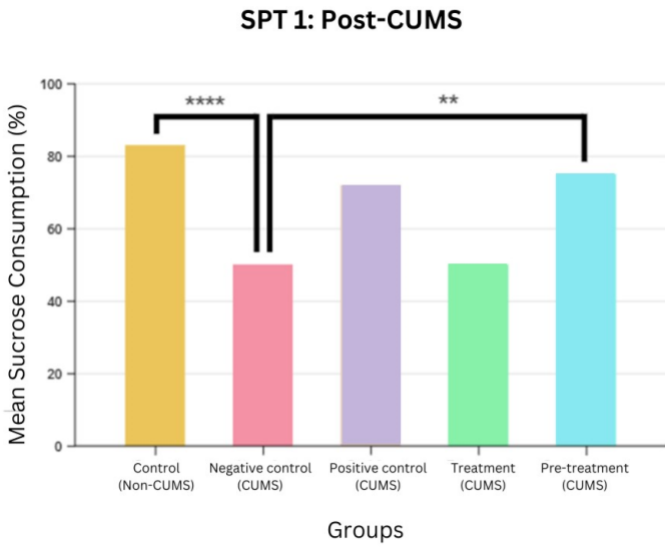

Figure 1: SPT 1 displays the effects of 56-day CUMS on Control (Healthy subjects, Group 1), negative Control (CUMS, Group 2), positive control (CUMS + FLX, Group 3), treatment (CUMS + NevGro® Forte, Group 4), and pre-treatment (NevGro® Forte + CUMS, Group 5). N=12 per group. The test was performed on day 57 which is 24 hours post-CUMS. Values are presented as mean ± SEM. The asterisk indicates $p<0.05$.

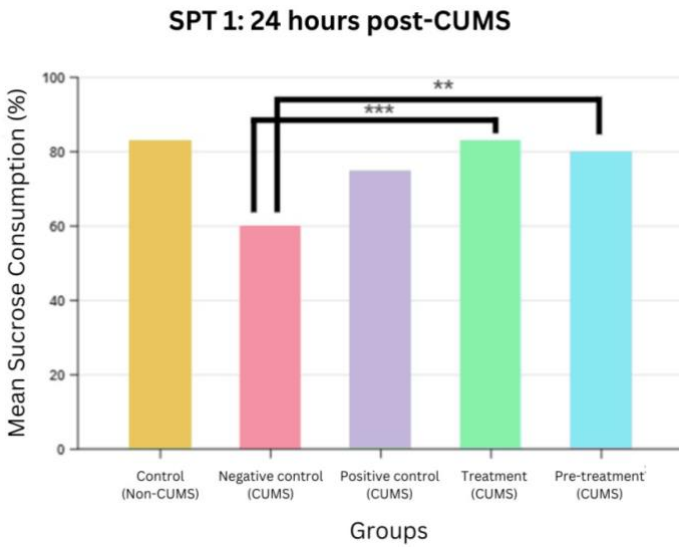

Figure 2: SPT 2 assessed the effects of treatments given to the animals that displayed depressive-like behaviours confirmed by SPT 1. SPT 2 was conducted at 24-hours post-completion of treatment on Control (Healthy subjects, Group 1), negative Control (CUMS, Group 2), positive control (CUMS + FLX, Group 3), treatment (CUMS + NevGro® Forte, Group 4), and pre-treatment (NevGro® Forte + CUMS, Group 5). N=12 per group. Data expressed as mean ± SEM. The asterisk indicates $p<0.05$.

3.2 Body weight measurement

The body weight of rats was measured throughout the experimental period. A decrease in body weight is indicated as one of the depressive symptoms. In this study, no significant changes were observed in the treatment and control groups (**Figure 3**).

3.3 Gross morphology of the brain

Gross morphological observation on the brain did not show any abnormality except a significant difference in the brain size and weight in the negative control group ($p<0.05$) as shown in **Figure 4** and **Figure 6**, as well as the size and structure differences in the hippocampus (**Figure 5**).

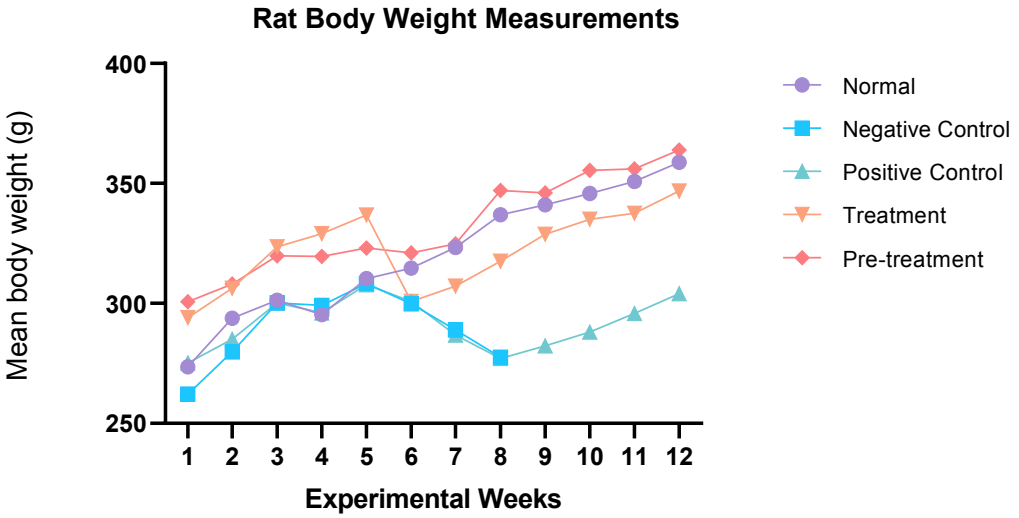

Figure 3: Mean weight of the experimental rats (g), N=12 per group. Data presented as mean ± SEM. Negative control ends at week eight of the study due to the CUMS protocol.

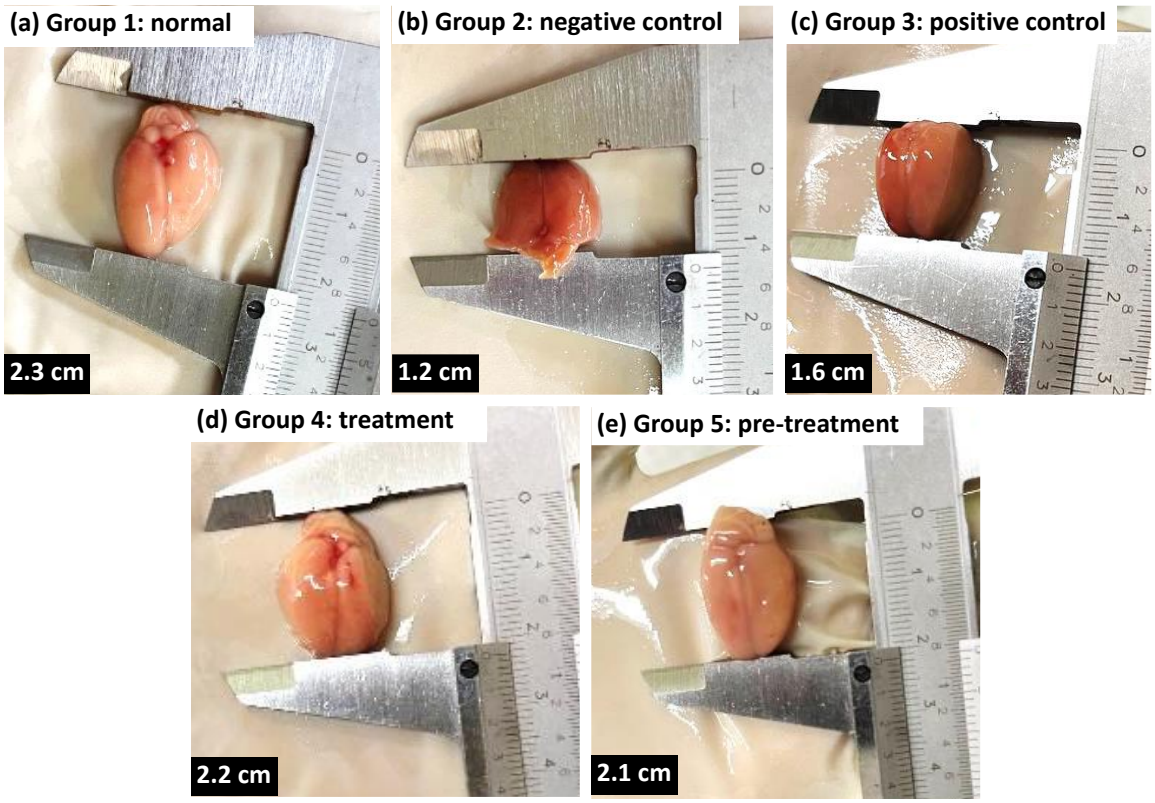

Figure 4: Gross morphology of the rats' fresh brains, **(a)** normal, **(b)** negative control (CUMS), **(c)** positive control (CUMS + FLX), **(d)** treatment (CUMS + NevGro® Forte), and **(e)** pre-treatment (NevGro® Forte + CUMS).

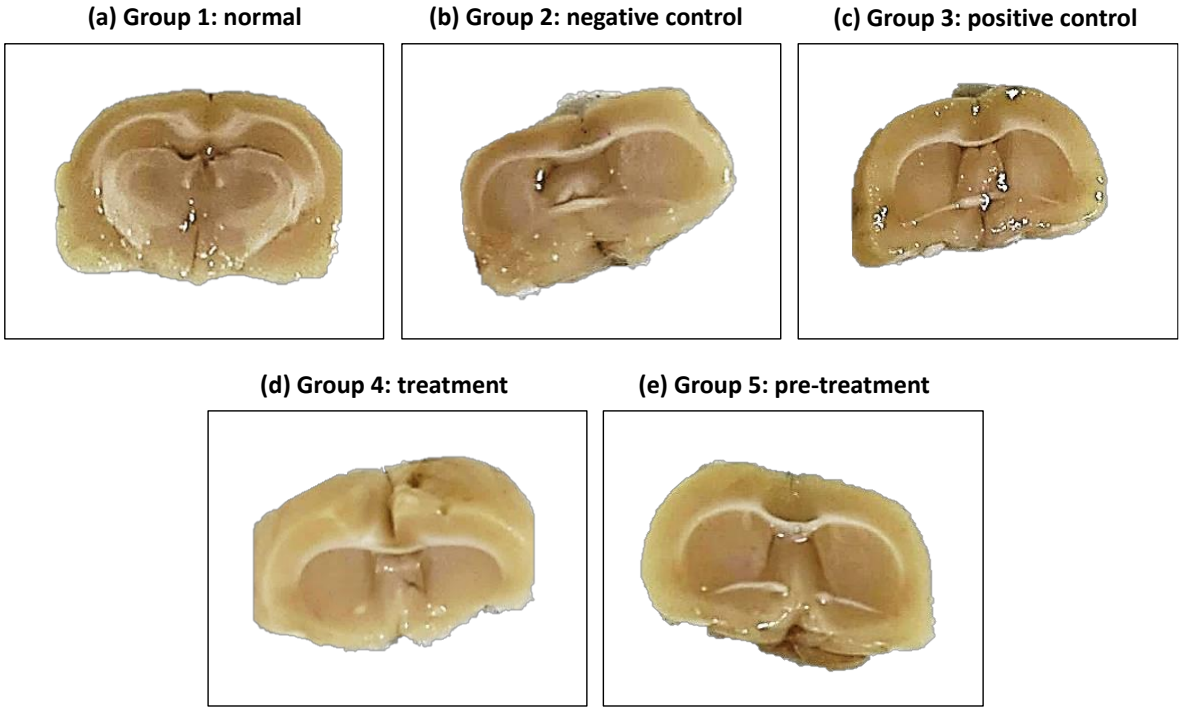

Figure 5: Gross morphology of the rats' brains cut into coronal sections after fixed in 10% formalin, **(a)** normal, **(b)** negative control (CUMS), **(c)** positive control (CUMS + FLX), **(d)** treatment (CUMS + NevGro® Forte), and **(e)** pre-treatment (NevGro® Forte + CUMS).

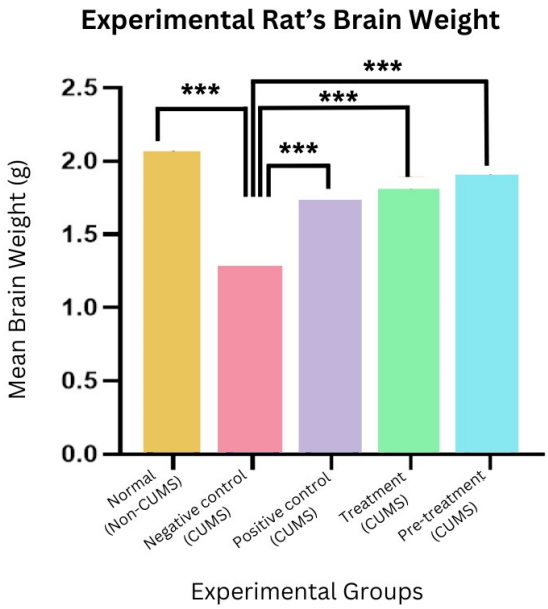

Figure 6: Mean weight of the experimental rats' brain (g), N=12 per group. Values are presented as mean ± SEM. The asterisk represents the significant value where the $p<0.05$.

3.4 Histological analysis

The brains were stained with H&E and cresyl violet to observe the brain structure and neurons. Based on the findings, the molecular layer (ML), granular cell layer (GCL), and pleomorphic layer (PL) can be observed in the dentate gyrus (**Figures 7 & 9**). Whereas pyramidal cell layer (PCL), stratum oriens (SO), stratum lucidum (SL), and neurites outgrowth (NG) of CA-3 region can be observed (**Figure 8 & 10**). Also, the thickness of GCL and PCL were determined. In the negative control group, the thickness of GCL and PCL are reduced and appeared smaller with scattered arrangements compared to the healthy control and treatment groups. Even among the treatment groups, NevGro® Forte shows a positive outcome compared to the positive control group that shows slightly scattered PCL arrangements.

Scanning electron microscopy (**Figure 11**) showed that the hippocampus surface in the negative control group (CUMS-induced) is flattened with a less dense surface composition compared to the healthy control group where the hippocampus surface is in denser composition which is literally like an active growth surface.

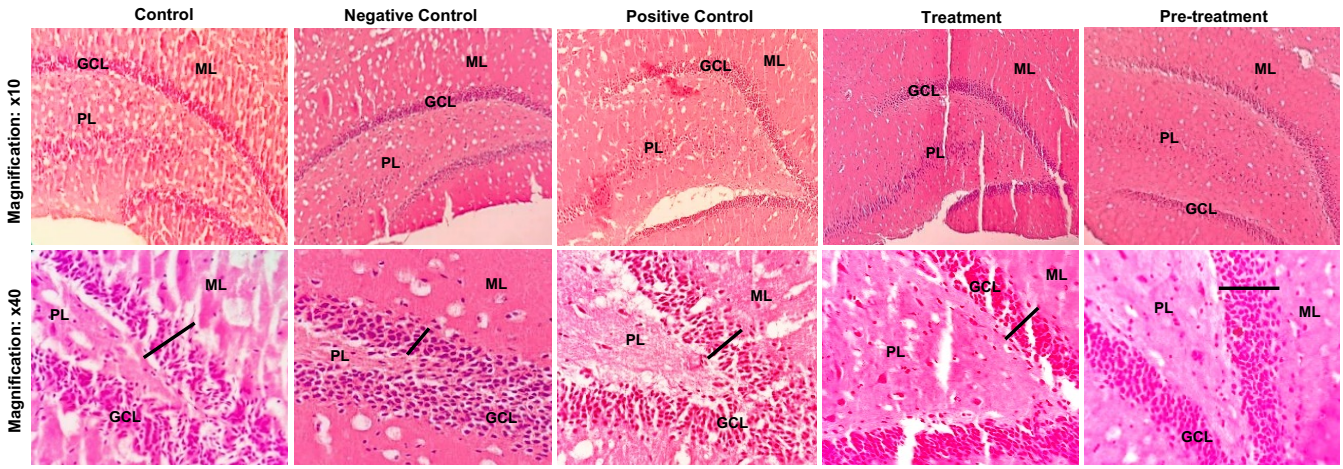

Figure 7: Dentate gyrus of rat hippocampus stained with H&E, observed under the optical light microscope with magnification of x10 and x40. ML, molecular layer; GCL, granular cell layer; PL, pleomorphic layers. Black line indicates the thickness of GCL.

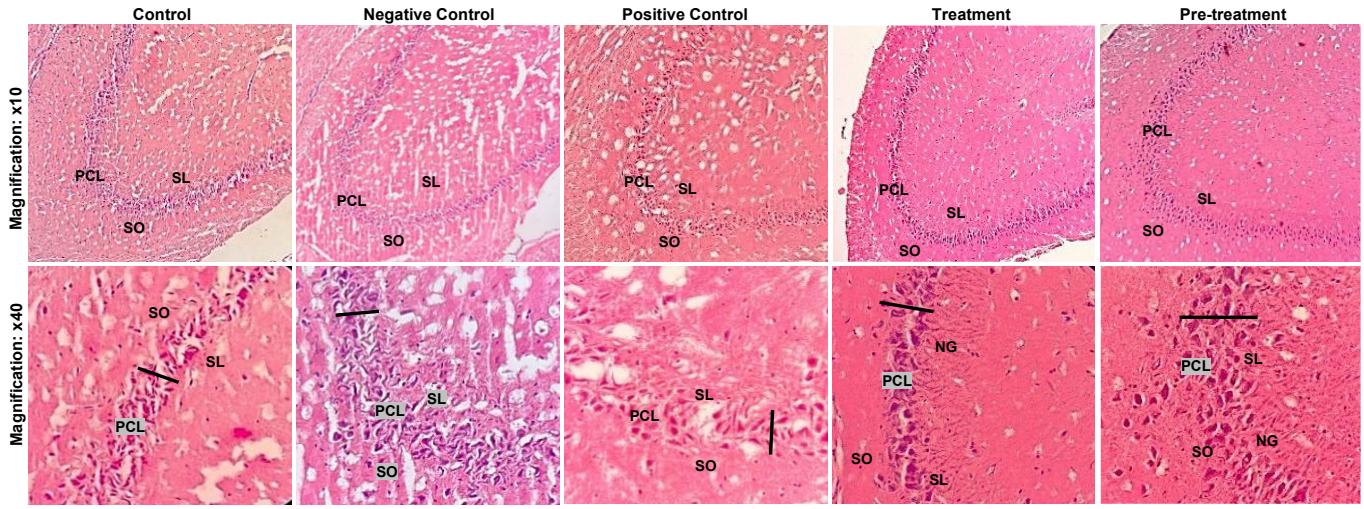

Figure 8: CA-3 region of rat hippocampus stained with H&E, observed under the optical light microscope with magnification of x10 and x40. PCL, pyramidal cell layer; SO, stratum oriens; SL, stratum lucidum. Neurites outgrowth (NG) can be seen in both NevGro® Forte-treated groups. Black line indicates thickness of PCL.

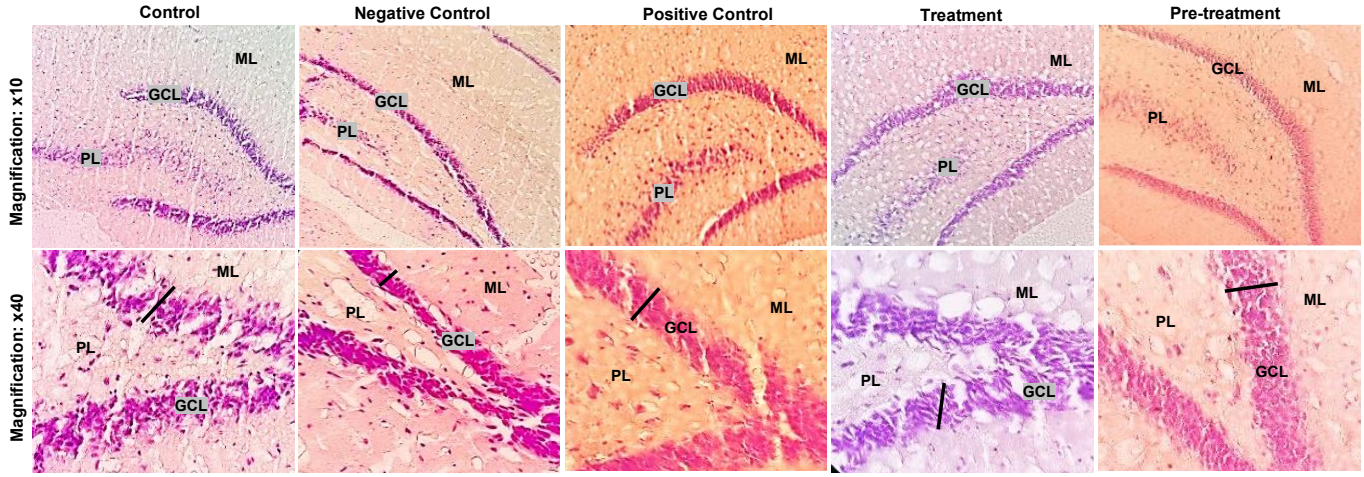

Figure 9: Dentate gyrus of hippocampus stained with cresyl violet, observed under the optical light microscope with magnification of x10 and x40. ML, molecular layer; GCL, granular cell layer; PL, pleomorphic layers. Black line: GCL thickness.

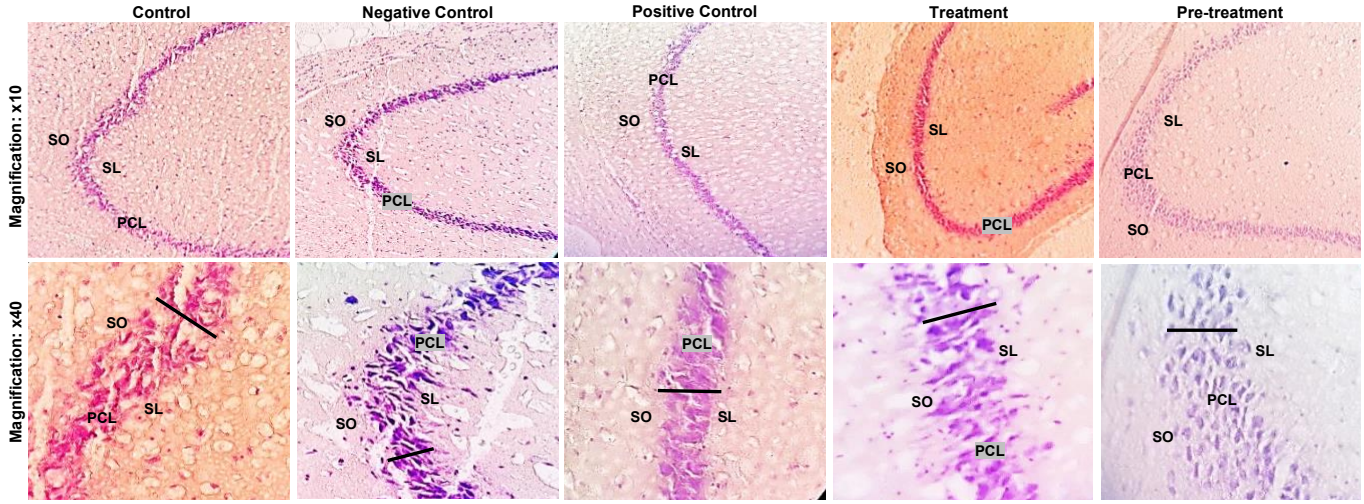

Figure 10: CA-3 of rat hippocampus stained with cresyl violet, observed under the optical light microscope with magnification of x10 and x40. PCL, pyramidal cell layer; SO, stratum oriens; SL, stratum lucidum. Black line indicates thickness of PCL.

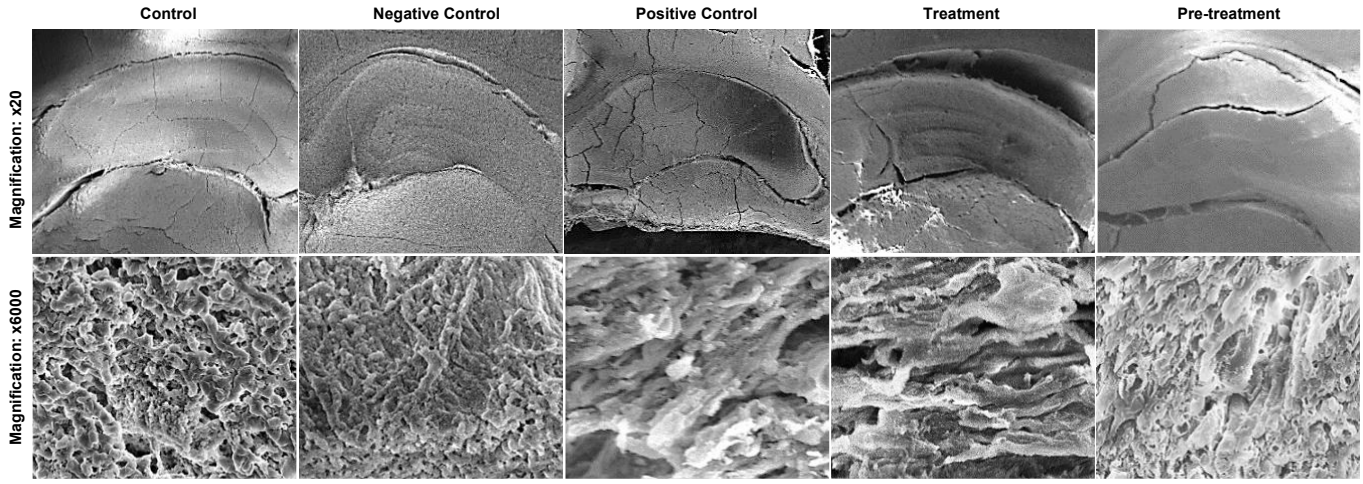

Figure 11: Surface of rat hippocampus were observed under the scanning electron microscope with magnification of x20 and x6000. CUMS-induced (negative control) group shows a flat surface with less dense of the hippocampus compared to the normal group as well as the FLX and NevGro® Forte treated groups that are compact surface.

4.0 DISCUSSION

The present study evaluates the antidepressant-like effects of NevGro® Forte in the CUMS model of depression in rats. NevGro® Forte is a product formulated with *Lignosus rhinocerotis*, *Ganoderma lucidum* and *Hericium erinaceus* extracts. Depression is a clinically significant and burgeoning public health crisis (Park & Zarate, 2019). The global prevalence of anxiety and depression increased by 25% in the first year of the COVID-19 pandemic (WHO, 2022). In the United States, the lifetime prevalence of severe depression is 21% of females and 11% to 13% of males (Beurel et al., 2020). This is primarily due to the disorder's chronicity or recurrence and a poor response to antidepressant therapy (Planchez et al., 2019). Antidepressants recommended for more severe depression are less likely to be effective due to the patient's characteristics, rather than anything inherent in the prescription (Alemi et al., 2021).

Conventional antidepressants have insufficient efficacy, and many have deleterious repercussions (Chong et al., 2021). NevGro® Forte affirms the possibility of becoming an alternate herbal-based treatment to antidepressants, underpinned by known health-promoting characteristics derived from medicinal mushrooms of *L. rhinocerotis, G. lucidum* and *H. erinaceus*. It is claimed to have a high concentration of both antioxidants glutathione and ergothioneine (Ba et al., 2021) with anti-inflammatory, antiviral and antibacterial properties (Ahmad, 2018; Kittimongkolsuk et al., 2021; Ryu et al., 2018).

We conducted the present study to investigate the therapeutic potential of medicinal mushrooms on the neurogenesis-dependent pathway of depression, thereby strengthening the neurological potential of these mushrooms to treat depressive symptoms by accelerating the formation of new neurons and neurite outgrowth.

4.1 Behavioural test: sucrose preference test

Figure 1 shows that there is a significant difference in sucrose preference between groups that were conditioned to CUMS and groups that were not. The CUMS groups exhibited decreased sucrose preference compared to the non-CUMS groups, indicating success in inducing depression in the required groups (Group 2, 3 and 4) before treatments were administered. Similar findings were obtained by Liu et. al (2017) who executed using similar protocols with minor modifications.

In the sucrose preference test, these animals have successfully developed depressive-like behaviours such as anhedonia, in which the animals have significantly lower sucrose preference over tap water (Frisbee et al., 2015). Significant differences in anhedonia were observed between the negative control and treatment groups after 28 days of treatment with NevGro® Forte and fluoxetine (**Figure 2**). A similar study by Chong et al. (2021) have demonstrated the same significant increase in sucrose preference among groups conditioned to CUMS following treatment with antidepressant and *H.erinaceus* respectively. The same *H. erinaceus* intervention group was also found to significantly reduce depressive-like behaviours and anxiety after oral administration (Chong et al, 2021) while commercialized

antidepressant-treated groups showcased a greater reduction in depressive-like behaviour following CUMS, further suggesting their effectiveness in reducing symptoms of depression (Benkert et al., 2000).

Similar findings were obtained by Liu et. al (2017) who executed the similar protocol with minor modifications. There is markedly reduced interest in sucrose consumption reflecting the loss of sensitivity towards pleasure or reward, which was observed after the administration of CUMS; as rodents are always known to possess sweet palate (Scheggi et al.2018). The condition of anhedonia or the loss of pleasure feelings following 56 days of CUMS suggests disturbances of the motivational or reward processes in the brain (Scheggi et al., 2018) and SPT which is calculated as the percentage of consumed sucrose solution over the amount of total liquid intake, is a reliable gustatory hedonic behaviour measure of the animal response to a sweet solution, which can be reversed with administration of antidepressants (Scheggi et al.2018).

4.2 Rats' body weight measurement

Loss of appetite and accompanying weight loss are typical symptoms of depression-melancholia (Konttinen et al., 2019). Depression is a complex set of symptoms that can be categorised into subtypes based on the accompanying presenting symptoms other than sad mood. It has recently been divided into two major subtypes: type 1 which is characterised by a loss of appetite and body weight, insomnia and suicidal ideation; type 2 which is also characterised by an increase in appetite, weight gain, leaden paralysis, hypersomnia and a persistently poor metabolic profile (Patsalos et al., 2021). Elevated depression feelings have been discovered to be negatively related to physical exercise, which is an effective weight loss behaviour (Vrany et al., 2018).

In **Figure 3**, the negative control rats showed the most diminished body weight, with only about 5.9% body weight increment compared to the normal group of rats that gained approximately 31.2% of body weight. It is known that the antidepressants ameliorate the symptoms of depression. Several studies have found that the ability of antidepressants to stimulate hippocampus neurogenesis in rats and non-human primates influences their effectiveness (Micheli et al., 2018). The positive control group which is the CUMS-induced rats treated with FLX, shows a 10.5% increment in body weight. A study in rats treated with either FLX medication or voluntary running demonstrated equivalent results in increased neurogenesis, dendritic spine density, and amelioration of depression-like behaviour after four weeks (Micheli et al., 2018).

However, antidepressant studies in people with moderate or severe depression found that currently available antidepressants could only improve depression symptoms in roughly 20% of individuals (Chong et al., 2019). Also, adverse effects such as headaches, dry mouth, nervousness, dizziness, weight gain, decreased interest in sex, and more side effects might frequently result in the failure to provide antidepressants to depressive individuals (Chong et al., 2019). This can be one of the reasons why only 10.5% body weight increment compared to the treatment and pre-treatment groups treated with NevGro® Forte which shows a multiplication of body weight with percentages of 17.9% and 21% respectively that is nearly to the normal healthy subjects. Medicinal mushrooms like *L. rhinocerotis, G. lucidum* and *H. erinaceus* has been discovered to enhance good nerve growth and brain health. Because it includes neurotrophic chemicals that can go through the blood-brain barrier, it has considerable potential for treating neurological problems (Chong et al., 2019).

4.3 Gross morphology of the brain

A reduction in hippocampus volume is one neurological difference strongly related to severe depression in adults, as proven by multiple meta-analyses (Barch et al., 2019). Based on the data analysis obtained regarding the brain weight measurement in **Figure 4** and **Figure 5**, we observed that the gross morphology of the negative control (CUMS-induced) brains were slightly smaller compared to other groups where the mean weight was significantly low as well (**Figure 6**). Besides, rats in the treatment groups show significant values when compared with the normal group of experimental rats, especially the treatment and pre-treatment groups that both use NevGro® Forte, nearly correspond to the normal group.

However, the nature of the relationship between major depression disorder (MDD) and hippocampal volume is controversial (Barch et al., 2019). Some investigations of amygdala and hippocampus volume reported no difference compared to controls, whereas others discovered a decrease (Bremner et al., 2000). Some claim that hippocampus volume reductions occur before the start of MDD and contribute to depression risk. It has been argued that early life adversity, poverty, and stress, for example, have been linked to disturbances in hippocampus structure and function, which in turn lead to dysregulated hypothalamic-pituitary-adrenal (HPA)

axis function and disordered emotional regulation, which in turn contribute to depression risk (Barch et al., 2019).

The pre-supplementary motor area, parietal-temporal regions, frontal cortex, temporal cortex, cingulate cortex, insular cortex, para-hippocampal gyrus, hippocampus, cerebellum, and orbitofrontal cortex have all been found to have lower grey matter volume in heterogeneity of MDD patients (Zheng et al., 2021). Also, several studies have found that persons suffering from depression have decreased thalamic volume, which is crucial for integrating sensory stimuli, emotions, and arousal (Rădulescu et al., 2021).

Additionally, following MDD remission, the volume loss in the subgenual anterior cingulate cortex of the brain region persisted. This could imply a reduction in the density of both nerve and glial cells (Niida et al., 2018). Such abnormalities in these brain regions have been demonstrated to be ameliorated by antidepressant medication (Nolan et al., 2020).

4.4 Histological analysis

Depression is linked to neurons shrinking in the cortical and limbic brain regions that control mood and emotion. Antidepressant therapy may promote neuroplasticity and reverse neuroanatomical defects identified in depressed patients (Rădulescu et al., 2021). Neuronal atrophy has been documented in chronic stress models in rodents and clinical post-mortem studies of depressed patients (Banasr et al., 2011) in the hippocampus and prefrontal cortex, resulting in a drop in hippocampal volume (Ali et al., 2017).

The negative effects of currently available antidepressants frequently contribute to patient noncompliance, therefore, new and safer antidepressants with few or no side effects are required (Ali et al., 2017). Actual medications like serotonin (5-HT) selective reuptake inhibitor (SSRI) antidepressants, take weeks to months to produce an apparent therapeutic effect (Rădulescu et al., 2021).

Our histological brain analysis showed a reduction in GCL thickness in the dentate gyrus (DG) (**Figure 7** and **Figure 9**) in the negative group (CUMS-induced) as well as smaller PL. Clinically, depressed patients have a 5% reduction in DG volume bilaterally and lower CA and subiculum volumes compared to healthy controls (Umschweif et al., 2019). MDD may result in significantly reduced DG and hippocampus volume by disrupting the HPA axis, causing neurotoxicity and higher levels of glucocorticoids, which can reduce neurogenesis, dendritic complexity and length, spine density, and glia cell counts (van Dijk et al., 2021). This lucidity of presentation was supported by Rădulescu et al (2021), where several structural neuroimaging investigations on depression have discovered dentate volume reductions and other substructure abnormalities. Umschweif et al (2019) have reported that a four-week chronic restraint stress paradigm in rats found a modest reduction in DG, CA3, and CA1 volumes and further reduction is dependent on the number of episodes and period of time (van Dijk et al., 2021).

Furthermore, we also observed that PCL of the negative control group is in scattered arrangement and the thickness is reduced compared to the normal group of animals (**Figure 8** and **Figure 10**). Similar findings were observed by Ayuob and Balgoon (2018), where the PL thickness in the CA3 region of the hippocampus of mice subjected to CUMS were considerably reduced when compared to unstressed mice. Loss of dendritic spines and retraction of apical dendrites of hippocampal CA3 pyramidal neurons have been observed in persistently stressed animals as well as in post-mortem tissue from depressive patients (Qiao et al., 2014). The CA3 possesses dense connection inside the hippocampus and functions as a hippocampal 'pacemaker,' crucial for information encoding and decoding. Smaller CA3 regions in MDD may contribute to the disorder's abnormal stress or HPA responses and the neurocognitive impairments observed in some MDD patients (Nolan et al., 2020).

Antidepressants stimulate neurogenesis and new cells in certain experimental models (Ali et al., 2017). We observed that fluoxetine and NevGro® Forte demonstrates efficacy in treating the CUMS-induced depression model in rats. Furthermore, the treatment and pre-treatment group (NevGro® Forte-treated) shows unforeseen effects where the PL is wider in DG and the GCL thickness are the same as the normal group. The arrangements of PCL in CA3 regions were well-organized compared to the positive group (FLX-treated). We also observed the neurite outgrowth indicating growing neurons in generating new axons and dendrites as they expand in response to stimuli. As the formation of axonal and dendritic processes is a distinguishing feature of neuronal cell morphology and a major driver of neuronal cell connection and function, neurite outgrowth has gained the greatest attention as an indicator of neurodevelopment and neuroregeneration *in vitro* (Meng et al., 2019).

Combination of three medicinal mushrooms (NevGro® Forte) may be a promising alternative medication for the treatment of depression by combating the neurotrophic and neurogenic pathophysiology of depression although the antidepressant effects of NevGro® Forte have not been verified or proven to be effective in comparison to conventional antidepressants.

Also, under the scanning electron microscopy observation in **Figure 11**, the surface of hippocampus in the negative control group (CUMS-induced) is flattened with a less dense surface composition compared to the normal group which could be due to the degeneration and neuronal atrophy that occurred in CUMS-induced group. The hippocampus is a temporal integral part of the cerebral cortex. Externally, it is characterized by a layer of densely compacted neurons that folds into an S-shaped structure, generating some of the greatest EEG waves related to learning and memory (Dhikav & Anand, 2012). The administration of NevGro® Forte at different concentrations have significantly improved the surface density with a compact topography and is comparable with animals treated with FLX.

5.0 CONCLUSIONS

The present study has shown the reliability of CUMS in inducing depressive-like behaviour in rat models. The administration of NevGro® Forte has successfully reversed the depressive symptoms induced by CUMS in rats.

The depressed brain tends to shrink in size especially the hippocampus region. On the other end, the treatment and pre-treatment groups, both of which are administered with NevGro® Forte, are remarkably equivalent to the healthy control group. Besides, our histological brain examination revealed a decreased thickness in GCL, DG in the negative group, and smaller PL. Also, we discovered that the PCL of the negative group is fragmented and thinner than the normal group of animals. Along with this, we found that fluoxetine and NevGro® Forte are effective in treating the CUMS-induced depression rat models. NevGro® Forte-treated groups exhibit unexpected outcomes in which the PL is broader in DG and the GCL thickness is the same as the control group. Furthermore, we found neurite outgrowth and well-organized PCL configurations in CA3 regions when compared to the fluoxetine-treated group.

Although the antidepressant benefits of NevGro® Forte have not been proven clinically, however based on the neurotrophic and neurogenic aetiology of depression, NevGro® Forte may be a possible alternative medicine for the treatment of depression and aid in the promotion of neurite outgrowth. Further investigations using other techniques for hippocampus morphological labelling, biochemical analysis and molecular studies are required to confirm the effect of NevGro® Forte as an alternative treatment for depression.

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References

Adebiyi, O., Adigun, K., Folarin, O., Olopade, J., & Olayemi, F. (2020). Administration of ethanolic extract of Erythrophleum ivorense (A Chev.) stem bark to male Wistar rats alters brain areas involved in motor coordination, behavior, and memory. *Journal of Ethnopharmacology*, *253*, 112650. https://doi.org/10.1016/j.jep.2020.112650

Ahmad, M. F. (2018). Ganoderma lucidum: Persuasive biologically active constituents and their health endorsement. *Biomedicine & Pharmacotherapy*, *107*, 507–519. https://doi.org/10.1016/j.biopha.2018.08.036

Alemi, F., Min, H., Yousefi, M., Becker, L. K., Hane, C. A., Nori, V. S., & Wojtusiak, J. (2021). Effectiveness of common antidepressants: a post market release study. *EClinical Medicine*, *41*, 101171. https://doi.org/10.1016/j.eclinm.2021.101171

Ali, S., Abd El Wahab, M., Ayuob, N., & Suliaman, M. (2017). The antidepressant-like effect of Ocimum basilicum in an animal model of depression. *Biotechnic & Histochemistry*, *92*(6), 390–401. https://doi.org/10.1080/10520295.2017.1323276

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). American Psychiatric Publishing.

Ayuob, N. N., & Balgoon, M. J. (2018). Histological and molecular techniques utilized to investigate animal models of depression. An updated review. *Microscopy Research and Technique*, *81*(10), 1143–1153. https://doi.org/10.1002/jemt.23105

Ba, D. M., Gao, X., Al-Shaar, L., Muscat, J. E., Chinchilli, V. M., Beelman, R. B., & Richie, J. P. (2021). Mushroom intake and depression: A population-based study using data from the US National Health and Nutrition Examination Survey (NHANES), 2005–2016. *Journal of Affective Disorders*, *294*, 686–692. https://doi.org/10.1016/j.jad.2021.07.080

Banasr, M., Dwyer, J. M., & Duman, R. S. (2011). Cell atrophy and loss in depression: reversal by antidepressant treatment. *Current Opinion in Cell Biology*, *23*(6), 730–737. https://doi.org/10.1016/j.ceb.2011.09.002

Barch, D. M., Tillman, R., Kelly, D., Whalen, D., Gilbert, K., & Luby, J. L. (2019). Hippocampal volume and depression among young children. *Psychiatry Research: Neuroimaging*, *288*, 21–28. https://doi.org/10.1016/j.pscychresns.2019.04.012

Benkert, O., Szegedi, A., & Kohnen, R. (2000). Mirtazapine compared with paroxetine in major depression. *The Journal of Clinical Psychiatry*, *61*(9), 656–663. https://doi.org/10.4088/jcp.v61n0911

Beurel, E., Toups, M., & Nemeroff, C. B. (2020). The bidirectional relationship of depression and inflammation: double trouble. *Neuron*, *107*(2), 234–256. https://doi.org/10.1016/j.neuron.2020.06.002

Bremner, J. D., Narayan, M., Anderson, E. R., Staib, L. H., Miller, H. L., & Charney, D. S. (2000). Hippocampal volume reduction in major depression. *American Journal of Psychiatry*, *157*(1), 115–118. https://doi.org/10.1176/ajp.157.1.115

Burstein, O., & Doron, R. (2018). The unpredictable chronic mild stress protocol for inducing anhedonia in mice. *Journal of Visualized Experiments*, *140*, e58184. https://doi.org/10.3791/58184

Cherubini, E., & Miles, R. (2015). The CA3 region of the hippocampus: how is it? What is it for? How does it do it? *Frontiers in Cellular Neuroscience*, *9*, 19. https://doi.org/10.3389/fncel.2015.00019

Chong, P. S., Fung, M. L., Wong, K. H., & Lim, L. W. (2019). Therapeutic potential of hericium erinaceus for depressive disorder. *International Journal of Molecular Sciences*, *21*(1), 163. https://doi.org/10.3390/ijms21010163

Chong, P. S., Poon, C. H., Roy, J., Tsui, K. C., Lew, S. Y., Phang, M. W. L., Tan, R. J. Y., Cheng, P. G., Fung, M. L., Wong, K. H., & Lim, L. W. (2021). Neurogenesis-dependent antidepressant-like activity of Hericium erinaceus in an animal model of depression. *Chinese Medicine*, *16*(1), 132. https://doi.org/10.1186/s13020-021-00546-8

Dhikav, V., & Anand, K. (2012). Hippocampus in health and disease: An overview. *Annals of Indian Academy of Neurology*, *15*(4), 239. https://doi.org/10.4103/0972-2327.104323

Dudek, K. A., Dion-Albert, L., Kaufmann, F. N., Tuck, E., Lebel, M., & Menard, C. (2019). Neurobiology of resilience in depression: immune and vascular insights from human and animal studies. *European Journal of Neuroscience*, *53*(1), 183–221. https://doi.org/10.1111/ejn.14547

Frisbee, J. C., Brooks, S. D., Stanley, S. C., & D'Audiffret, A. C. (2015). An unpredictable chronic mild stress protocol for instigating depressive symptoms, behavioral changes and negative health outcomes in rodents. *Journal of Visualized Experiments*, *106*, e53109. https://doi.org/10.3791/53109

Jonas, P., & Lisman, J. (2014). Structure, function, and plasticity of hippocampal dentate gyrus microcircuits. *Frontiers in Neural Circuits*, *8*, 107. https://doi.org/10.3389/fncir.2014.00107

Kim, Y. K., & Park, S. C. (2021). An alternative approach to future diagnostic standards for major depressive disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *105*, 110133. https://doi.org/10.1016/j.pnpbp.2020.110133

Kittimongkolsuk, P., Pattarachotanant, N., Chuchawankul, S., Wink, M., & Tencomnao, T. (2021). Neuroprotective effects of extracts from tiger milk mushroom Lignosus rhinocerus against glutamate-induced toxicity in HT22 hippocampal neuronal cells and neurodegenerative diseases in Caenorhabditis elegans. *Biology*, *10*(1), 30. https://doi.org/10.3390/biology10010030

Konttinen, H., van Strien, T., Männistö, S., Jousilahti, P., & Haukkala, A. (2019). Depression, emotional eating and long-term weight changes: a population-based prospective study. *International Journal of Behavioral Nutrition and Physical Activity*, *16*(1), 28. https://doi.org/10.1186/s12966-019-0791-8

Lew, S. Y., Teoh, S. L., Lim, S. H., Lim, L. W., & Wong, K. H. (2020). Discovering the potentials of medicinal mushrooms in combating depression – A review. *Mini-Reviews in Medicinal Chemistry*, *20*(15), 1518–1531. https://doi.org/10.2174/1389557520666200526125534

Liu, C., Gu, J. Y., Han, J. H., Yan, F. L., Li, Y., Lv, T. T., Zhao, L. Q., Shao, Q. J., Feng, Y. Y., Zhang, X. Y., & Wang, C. H. (2017). Enriched environment combined with fluoxetine ameliorates depression-like behaviors and hippocampal SYP expression in a rat CUS model. *Brain Research Bulletin*, *135*, 33–39. https://doi.org/10.1016/j.brainresbull.2017.09.009

Meng, P., Zhu, Q., Yang, H., Liu, D., Lin, X., Liu, J., Fan, J., Liu, X., Su, W., Liu, L., Wang, Y., & Cai, X. (2019). Leonurine promotes neurite outgrowth and neurotrophic activity by modulating the GR/SGK1 signaling pathway in cultured PC12 cells. *NeuroReport*, *30*(4), 247–254. https://doi.org/10.1097/wnr.0000000000001180

Micheli, L., Ceccarelli, M., D'Andrea, G., & Tirone, F. (2018). Depression and adult neurogenesis: Positive effects of the antidepressant fluoxetine and of physical exercise. *Brain Research Bulletin*, *143*, 181–193. https://doi.org/10.1016/j.brainresbull.2018.09.002

Niida, R., Yamagata, B., Matsuda, H., Niida, A., Uechi, A., Kito, S., & Mimura, M. (2018). Regional brain volume reductions in major depressive disorder and bipolar disorder: An analysis by voxel-based morphometry. *International Journal of Geriatric Psychiatry*, *34*(1), 186–192. https://doi.org/10.1002/gps.5009

Nolan, M., Roman, E., Nasa, A., Levins, K. J., O'Hanlon, E., O'Keane, V., & Willian Roddy, D. (2020). Hippocampal and amygdalar volume changes in major depressive disorder: A targeted review and focus on stress. *Chronic Stress*, *4*, 247054702094455. https://doi.org/10.1177/2470547020944553

Park, L. T., & Zarate, C. A. (2019). Depression in the primary care setting. *New England Journal of Medicine*, *380*(6), 559–568. https://doi.org/10.1056/nejmcp1712493

Patsalos, O., Keeler, J., Schmidt, U., Penninx, B. W. J. H., Young, A. H., & Himmerich, H. (2021). Diet, obesity, and depression: A systematic review. *Journal of Personalized Medicine*, *11*(3), 176. https://doi.org/10.3390/jpm11030176

Planchez, B., Surget, A., & Belzung, C. (2019). Animal models of major depression: drawbacks and challenges. *Journal of Neural Transmission*, *126*(11), 1383–1408. https://doi.org/10.1007/s00702-019-02084-y

Polesza k, E., Wośko, S., Sławińska, K., Wyska, E., Szopa, A., ŚWiąder, K., Wróbel, A., Szponar, J., Doboszewska, U., Wlaź, P., Wlaź, A., & Serefko, A. (2020). Influence of the endocannabinoid system on the antidepressant activity of bupropion and moclobemide in the behavioural tests in mice. *Pharmacological Reports*, *72*(6), 1562–1572. https://doi.org/10.1007/s43440-020-00088-0

Qiao, H., An, S. C., Ren, W., & Ma, X. M. (2014). Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression. *Behavioural Brain Research*, *275*, 191–200. https://doi.org/10.1016/j.bbr.2014.08.040

Rădulescu, I., Drăgoi, A., Trifu, S., & Cristea, M. (2021). Neuroplasticity and depression: Rewiring the brain's networks through pharmacological therapy (Review). *Experimental and Therapeutic Medicine*, *22*(4). https://doi.org/10.3892/etm.2021.10565

Ryu, S., Kim, H. G., Kim, J. Y., Kim, S. Y., & Cho, K. O. (2018). *Hericium erinaceus* extract reduces anxiety and depressive behaviors by promoting hippocampal neurogenesis in the adult mouse brain. *Journal of Medicinal Food*, *21*(2), 174–180. https://doi.org/10.1089/jmf.2017.4006

Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M., Abbafati, C., Adolph, C., Amlag, J. O., Aravkin, A. Y., Bang-Jensen, B. L., Bertolacci, G. J., Bloom, S. S., Castellano, R., Castro, E., Chakrabarti, S., Chattopadhyay, J., Cogen, R. M., Collins, J. K., . . . Ferrari, A. J. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *The Lancet*, *398*(10312), 1700–1712. https://doi.org/10.1016/s0140-6736(21)02143-7

Scheggi, S., De Montis, M. G., & Gambarana, C. (2018). Making sense of rodent models of anhedonia. *International Journal of Neuropsychopharmacology*, *21*(11), 1049–1065. https://doi.org/10.1093/ijnp/pyy083

Tang, M., Huang, H., Li, S., Zhou, M., Liu, Z., Huang, R., Liao, W., Xie, P., & Zhou, J. (2019). Hippocampal proteomic changes of susceptibility and resilience to depression or anxiety in a rat model of chronic mild stress. *Translational Psychiatry, 9*(1), 260. https://doi.org/10.1038/s41398-019-0605-4

Umschweif, G., Greengard, P., & Sagi, Y. (2019). The dentate gyrus in depression. *European Journal of Neuroscience*, *53*(1), 39–64. https://doi.org/10.1111/ejn.14640

Van Dijk, M. T., Cha, J., Semanek, D., Aw, N., Gameroff, M. J., Abraham, E., Wickramaratne, P. J., Weissman, M. M., Posner, J., & Talati, A. (2021). Altered dentate gyrus microstructure in individuals at high familial risk for depression predicts future symptoms. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *6*(1), 50–58. https://doi.org/10.1016/j.bpsc.2020.06.006

Vrany, E. A., Hawkins, M. A., Wu, W., & Stewart, J. C. (2018). Depressive symptoms and weight loss behaviors in U.S. adults. *Eating Behaviors*, *29*, 107–113. https://doi.org/10.1016/j.eatbeh.2018.03.006

Vos, T., Lim, S. S., Abbafati, C., Abbas, K. M., Abbasi, M., Abbasifard, M., Abbasi-Kangevari, M., Abbastabar, H., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abolhassani, H., Aboyans, V., Abrams, E. M., Abreu, L. G., Abrigo, M. R. M., Abu-Raddad, L. J., Abushouk, A. I., . . . Murray, C. J. L. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396*(10258), 1204–1222. https://doi.org/10.1016/s0140-6736(20)30925-9

WHO. (2019). *Depression*. Retrieved October 21, 2023, from https://www.who.int/health-topics/depression#tab=tab_1

WHO. (2022, March 2). *COVID-19 pandemic triggers 25% increase in prevalence of anxiety and depression worldwide.* Retrieved October 21, 2023, from https://www.who.int/news/item/02-03-2022-covid-19-pandemic-triggers-25-increase-in-prevalence-of-anxiety-and-depression-worldwide

Wyska, E. (2019). Pharmacokinetic considerations for current state-of-the-art antidepressants. *Expert Opinion on Drug Metabolism & Toxicology*, *15*(10), 831–847. https://doi.org/10.1080/17425255.2019.1669560

Zheng, R., Zhang, Y., Yang, Z., Han, S., & Cheng, J. (2021). Reduced brain gray matter volume in patients with first-episode major depressive disorder: A quantitative meta-analysis. *Frontiers in Psychiatry*, *12*, 671348. https://doi.org/10.3389/fpsyt.2021.671348