e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(7); 05-12
Original Research Article

Evaluation of the Effect of Niacin in Chronic Unpredictable Stress Induced Depression in Wistar Rats

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Received: 18-03-2023 / Revised: 21-04-2023 / Accepted: 26-05-2023
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Conflict of interest: Nil

Abstract:
Everyday functioning is impacted by the common mood disorder known as depression. In addition to the limitations of first-line treatment for depression, it is believed to be ineffective in causing remission of depression. As a result, finding novel targets for the therapy is necessary. One potential new target is brain derived neurotrophic factor (BDNF). Niacin was found to increase the BDNF level in several preclinical study. Present study was designed to validate the efficacy of Niacin as an anti-depressant in chronic unpredictable mild stress (CUMS) model in male Wistar rats with behavioral and biochemical parameters. After obtaining approval from ethics committee, the standardization was carried out. 24 male Wistar rats at random were placed into 3 groups of 8 animals each: Normal saline, Fluoxetine, and Niacin, administered per orally, on each day. Depression was induced by CUMS for 28 days. On the 29th day, behavioural tests were undertaken followed by estimation of serum BDNF via ELISA. Results highlighted a significant difference in Forced swim test and sucrose preference test with the Niacin group and Fluoxetine compared to VC ($p<0.001$). Results in BDNF-ELISA were significantly higher than those in VC. However, no significant difference was observed between the Fluoxetine and niacin groups ($p>0.05$), signifying the comparable results. Niacin has shown to have anti-depressive effects evidenced by behavioral tests and the ability to alter BDNF levels.
Keywords: Niacin; Brain-Derived Neurotrophic Factor; Neuroplasticity, Chronic Unpredictable Mild Stress.

Introduction

Depression is one of the most frequently encountered neuropsychiatric conditions. [1] The patients frequently have persistently pervasive low mood, anhedonia, a sense of worthlessness, low energy, poor attention, abnormal eating habits, psychomotor slowness or agitation, and sleep difficulties or suicidal thoughts. According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) a patient is depressed if five of the aforementioned symptoms are present. [2,3] The worldwide burden of depression is expected to reach 280 million patients in 2021. According to WHO estimates, 3.8% of people have experienced depression at some point in their lives. Around 7,000,00 individuals with depression commit suicide each year. [4] Depression remains a key barrier to living a good life owing to the declining standard of living, burdensome financial obligations, and aberrant bodily and psychological health.

There is still a little understanding of the pathogenic mechanisms of depression. The monoamine theory, neuroendocrine processes, neuroimmune, and cytokine hypothesis are only a few of the theories that have been put up to explain the mechanism of depression. [5]

The connection between neural plasticity and depression has been evaluated by recent research. [6,7] The pathophysiology of major depressive disorder (MDD) involves several neurotropins that bind to common tyrosine kinase receptors, such as Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF). A protein called BDNF is produced in the brain and broadly distributed throughout the central nervous system (CNS) and peripheral nervous system (PNS), including the cortex, hippocampus, hypothalamus, and other brain regions. [8] BDNF has a critical role in the development, growth, differentiation,

and survival of neurons as well as the structure and function of the nervous system. [8,9] The most significant neurotropin- BDNF, which suggests that variations in BDNF levels in the brain are strongly related to the pathogenesis of MDD. [10]

Pharmacological therapy includes second generation antidepressants like selective serotonin reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs), while non-pharmacological modalities include psychotherapy and Cognitive Behavioral Therapy (CBT). Clinicians offer either psychotherapy or second-generation antidepressant as an initial treatment for depression. [11] Today's pharmacotherapy, however, comes with several shortcomings, including intolerance, decreased compliance, treatment failure, delayed response, and recurrence and several common adverse events including nausea, headache to grievous effects of sedation, seizure, and sexual dysfunction. Even with improved care and best use of pharmacotherapy, a complete recovery cannot be achieved in 20–30% patients of MDD. [12] This suggests that a quick and thorough search for innovative treatments is necessary.

We have seen researchers working continuously to find an alternate option for treating depression. One of the vitamin-B3's in the B complex family is niacin. A growing body of evidence suggests Niacin's potential role in raising the BDNF levels. One such preclinical study was conducted in a stroke model in rats.

Synaptic plasticity and axon growth induced by Niacin treatment of stroke was mediated by HDL-induced upregulation in BDNF/ TrkB axis. Niacin helping in upregulation of BDNF, and other neurotrophic factors could contribute to the long-term adaptations that are required for the therapeutic actions of anti-depressants. Thus, it is possible to hypothesize that drugs like niacin, which raises the brain's BDNF level, may also promote synaptic plasticity. [13]

A thorough literature search revealed out very few research examining the anti-depressant potential of niacin. So, using the chronic unpredictable mild stress (CUMS) model, we conducted this study to determine if niacin has an anti-depressive effect. Extended stress exposure results in the development of depression, anxiety, cognitive problems, changes in neurological and biochemical indicators, and more. [14,15] Compared to previous animal models testing the anti-depression potential, the CUMS model has demonstrated a higher face validity and construct validity. [16]

The objectives of the study were to evaluate the effect of Niacin in CUMS model of depression. Comparing the effects of Niacin and Fluoxetine in CUMS utilizing behavioral measures like Forced Swim Test (FST) and Sucrose Preference Test (SPT) and with serum BDNF measurement were the secondary goals. We also aimed to understand the mechanism of action of Niacin. We believe that the study will add crucial evidence aiding the future researchers.

Materials and Methods:

The Institutional Animal Ethics Committee (AEC/11/2018) granted clearance for the study to proceed. In accordance with the recommendations of the Committee of Control and Supervision of Experimentation in Animals (CCSEA), animals were bred at random in the Centre for Animal Studies of the Seth GS Medical College and KEM hospital in Mumbai. A total of 42 male Wistar rats, aged 4-6 weeks, were used in the course of study. Twenty of the 42 rats were utilised in Phase I (model standardisation) while the remaining were employed in Phase II.

These animals were kept in controlled environments with a temperature range of 23ºC ± 4ºC and humidity range of 30–70%. We housed individual rat in polypropylene cages with stainless steel top grill and giving an access to clean food pellets (Chakan oil mills, Maharashtra) and UV filtered drinking water. We ensured a 12-hour light: dark cycle.

Niacin was used as the test drug in the study, and Fluoxetine served as the active control. Both drugs were purchased from Sigma Aldrich in Mumbai. From earlier investigations, a dose of 40 mg/kg niacin and 5 mg/kg fluoxetine was calculated. [18,19] As a disease control, we utilised normal saline, 5mL/kg. Every drug was given orally once a day (OD). GENLISA® ELISA kits were purchased from KRISHGEN BioSystems for the estimation of the serum BDNF levels, and the levels were determined using the Sandwich ELISA technique.

Prior to conducting the actual research, the CUMS model was standardised in our institution for a period of 28 days. This widely used CUMS paradigm model involves subjecting animals to a number of mild stressors over the course of four weeks in a random, intermittent, and unexpected fashion in order to avoid the apprehension towards the impending stressors. The stressors included a 12-hour period of no access to food or water, an 18-hour period of a 45° cage tilt, a 12-hour period of damping the sawdust bedding, a 12-hour period of group housing (10 rats in one cage), a 10-minute period of shaking the cage in an orbital shaker with 150 rpm, and a 24-hour period of continuous lighting of rat cages.

Figure 1 shows the distribution of stressors. [14] These rats underwent the Sucrose Preference Test (SPT) on day 29. The rats were evaluated for their

ability to consume sucrose and water within an hour after being deprived of food and water for 16 hours on at least three separate occasions (days 26, 27, and 28) in their home cage.

Rats were offered either sucrose or water to quickly quench their thirst.SPT was computed as an average for the testing period as a proportion of the volume of sucrose consumption over the total volume of fluid intake. [20]

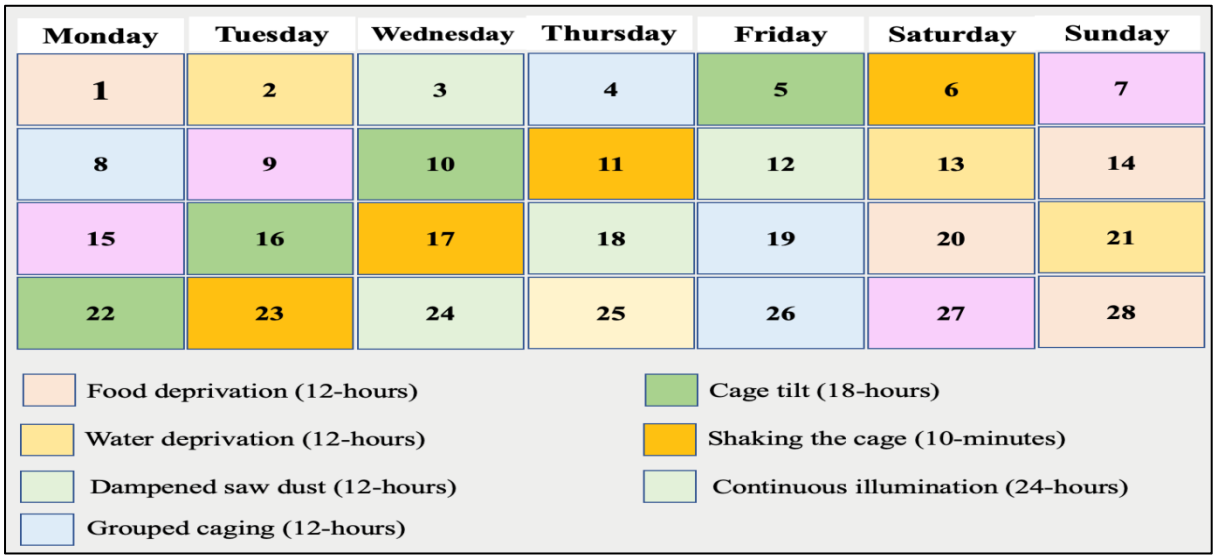

Figure 1: Stressor distribution

Figure 2 describes the phase 1 of standardisation with animal distribution.

Serial number	Group (n=10/ group)	Drugs administered (Per oral)
1	Control	Normal saline
2	Chronic unpredictable mild stress	Normal saline

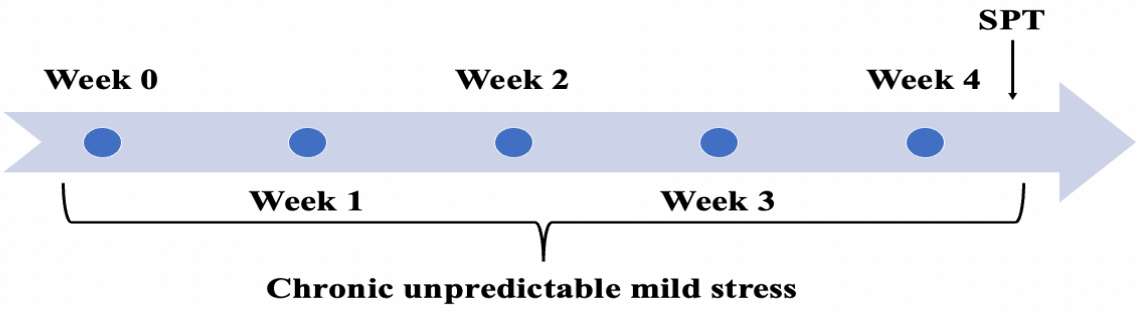

Figure 2: Description of Phase I

After that, Phase II was carried out using 24 animals in Phase IIA and Phase IIB. Animals were given the study drugs orally for 28 days at specific dosages to assess for efficacy. Figure 3 shows the Phase IIA and Phase IIB methodologies. The eight Wistar rat each was randomized to disease control group (Normal saline: 5ml/kg per oral), positive control (fluoxetine: 5mg/kg per oral) and test group (niacin: 40mg/kg per oral).

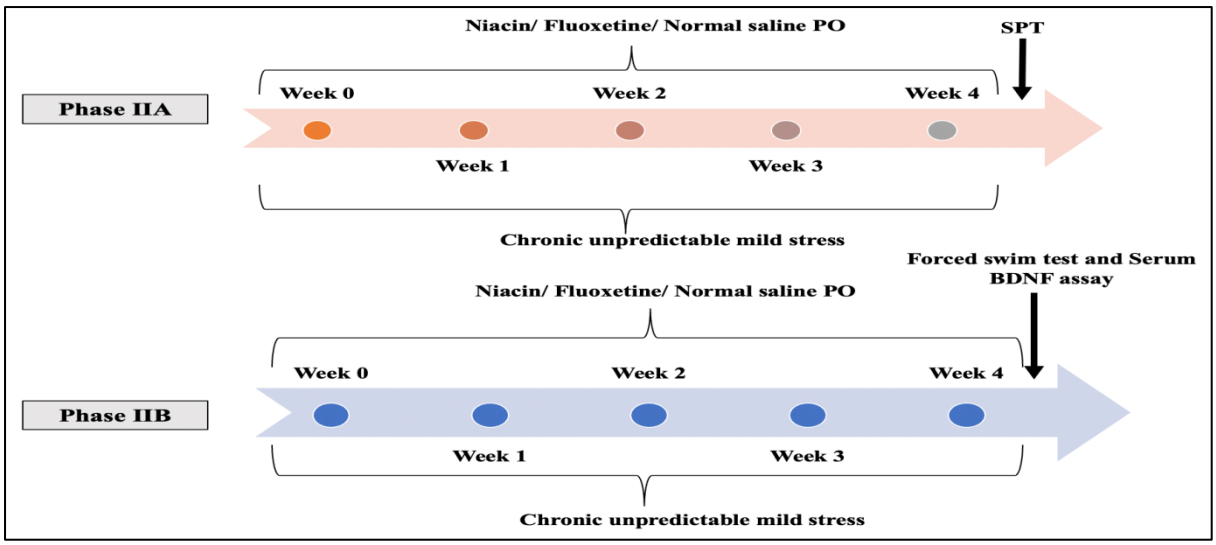

Figure 3: Description of Phase II

Behavioral assessment:

To discriminate the immobility behavior of rats in FST due to antidepressant effect from that of general behavioral stimulation (false positive), we conducted Open Field Test (OFT) for a period of 5 minutes.

Locomotion (number of lines crossing within 5 min) and rearing frequencies (number of times an animal stands on the hind-limbs) were evaluated using the Maze Master 2.0 software. The depressive behavior was assessed via FST which was conducted in a dark room after allowing the animal to acclimatize for a period of 30-minutes. Each rat was placed in an inescapable transparent cylindrical tank filled with water (±24°C), for 5 minutes.

We ensured that a 15-minute pre-conditioning of all the animals were carried out to induce despair. The total duration of immobility in a 5-minute trial was analyzed using a stopwatch.

Statistical analysis: Data were analyzed using IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. The data was expressed as mean and standard. The level of significance was set at $P < 0.05$. Normality was tested by Shapiro–Wilk test. Parametric data for standardization were analyzed using unpaired t-test and that for the study were analyzed using single-way ANOVA followed by post hoc Tukey's test.

Results and discussion:

Results of standardization:

The standardization procedure was carried out with the intention to establish the CUMS model at our institution. SPT was performed on day-28, as was described in the section above. We observed a statistically significant decrease ($p<0.05$) in the CUMS group in comparison with the control group. Figure 4 summarizes the results of standardization.

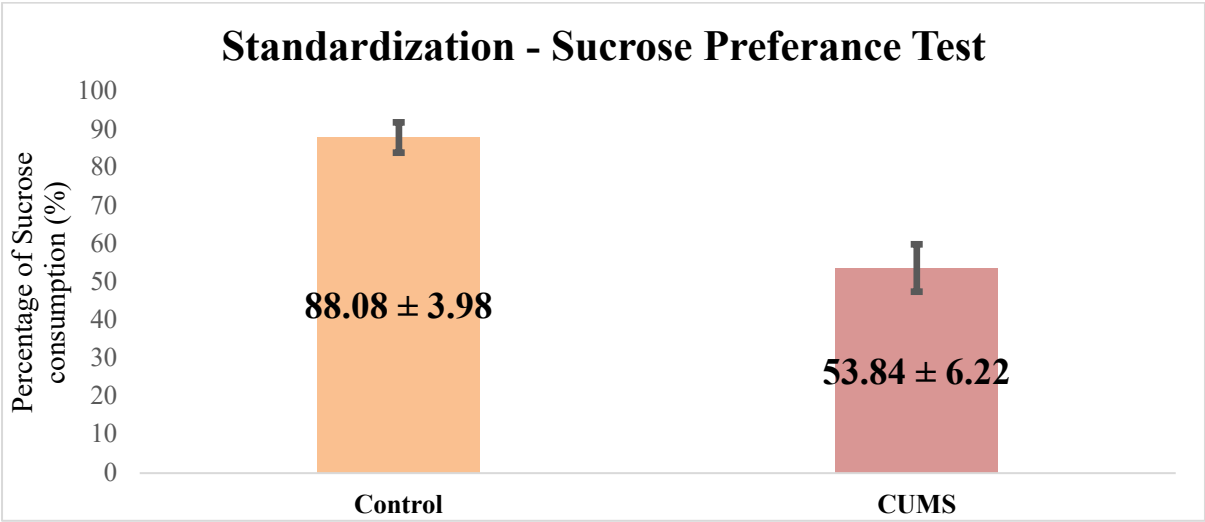

Figure 4: Graphical representation of variables of Sucrose Preference Test (n=10/group).

"*" represents p< 0.05 versus control group, using unpaired t-test.

Results of Part IIA: Figure 5 depicts the values represented as mean and standard deviations of data that passed the test for normality. Application of one-way ANOVA followed by post hoc Tukey's test showed statistically significant increase (p<0.001) in sucrose consumption in fluoxetine and niacin group compared to disease control at Day-28. Additionally, we found that there was not a significant statistical difference between Niacin and Fluoxetine in SPT, making them comparable.

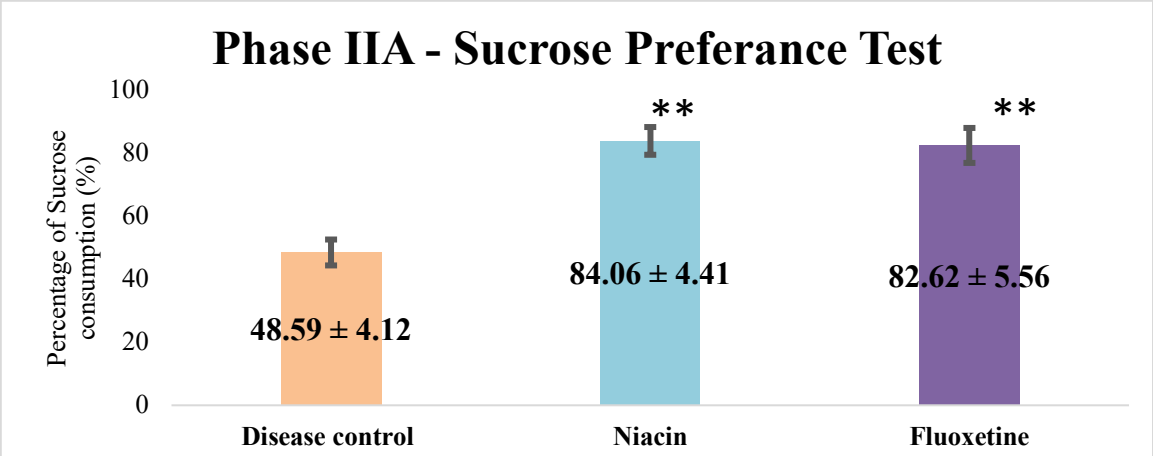

Figure 5: Graphical representation of variables of Sucrose Preference Test (n=8/group)

"**" represents p< 0.001 versus Disease control group, using ANOVA and post-hoc Tukey's test.

Results of Part IIB: We conducted the FST to assess the effect of niacin and fluoxetine on the CUMS model of depression, and BDNF-ELISA was carried out to gain an understanding of niacin's mechanism of action. In all groups, the locomotor activity of rats that had undergone FST was assessed using the OFT. The locomotor score, rearing score, and defecation score measurements showed no statistically significant change across the test groups, indicating that rats were not generally stimulated in their behavior.Additionally, the antidepressant effects of the test drugs were responsible for the shorter period of immobility. Table 1 summarizes the results of the OFT.

Table 1: Results of Open field test

Groups (n=8/ group)	Locomotion score (Mean ± SD)	Rearing score (Mean ± SD)	Defecation score (Mean ± SD)
Disease Control	24.25 ± 3.05	19.87 ± 1.24	2.5 ± 1.06
Niacin	23.37 ± 2.87	19. 5± 2.32	2.37 ± 1.50
Fluoxetine	24.75 ± 3.69	20.62 ± 1.99	2.25 ± 0.70

On FST, we found a statistically significant decrease (p<0.001) in immobility time on FST in fluoxetine and niacin group compared to disease control on Day-28. Additionally, we found that niacin and fluoxetine did not significantly differ in FST, making them comparable. The results have been depicted in **figure 6.**

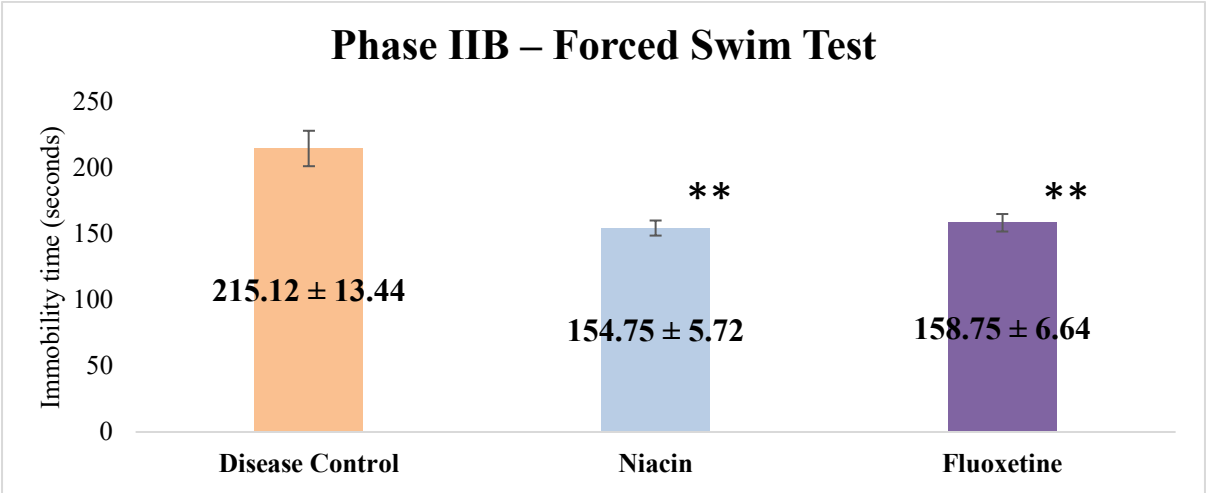

Figure 6: Graphical representation of variables of Forced Swim Test (n=8/group)

"**" represents p< 0.001 versus Disease control group, using ANOVA and post-hoc Tukey's test.

On estimating the serum BDNF levels, we found a statistically significant increase (p<0.001) in serum BDNF-ELISA in fluoxetine and niacin group compared to disease control on Day-28. Niacin and fluoxetine did not significantly differ in Serum BDNF assay; hence they were comparable according to our analysis (Figure 7).

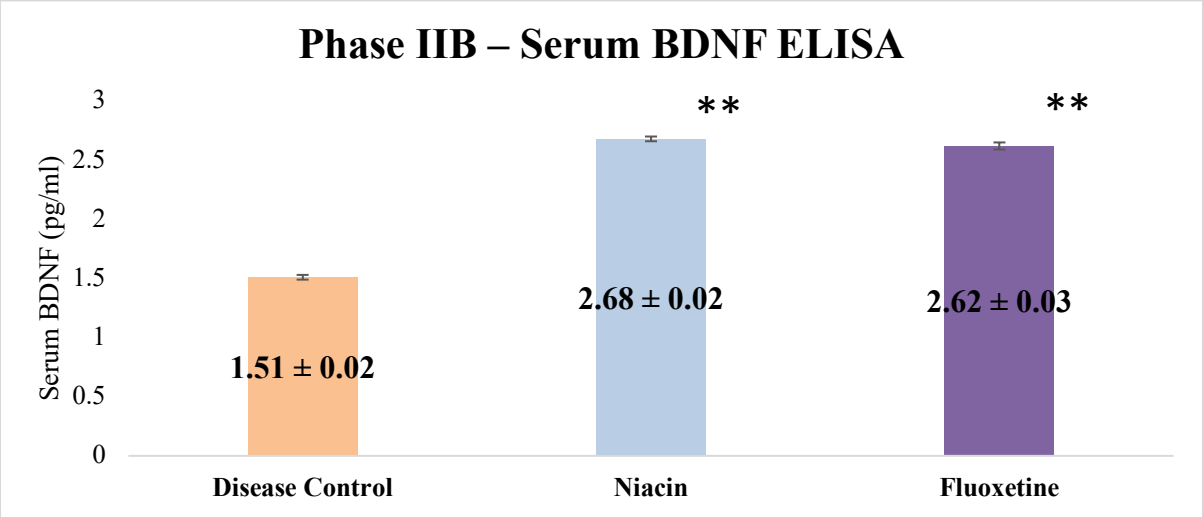

Figure 7: Graphical representation of variables of Serum BDNF-ELISA (n=8/group).

"**" represents p< 0.001 versus Disease control group, using ANOVA and post-hoc Tukey's test.

Globally, mental illnesses place a heavy burden on the healthcare system. Depressive disorders are among the top 25 illnesses overall, according to the Global Burden of illnesses, Injuries, and Risk Factors Study (GBD) 2019, which adds to the significant impact. [21] Niacin is hypothesized to promote neurogenesis and neuroplasticity, reduce oxidative stress, and glutamate excitotoxicity and attenuate decrease of serotonin, dopamine, norepinephrine, and their derivatives. Due to all the above-mentioned mechanisms, niacin is thought to play a major role in the future for the treatment of various neuropsychiatric disorders and our results are in line with previous studies. [22]

In the first phase of our research, CUMS was standardized, and the results revealed a significantly significant decrease (p<0.001) in the amount of sucrose consumed (53.84 ± 6.22%) compared to normal control on SPT in rats. Niacin had a substantial (p<0.05) antidepressant effect on SPT in rats in part-IIA when compared to disease control (Niacin: 84.06 ± 4.4% vs Disease control: 48.59 ± 4.12 In the part IIB, there was no evidence of behavioral stimulation of the rats based on the locomotor activity evaluated by the OFT, which did not indicate a significant difference (p>0.05) among the test groups.

This suggests that the reduction in immobility time caused by niacin was solely owing to the antidepressant effect and not because of the impact of central nervous system stimulation. On the FST, we saw a significant decrease in total immobility time in the niacin group (p<0.05) in comparison to the Disease control group (Niacin: 154.75 ± 5.72 seconds vs Disease control: 215.12 ± 13.44 seconds) suggesting an antidepressant effect of niacin. The serum BDNF levels were also significantly higher (p<0.05) in the niacin group when compared to Disease control (Niacin: 2.68 ± 0.02 pg/mL vs Disease control: 1.51 ± 0.02 pg/mL). This demonstrates that niacin is crucial in raising BDNF levels, which further helps alleviate depressed symptoms. The levels of fluoxetine (positive control) showed no statistically significant difference with the niacin group in all the parameters, however we noticed a statistical difference with the Disease control group.

Our findings were consistent with the 2020 research by Liu Z et al. The investigators set out to determine if sirtuin-1 (SIRT1) suppression by nicotinamide (vitamin B3) could reduce depressed symptoms in a 24-hour restraint mouse model. The study was evidenced by an increase in sucrose preference test in OFT and decrease in immobility time in FST. However, scientists were unable to precisely establish the way that vitamin B3 reduced depression. [23]

A further study was carried out by Xiaoxian X et al. to determine if Nicotinamide could reverse accelerated ageing and deficiencies in ATP synthesis in stress-induced depression by activating SIRT3. The depression was induced by chronic corticosterone (CORT) exposure in male C57/BL6 mice. The study showed that NMN administration alleviated depression-like behavior with a statistically significant reduction in immobility on FST and Tail suspension test (TST) and other objective parameters like ELISA of Nicotinamide

phosphoribosyl transferase (NAMPT) and SIRT3 activity. This study also shed insight on the positive effects of niacin and its potential use as an antidepressant in the future. [24]

Niacin's effects were also investigated in a case study with a 47-year-old woman with anxiety and depression, no flush niacin (3,000 mg) had a positive impact on the depression, and no instances of anxiety were seen. As a result, after one month of treatment, there was an improvement, and after five months, the female had reached clinical remission. She was further given a probiotic containing niacin and 300 mg of gamma-amino butyric acid (GABA).

Therefore, it is impossible to say if the GABA and probiotics benefited this patient's depression. [25] However, the study produced weak empirical evidence and to ascertain the actual therapeutic benefits and side effect profile of Niacin for depression, carefully designed controlled studies must be developed.

The strength of the study was conducted following the standardization of the depression model, and it was supported by an analysis of the antidepressant impact using two separate behavioral tests.

Serum BDNF ELISA was performed to understand the mechanism of Niacin as an antidepressant, and it was also confirmed that the reduction in immobility achieved in the FST was due to the drugs' antidepressant-like activity and was not a false positive by measuring locomotor activity using the open field test.

However, the limitations included that Niacin’s dosage ranges and the combination with fluoxetine were not assessed in our study. ELISA was used to evaluate the blood levels of BDNF. Although it would have been ideal to measure BDNF using hippocampus tissue.

The experimental data concluded that the chronic unpredictable mild stress model causes behavioral and biochemical changes with Niacin 40mg/kg and showed an improvement in the depressive symptoms with male Wistar rats. The antidepressant effects of Niacin were comparable to those of Fluoxetine.

The study has extended the horizon and scope for the future pre-clinical research and randomized trials to evaluate the potential of Niacin as an anti-depressant. Studies ought to determine if including niacin in antidepressant regimens enables dosing decrease of the current antidepressants.

Financial support and sponsorship: The Research Society, Seth GSMC and KEM Hospital, Mumbai, India

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