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VITAMIN-D ATTENUATES CHRONIC UNPREDICTABLE MILD STRESS INDUCED DEPRESSION LIKE BEHAVIOR IN RATS

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Abstract

Depression is very common brain disorder having characteristics like mood swings, anxiety, insomnia, cognitive impairment with suicidal tendency. Fluoxetine is a precursor of selective serotonin reuptake Inhibitors (SSRIs) which is very popular member of this category used for the treatment of this disorder. Vitamin-D generally requires for normal growth of bone. The exact biological mechanisms linking to Vitamin-D and depression are not fully understood but some studies reported that it has some biological activity in neurological development and function. This research was carried out to investigate the neuroprotective effects of Vitamin-D. We used chronic unpredictable mild stress (CUMS) animal model for depression where animals are constantly exposed to a sequence of unpredictable mild stressors which trigger number of life stress events. Undoubtedly there are so many characteristic symptoms and neurological deformity observes in CUMS-induced animals which are identical to those found in human depressed patients. Depression like behaviors were recorded as open-field test (locomotor activity), force swim test (immobility time), tail suspension test (immobility time), splash test (grooming time) in rats. Brain homogenate was used to estimate oxidative stress (glutathione-GSH, superoxide dismutase-SOD, lipid peroxidation-TBARS, catalase, nitrite oxide and total protein). CUMS induced rats displayed depression like behaviors, cognitive impairment and various biochemical changes in brain. However, treatment with the combination of Fluoxetine and Vitamin-D significantly restored the depression like behaviors and biochemical impairments in rats. Vitamin-D in combination of fluoxetine offered neuroprotective effects against CUMS induced depression like behaviors in rats.

Keywords: SSRIs, CUMS, immobility time, grooming time, TBARS.

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1. INTRODUCTION

Depression is a complex neuropsychiatric disease with extensive morbidity and dominant cause of suicide in the world. The global incidence of depression has been reported as high as 4.4%.[1] More than 300 million people worldwide suffer from depression.[2-3] According to the World Health Organization report, nearly 800,000 people die by suicide every year.[4] Depression reduces the quality of life and has become a health burden for families and society.[5] Chronic unpredictable mild stress (CUMS) is one of the most commonly used preclinical models for understanding the onset and progression of depression in rat.[6] CUMS can well induce several features of human pathology, such as altered circadian rhythms, anhedonia, and increased anxiety and hopelessness that was linked with depressive behavioral tests such as sucrose preference test (SPT), tail suspension test (TST), forced swimming test (FST), and open-field test (OFT) are commonly used to assess the depression phenotype. The pathogenesis of depression is still unclear, treatment methods are limited, and complete remission or cure rates for depression are very low. Therefore, it is critical to obtain an understanding of the pathogenesis of and effective treatment for depression.

Vitamin-D receptors are highly expressed in the hippocampus, hypothalamus, thalamus and cortex, here it involves in normal brain development while its deficiency causes morphological changes in brain (enlarged ventricles and decreased cortical thickness), and neurological disorders.[7] Other risk factors due to the deficiency of vitamin-D reported as schizophrenia, psychosis, multiple sclerosis. A study found that the brain's neurotransmitters (acetylcholine, serotonin, dopamine, norepinephrine, glutamate, and gamma-aminobutyric acid- GABA), which are involved in perception, cognition, movement, and emotion, change in depression. Vitamin-D is also involved in the regulation of the synthesis of acetylcholine (Ach), dopamine (DA), serotonin (5HT3), and GABA through the neural growth factor (NGF) in the septo-hippocampal pathways. It was proved that low-level of vitamin-D have been associated with depression.[8] Incidence of depression was higher (8–14%) in people with vitamin-D deficiency.[9]

Fluoxetine, is a selective serotonin-reuptake inhibitors (SSRIs) and mainly employed for depression *via* inhibiting presynaptic reuptake of serotonin at the serotonin transporter.[10-11] SSRIs are frequently taken alone or with other pharmaceuticals such as mirtazapine in psychosis, anxiety, autism, bipolar disorder and depression like behaviors.[12,13,14,15,16-17] However, the role of Vitamin-D alone and in combination with fluoxetine were not studied in CUMS induced depression like behaviors. So, we hypothesized that Vitamin-D or its combination with fluoxetine may have potential to prevent depression like behavior.

2. MATERIALS AND METHODS

2.1 Animals and ethics statement

Male Wistar Albino rats (200-250g; 3 to 5 months old) were purchased from the Indian Veterinary Research Institute in India and were kept in a typical lab setting with unlimited access to water. The Institutional Animals Ethics Committee (IAEC) gave its approval to the protocol (1024/PO/Re/S/08/CPCSEA). To ensure that no animals used in experiments suffered at any point during the process, IAEC criteria were followed.

2.2 CUMS induced depression like behaviors

CUMS is the most conventional animal model for depression. Originally designed for 21 days by Willner et al. (1992) on rats followed by different mild and unpredictable stressors.[18] All these stressors are repeated, corporal and

psychological stressors which executes as that animal are constantly exposed to a sequence of unpredictable mild stressors which triggers number of stress events.

S. No.	Stressor	Duration
1	food deprivation	24 Hrs.
2	water deprivation	24 Hrs.
3	empty water bottles	24 Hrs.
4	slope cage (45ºC)	14 Hrs.
5	wet bedding	16 Hrs.
6	tail pinch	60 Sec.
7	cold swimming	5 Min.
8	cage shaking	10 Min.
9	group housing	12 Hrs.

Table 1. Different stressors

2.3 Drugs and experimental protocol

All medications and chemicals were bought from well-known suppliers. From previously published studies, dosages of Vit.D (5.0 mg/kg s.c.) and fluoxetine (0.5 mg/kg s.c.) were used.[19-20] In total, eight groups with a total of six Male Wistar Albino rats each were employed in this study.

2.4 Experimental protocol

Total 48 animals were used in the study. There were 8 groups as given below and each group contains 6 animals.

Group I- Control: The animals were treated (10 ml/kg i.p) normal saline for 21 days.

Group II and III- Fluoxetine and Vitamin-D *per se*: The Animals were administered Fluoxetine (0.5 mg/kg s.c) and Vitamin-D (5.0 mg/kg s.c.) for 21 days.

Group IV- CUMS: The Animals were treated with different mild stressors to induce CUMS for 21 days.

Group V- CUMS + Fluoxetine: Fluoxetine (5.0 mg/kg s.c) was administered to the CUMS treated rats starting from day 1 to day 21.

Group VI- CUMS + Vitamin-D Dose 1: Vitamin-D (2.5 mg/kg s.c) was administered to the CUMS treated rats from day 1 to day 21.

Group VII- CUMS + Vitamin-D Dose 2: Vitamin-D (5.0 mg/kg s.c) was administered to the CUMS treated rats from day 1 to day 21.

Group VIII- CUMS + Fluoxetine + Vitamin-D Dose 1: The CUMS treated rats were administered combi. of Fluoxetine (5.0 mg/kg s.c) and Vitamin-D (2.5 mg/kg s.c)

2.5 Assessment of Behavior Parameter

To evaluate several behavioural parameters, all the animals (n=48) in their respective groups underwent the tail suspension test, forced swim test, splash test, and open field.

2.5.1 Tail Suspension Test (TST)

The TST was executed at first day, on day 1, 7, day 14, and day 21 during the CUMS operations. All animals were swung above the 15 cm of floor by their tail tip (I cm) stick to the lever. The immobility time was recorded for 6 min., whenever the rat was passively suspended or in fully motionless state, it could be regarded as immobile.[21]

2.5.2 Forced Swimming Test (FST)

At the end of the CUMS methods FST was performed on animals. Each rat was putted one by one to the device contains a cylinder (47 cm long; inside diameter 38 cm), filled with normal water and temp. was maintained at 25 ± 2°C. All animals were forced for swimming for 6 min. now the immobility time was note down. The immobility state is that when the animal without movement floated in the water by putting the head above the water level.[22]

2.5.3 Splash Test

The rats were separately kept in the cage which restoring the normal environment, and adjusted 30 min. before to the experimental environment. After that, the 30% sucrose sol. was sprayed to the rat gently to their back and neck for three times. The frequency of grooming was noted. To avoid any impacts on the next test, the cage was splashed with 75% ethyl alcohol and padding was changed.[23]

2.5.4 Open-Field Test (OFT)

This test was performed to determine the chronic stress effects on animals. Test was executed on the last 2 days before completion of the experiment. The apparatus is a rectangular container (80 cm× 80 cm × 50 cm), flooring of container is equally splits into 25 (16 cm× 16 cm) uniform squares. The rats will put in the middle of the open-field container and allow to openly exploring for three min. Two motor parameters were quantified all over this test: (1) locomotion frequency (numbers of lines which the rat crossed one of the grid lines with all four paws) (2) rearing frequency (times of rearing with hind legs). The animals were acclimatized to the experimental area for min 2 hrs before start of the test. The OFT test was carried out in a room which was sound proof and there was no human interference, also the room cleaning was done with a 5% water-ethanol solution, prior to behavioral testing for removal of bias because of the odors leaved by former rats. The assessment of rat's motivational behaviors was performed and noted by two independent observers in a blinded manner.[24]

2.6 Estimation of Biochemical Parameter

2.6.1 Isolation of brain and homogenization

After behavioral studies, rats in their respective groups (n=48) were sacrificed, isolation of the brain and preparation of the homogenate were done as per formerly documented reports.[25] The supernatants were used for biochemical assessments.

2.6.2 Measurement of MDA

Ohkawa et al. (1979) method was used for the measurement of the Lipid peroxide content.[26] MDA was estimated with lipid peroxidation, & were measure by spectrophotometrically used as method of 1,1,1,3 tetraethoxypropane. MDA was expressed in named of nano moles per mg of protein. 500 µl Tissue homogenates in a phosphate buffer (pbs 7.4) added 300 µl 30% Trichloroacetic acid (TCA). 150 µl 5N HCL & To 300 µl of 2% (w/v) 2-thiobarbituric acid (TBA) added to reactant mix be heated at 90°C for 15min. The blend was centrifugated at 12,000 × g for 10 min. pink color supernatant obtained & Measured the Absorbance 532 nm by UV spectrophotometrically.

2.6.3 Estimation of GSH

Ellman (1959) method readjusted by Sedlak and Lindsay (1968) was adapted to figure out level of reduced glutathione.[27-28] GSH was calculated by its 5,5' dithiobis (2-nitrobenzoic acid) reaction with yellow chromophore & measured by UV spectrophotometrically at 412 nm. 1 ml tissue homogenate added 1 ml of 10 % TCA for deproteinization centrifuged at 2000× g for 5 min at 4°C & clear supernatant was estimated the GSH. 0.1ml tissues sample (pH 8.4) added 0.5ml 5,5' dithiobis (2-nitrobenzoic acid), 0.4ml double distilled water with continuous stirring.[29]

2.6.4 Estimation of Superoxide Dismutase (SOD)

Kono et al, 1978 method was adopted to determine the SOD.[34] The method was measured by SOD activity and 1mM of tissues were homogenized in chilled 0.1 mM ,50mM sodium carbonate, 96mM nitro blue tetrazolium (NBT). 2ml above mixture, 0.05ml of hydroxylamine, 0.05 ml of clear supernatant & the auto were measured spectrophotometrically for 2 min at 30 sec at 560 nm.[30-31]

2.6.5 Estimation of Catalase

Luck, 1971 method was adopted to assay the catalase activity.[32-33] The method

by which breakdown of hydrogen peroxide was estimated by catalase activity 3ml of hydrogen peroxide, phosphate buffer was added 0.05ml of supernatant of tissue homogenate was added as enzyme source, and the contents were mixed well. Absorbance at 240 nm was reported 20 s for 3 min.[34]

2.6.6 Estimation of Nitrite Oxide

Nitrite, the stable end product of nitric oxide metabolism (as RNI) was measured in plasma by Griess method.[35] Nitrite was measured in the rat brain using Griess reagent and acts as an indicator of nitric oxide production. 100µl of Griess reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid & .1% naphthylamine diamine dihydrochloric acid in hydrogen peroxide and 100µl of clear supernatant. 542 nm of Absorbance was measured.[36]

2.6.7 Total Protein Estimation

For estimation of protein in tissue homogenates was performed by the method of Lowry et al. (1951).[37] Protein was measured in all brain samples for according to the method of Lowry using bovine serum albumin (BSA) (1 mg/ml) as standard.

2.7 Statistical Analysis-

Statistical analysis was done by using software GraphPad Prism. All results were shown as the mean and standard error of the mean-S.E.M. One-way analysis of variance (ANOVA) was used to assess all the results, followed by Tukey's multiple comparison tests. In terms of statistics, a value of $p<0.05$ was considered significant.

3. RESULTS

3.1 Assessment of Behavioral Parameters

3.1.1 Tail Suspension Test:

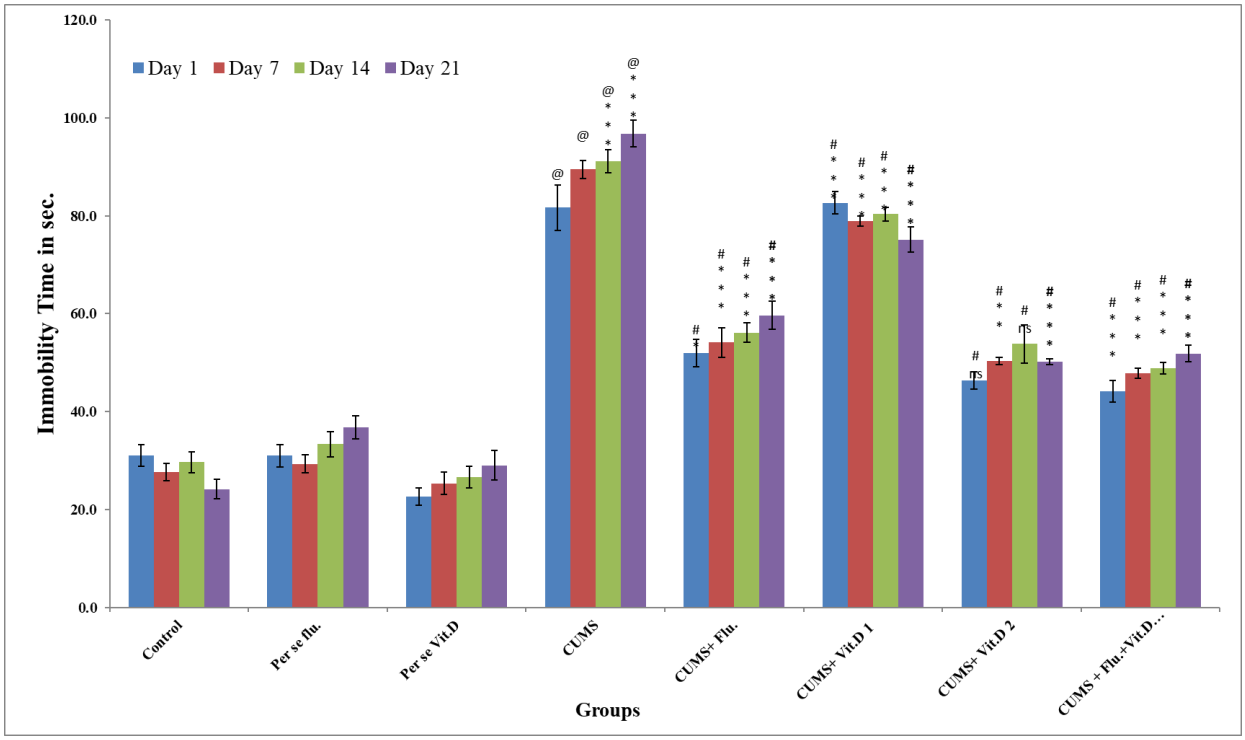

Figure 1. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D 1) on TST

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's test using software Graph Prism Pad.

Repeated CUMS exposure on day 1, 7, 14, 21 significantly ***@ [p<0.05] increased immobility time when compare to control group. However, administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c), and Flu. (5.0 mg/kg s.c) + Vit.D (2.5 mg/kg s.c) significantly ***# [p<0.05] decreased the immobility time when compare to CUMS treated group **(Figure 1).** TST- tail suspension test; Vit.D: Vitamin-D, Flu: Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous, ***@ [p<0.05] vs. control group, ***# [p<0.05] vs. CUMS treated group.

3.1.2 Forced Swim Test:

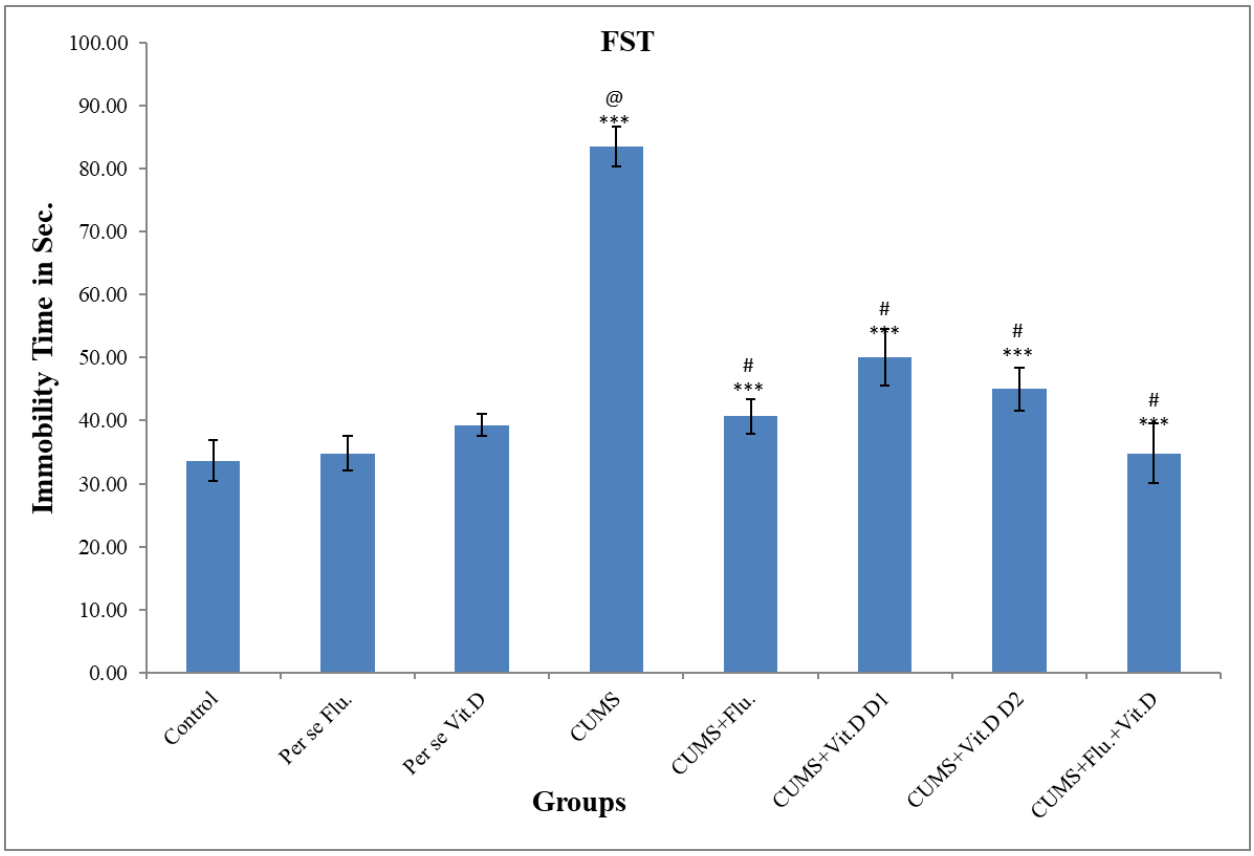

Figure 2. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on FST

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison test using software Graph Prism Pad.

Repeated CUMS exposure on day 21 significantly ***@ [p<0.05] increased immobility time when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. [D1 (5.0 mg/kg s.c), D2 (10.0 mg/kg s.c)] and combi. Flu. D1 (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [p<0.05] decreased the immobility time when compare to CUMS treated group **(Figure 2).** Vit.D- Vitamin-D, Flu: Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous. ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group; FST- force swim test.

3.1.3 Splash Test:

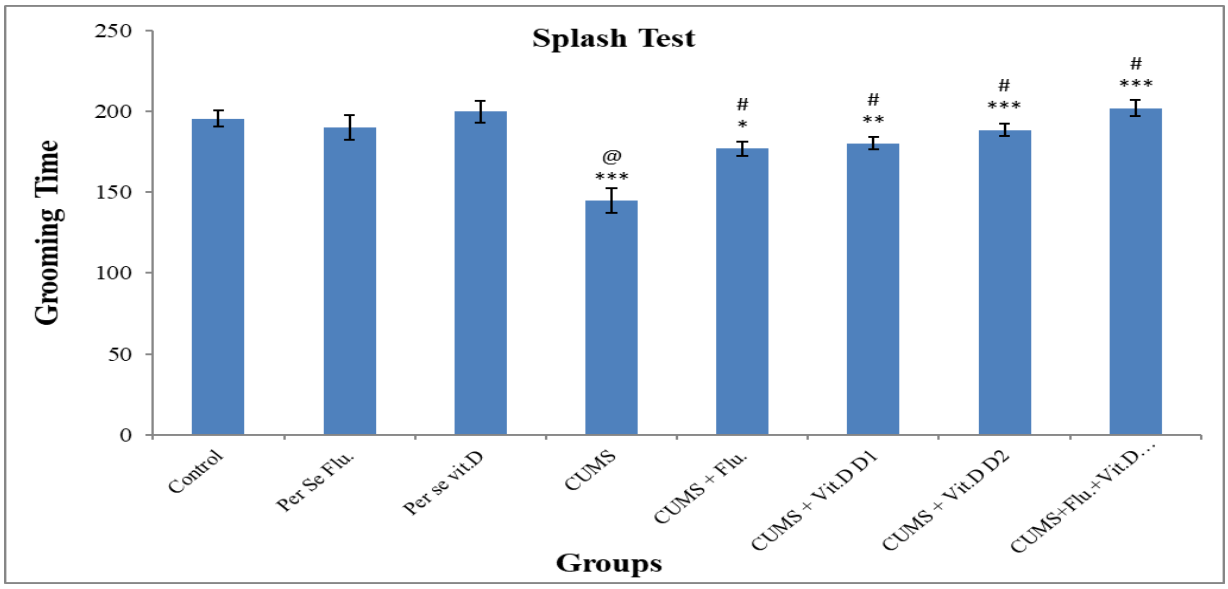

Figure 3. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on Splash Test

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison tests using software GraphPad Prism.

Repeated CUMS exposure on day 21 significantly ***@ [p<0.05] decreased grooming time when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c), and Flu. (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [p<0.05] increased the grooming time when compare to CUMS treated group (**Figure 3).** Vit.D- Vitamin-D, Flu.- Fluoxetine, D- Dose, CUMS-chronic unpredictable mild stress, s.c-subcutaneous ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group

3.1.4 Open Field Test:

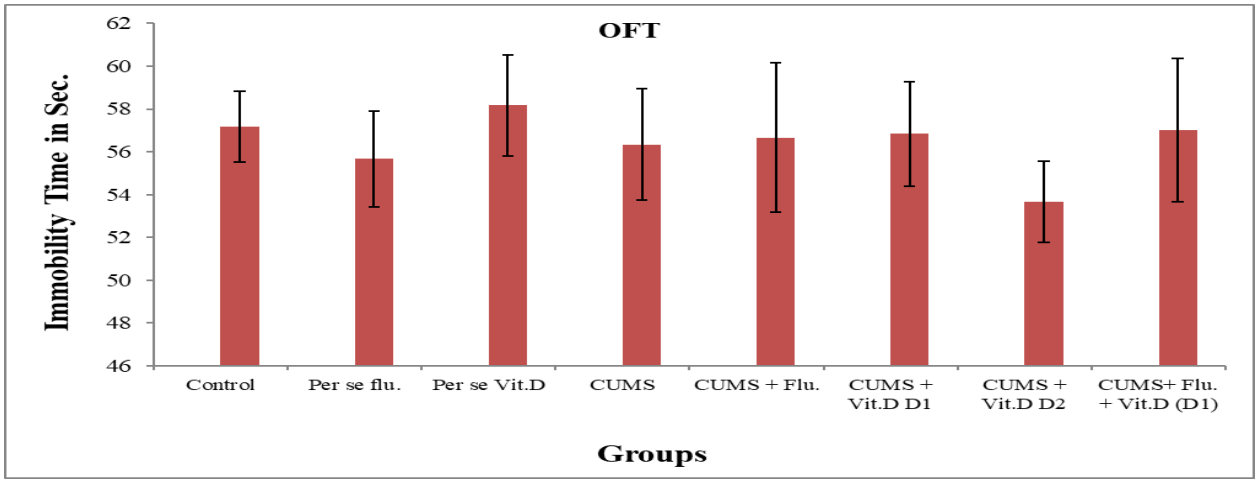

Figure 4. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on OFT

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison test using software GraphPad Prism.

Repeated CUMS exposure on day 21, and administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c) and Flu. (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c). No significant [p<0.05] change observed on comparison of all group **(Figure 4).** OFT- open field test, Vit.D- Vitamin-D, Flu.- Fluoxetine, D-Dose (Fluoxetine 5.0 mg/kg + Vitamin-D 2.5 mg/kg), CUMS- chronic unpredictable mild stress, s.c- subcutaneous ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group SEM- standard error mean.

3.1.5 Body Weight

There was no significant difference in body weight between different groups.

3.2 Estimation of Biochemical Parameter

3.2.1 Estimation of GSH:

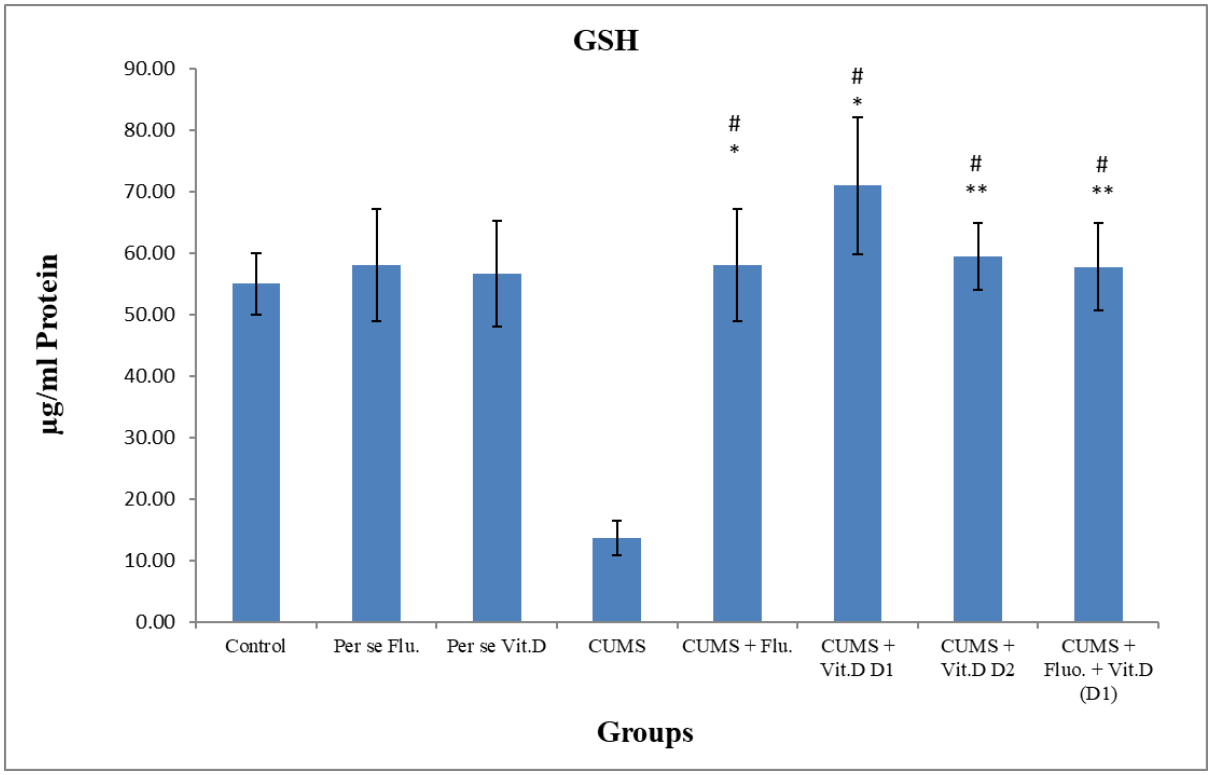

Figure 5. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on GSH

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison test using software GraphPad Prism.

After repeated CUMS exposure experiment there was significantly ***@ [p<0.05] decreased level of brain GSH when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c), and Flu. (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [p<0.05] increased the level of brain GSH as compare to CUMS treated group **(Figure 5).** SEM- standard error mean, GSH- reduced glutathione, Vit.D- Vitamin-D, Flu.- Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group.

3.2.2 Estimation of MDA:

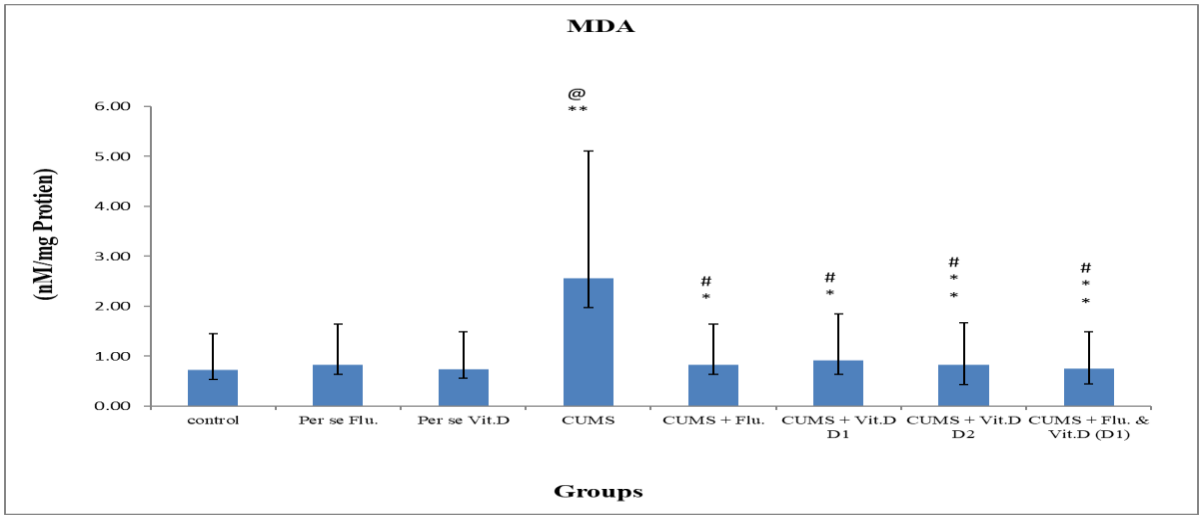

Figure 6. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on MDA

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison test using software GraphPad Prism.

After repeated CUMS exposure experiment there was significantly ***@ [p<0.05] increased level of brain MDA when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c), Flu. (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [p<0.05] decreased the level of brain MDA as compare to CUMS treated group **(Figure 6).** SEM- standard error mean, MDA- malondialdehyde, Vit.D- Vitamin-D, Flu.- Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group.

3.2.3 Estimation of Nitrite:

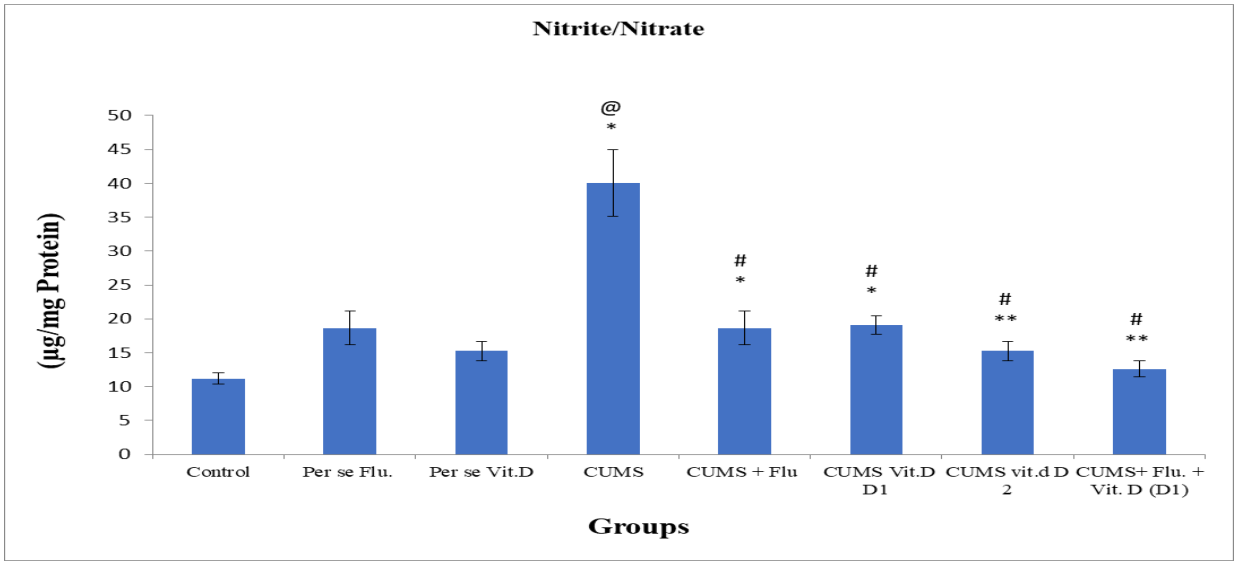

Figure 7. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on Nitrite/nitrate

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison test using software GraphPad Prism.

After repeated CUMS exposure experiment there was significantly ***@ [p<0.05] increased level of brain Nitrite when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c), Flu. D1 (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [p<0.05] decreased the level of brain Nitrite as compare to CUMS treated group **(Figure 7).** SEM- standard error mean, Vit.D- Vitamin-D, Flu: Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group.

3.2.4 Estimation of Catalase:

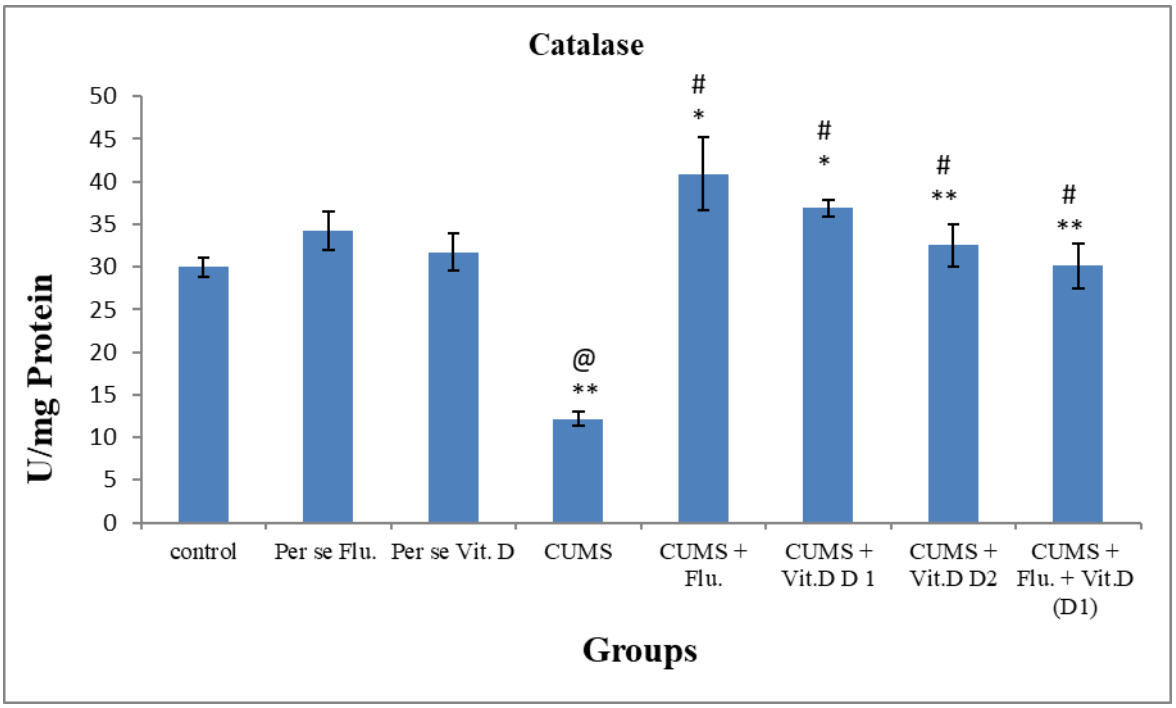

Figure 8. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on Catalase

Results were expressed as mean ± SEM, one way ANOVA followed by tukey's multiple comparison test using software GraphPad Prism.

After repeated CUMS exposure experiment there was significantly ***@ [p<0.05] decreased level of brain catalase when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c), and Flu. (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [p<0.05] increased the level of brain catalase as compare to CUMS treated group **(Figure 8).** SEM- standard error mean, Vit.D- Vitamin-D, Flu.- Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous ***@ [p<0.05]- control group, ***# [p<0.05]- CUMS treated group.

3.2.5 Estimation of SOD:

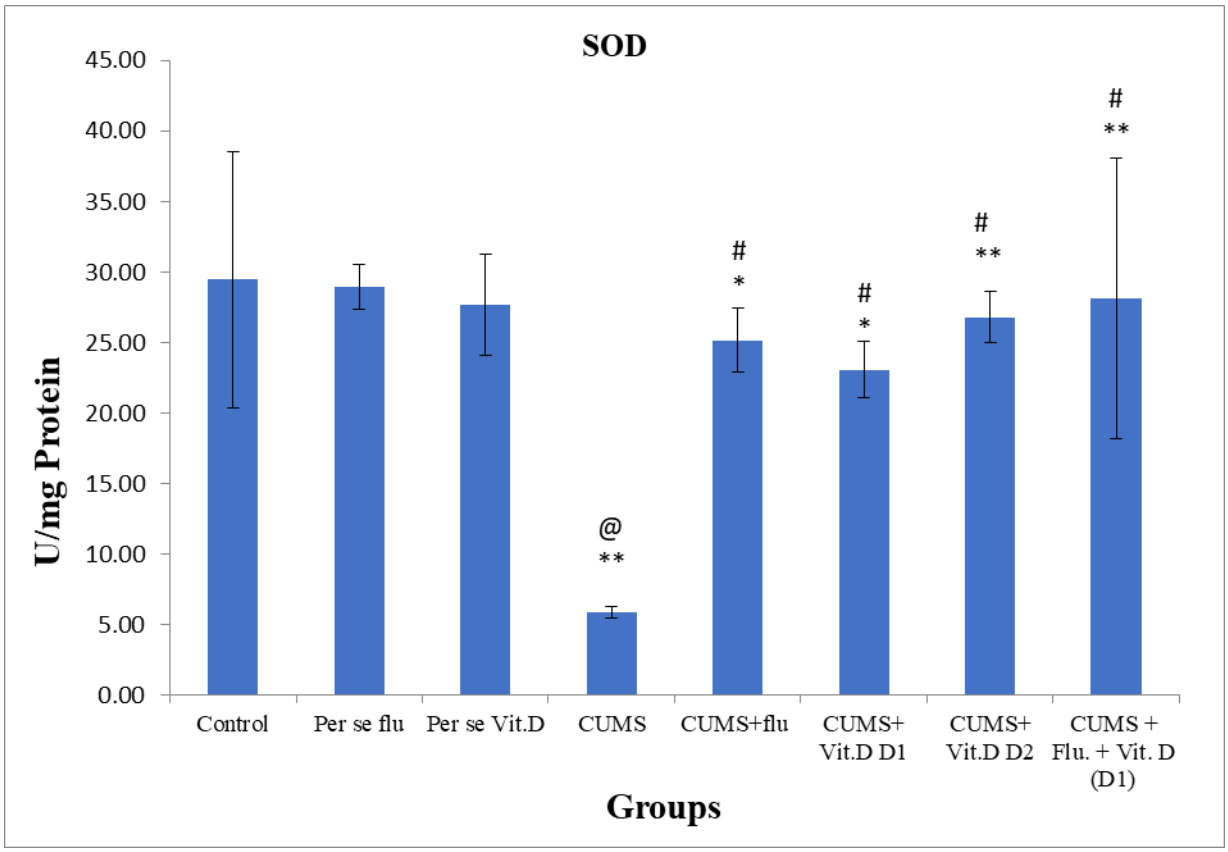

Figure 9. Effects of Vitamin D, Fluoxetine and Fluoxetine + Vitamin D (D1) on SOD

Results were expressed as mean ± SEM, one-way ANOVA followed by tukey's multiple comparison test using software GraphPad Prism.

After repeated CUMS exposure experiment there was significantly ***@ [$p<0.05$] decreased level of brain SOD when compare to control group. While administration of drug Vit.D [D1 (2.5 mg/kg s.c), D2 (5.0 mg/kg s.c)], Flu. (5.0 mg/kg s.c) and Flu. (5.0 mg/kg s.c) + Vit.D D1 (2.5 mg/kg s.c) significantly ***# [$p<0.05$] increased the level of brain SOD as compare to CUMS treated group **(Figure 9).** SEM- standard error mean, SOD- Superoxide dismutase, Vit.D- Vitamin-D, Flu: Fluoxetine, D- Dose, CUMS- chronic unpredictable mild stress, s.c- subcutaneous ***@ [$p<0.05$]- control group, ***# [$p<0.05$]- CUMS treated group.

4. DISCUSSION

The represented study was formulated for analysis of antidepressant like effects of Vitamin D at low and high dose in chronic unpredictable mild stress (CUMS) animal model, moreover to examine the beneficial effect of drug of Vit. D with Fluoxetine. The results of this study give a way that the CUMS regimen produced a significant declined state and effects are reversed by administering the Fluoxetine with Vitamin D. But there was no significant effect on loco-motor activity and body weight of rats. All drugs administered prevented the stress-induced reduction in grooming time in splash test, decrease the immobility time in tail suspension method, and reduce the immobility time in force swim test.

Stress is very authenticating animal method to generate the depression like behavior.[38] Furthermore chronic stress caused suppressed neurogenesis, diminished cell proliferation and survival etc.[39] SSRI like fluoxetine reversed the behavior effects of CUMS group.[40-41] Tail suspension test and force swim test are behavior distress widely used to examine depression like behavior in animals. After exposure to CUMS immobility time imitate as depression in human being. The investigated data exhibit that rats exposed to chronic stress presented a significant prolongation of immobility time in both tail suspension test (TST) and force swimming test (FST) and the Vitamin D administration significantly reversed the duration of immobility in rats it is the indication of antidepressant like effect.

Open field test (OFT) is very extensively used method for the evaluation of the loco-motor behavior in the study animals, in this test, CUMS group rats showed decreased crossing & the rearing, it is the indication of reduced exploration and the insensitivity in the animals so Vitamin D has the ameliorative effect on the loco-motor behavior of animal. Splash Test is another widely used test to examine the grooming time in experimental animal. When the rats were exposed to CUMS group showed decreased grooming time and Vitamin D administration reverse this effect, it indicated that, Vitamin D is effectively shoed positive results.

In Biochemical estimation malondialdehyde (MDA) was measured by using the okhawa method on exposure to CUMS it showed the increased level in rats. As in stress state free radicals produced the lipid-peroxidation in brain which is one of the resultants of polysaturated fatty acids peroxidation in the cell. Overproduction of free radicals caused increased the level of MDA. Vit.D administration reduced the level of MDA as compare to CUMS group.[42] Glutathione (GSH) was examined by the Ellman's method. It is the main free radical scavenger in the brain; reduced level elevates cell vulnerability toward the oxidative stress. When animal exposed to CUMS GSH level reduced and administration of Vit.D significantly maintained GSH level.[43] Nitrite oxide was measured by using Griess method, it is diffusible gaseous component and main messenger of CNS its level increased when rats were exposed to CUMS. Overproduction can cause cell death in Nervous System and Vit.D significantly decreased its level when compare to CUMS group animal.[44] Superoxide dismutase (SOD) is measured by using Kono method; in inflammation on stress state its level decreased on administration of CUMS rats, Vit.D significantly increased SOD level as compare to CUMS group.

5. CONCLUSION

Fluoxetine, Vit.D and fluoxetine with Vit.D reversed the stress-induced effects following the CUMS procedures. But the study suggested that, there were more beneficial effects of combination drug which can lessen the dose of individual drug. The results of this study supported the hypothesis that Vitamin D can be beneficial as fluoxetine for the treatment of depression. But some preclinical and clinical studied required to support this presented mechanism. So, it can be a good candidate drug for the treatment of depression in future.

6. FUNDING

Self-Financed

7. CONFLICTS OF INTEREST

No conflict of interests has been reported by the authors in this work.

8. AUTHOR CONTRIBUTIONS

Each author made an active contribution in developing, collecting data or analysis and interpretation of the findings. The authors have also made significant contributions in the design and revision of this article. As indicated by the International Committee of Medical Journal Editors, each author may be designated as an Author in accordance with guidelines and requirements.

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