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ORIGINAL ARTICLE

Effect of Vitamin D Supplementation on Neurological Function and Gastric Emptying in Rat Model of Depression

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ABSTRACT
Background: By 2030, depression is predicted to rank among the top three diseases in terms of burden. Previous research showed controversies about vitamin D's impact on depression and its correlation with altered cognitive function and stomach emptying. We therefore carry out this research to look into this effect. We aimed in this work to investigate the influence of vitamin D on behavior and neurochemical parameters and gastric emptying in a rat model of restraint stress induced depression.
Methods: This study was conducted in in the animal unit of the physiology department, faculty of Medicine, Zagazig University on 36 healthy, adult male Wistar albino rats. Rats were divided into group 1 (n=9): Sedentary intact, group 2 (n=9): Depression induced by restraint stress, group 3 (n=9): (depression induced+ prophylactic vitamin D) and group 4 (n=9): (depression induced+ curative vitamin D).
Results: Group 2 (restraint induced depression group) showed a significant decrease in gastric emptying when compared to that of group 1. Group 3 (preventive vitamin D group) showed a significant increase in gastric emptying when compared to that of group 2 and no significance when compared to that of group 1.
Conclusions: Vitamin D has a preventative impact against chronic restraint stress-induced behavioral and biochemical abnormalities and relieving somatic symptoms as improvement of gastric emptying.
Keywords: Vitamin D, gastric emptying, rat depression.

INTRODUCTION

A very low mood, interest loss, cognitive impairment, and a persistent sense of hopelessness are all signs of depression, a serious mood disorder. It significantly impacts the impacted person's ability to function on an individual, biological, and social level **[1].**

Depression is a highly prevalent disorder, although it can be difficult to treat medically because it is a significant public health concern since many patients who recover experience relapses or recurrences of their symptoms. Furthermore, many of these patients are unable to attain treatment-induced remission **[2].**

One common complaint from people with major depressive disorder (MDD) is somatic symptoms, gastrointestinal problems that are often mentioned and associated with worse results **[3].** Despite the abundance of evidence, the relationship between depressive episodes and gastrointestinal dysfunctions still has to be confirmed **[4].**

Since drug side effects induce poor adherence and some patients may not see a clinically significant response with high recurrence rates, it is imperative to find alternate methods for treating and preventing depression.

One unique neurosteroid hormone that controls the homeostasis of bones in the body is vitamin D. As it crosses the blood-brain barrier, it is also implicated in brain activities **[5].**

Furthermore, the fact that vitamin D plays a

major role in brain functions like neuroplasticity and neuroimmunomodulation raises the possibility that it may also play a part in mental illnesses like depression **[6].**

It is interesting to note that it has anti-inflammatory properties that obviously participate in preventing neuroinflammation and antioxidant properties that shield cells from oxidative damage **[7].** Furthermore, **Al-Ramadhan et al. [6]** demonstrated that the antidepressant effects of vitamin D are comparable to those of prescription antidepressants. However, **Choukri et al. [8]** found that after taking vitamin D supplements, there were no differences in anxiety or depression outcomes between the intervention and control groups.

There are very few scientific trials evaluating the effectiveness of vitamin D supplementation in the management of anxiety and depression, and the conclusions of those that are conducted can be contentious. Future research is necessary in light of the inconsistent results in order to develop a vitamin D supplementation program that effectively prevents or reduces symptoms of anxiety and depression **[9].**

Interestingly, this is the first study to examine the effect of vitamin D on gastric emptying and brain functions in restraint induced rat model of depression and to explore possible role for prevention and treatment of MDD.

METHODS

Animals:

A total of 36 mature male Wistar albino rats in good health, weighing between 150 and 200 grams, were acquired from the Animals House at Faculty of Veterinary Medicine, Zagazig University.

The animals were housed in plastic rodent cages with six rats per cage in hygienic conditions in the animal unit of the physiology department of Faculty of medicine at Zagazig University. The cage is 45 cm by 28 cm by 20 cm. Prior to the start of the trials, the rats were given two weeks to get used to living in an animal house.

The animals had free access to food and water and were fed a regular diet that was provided by Zagazig Faculty of Agriculture. The Zagazig University Animal Care and Use Committee and the Physiology Department approved the experimental protocol (ZU-IACUC). The approval number is ZU-IACUC/3/F/9/2023.

Grouping of animals

Rats were split into four equal groups at random: Group 1 (n=9): Sedentary control intact controls will serve to establish basal levels of studied parameters (Behavioral, Pathological, and Biochemical). Group 2 (n=9): Depression induced by restraint stress as the rat is restrained from 09.00 am to 12.00 pm daily for 28 days; Group 3 (n=9): (depression induced+ prophylactic vitamin D) 500IU/kg by oral gavage once daily for 28 days of induction; and Group 4 (n=9): (depression induced+ curative vitamin D) 100 IU/kg by oral gavage for 7 days following induction.

Methods:

1- Induction of depression

To create restraint stress, the rat was restrained every day for 28 days from 9 am to 12 pm. Each rat was put into a cylindrical plastic confinement tube with holes in it for ventilation. This cylinder's diameter was designed to match the mouse's body, preventing it from turning and moving in either direction. The restrainer can be manufactured in a lab or bought commercially. Following protocol, the animals were returned to their individual cages **[10].**

2. Vitamin D supplementation protocol

Rats were given 500 IU/kg of vitamin D orally once a day via gavage. the oral delivery of vitamin D (100 IU/kg) for seven days following the induction of depression **[11].** For body weight measurement, throughout the restraint stress modeling process and vitamin D treatment, all the rats were weighed once a week at a set time.

Measurement of percentage of gastric emptying was done by the method mentioned by **Wang et al. [9].** After feeding the fed rats for an hour, the food and water were taken away. Rats were beheaded, a midline laparotomy was performed, the stomach was taken out, clamped on both ends, and it was weighed after an hour. Next, the stomach was opened, the contents were removed using tap water, and the weight of the stomach wall was measured.

The difference between the stomach weight with and without its contents was used to calculate the amount of food (in grams) that was inside it. The container weight before and after one hour of food exposure was used to calculate how much food the rats consumed throughout the one-hour refeeding session. Additionally, the amount of water consumed by each rat was weighted and added to the amount of food consumed. Water intake was recorded both before and after drinking. The following formula was used to determine the percentage of stomach emptying that occurred during the study period of one hour: weight of food consumed / moist weight of food retrieved from the stomach equals [1 for gastric emptying (%)] × 100 **[12].**

3. Evaluation of Behavioral Parameters

1- Forced swim test (FST) Principle

Procedures:

For the rat FST, two swimming sessions spaced out by a day were recommended. A 5-min test stage session was held 24 hours after the first session, which lasted 15 minutes as a pre-test.

1) **Habituation**: To give the rats enough time to adjust to the testing environment, move them from their home cages to the waiting area at least half an hour before the behavioral test.
2) In order to keep the rats' hind legs from reaching the bottom of the container, fill the cylinders with tap water at 23 ± 1 °C and regulate the water depth according to the size of the rats.
3) Each rat should be kept in the water-filled cylinder container for fifteen minutes.
4) Place the rat in the makeshift drying cage with a heat pad underneath and a heat lamp above after 15 minutes.
5) After every session, replace the water to avoid any impact on the next rat.

Sixteen hours later, the five-minute test phase starts.

7) After turning on the video camera, immerse the rat in the cylindrical container filled with water for five minutes.

8) After every session, replace the water to avoid any influence on the subsequent rats.

Result analysis

Behavioral coding in the initial five-minute test phase

1) If the rat is floating in the water and moving only enough to keep its snout above the surface, note how long it was "Immobile."
2) If rapid forelimb movement is seen, note the amount of time spent as "Struggling/climbing."
3) If the front or rear limbs are moving in a paddling motion, note how long was spent "swimming."

2. Open field maze test (OFT)

Procedure:

1) Instrument acclimation

First, move the rats from their home cages into the testing area so they have about ten minutes to get used to the new environment before the test.

2) Clean the open field: To ensure that every animal can detect the disinfectant equally, it is imperative to clean the open field, even if it was clean prior to the initial trial.

3) Test (3-minutes session)

-With careful handling of its body, remove the test rat from its home cage and set it on the corner square facing the open field box's corner.

-Position the camera over the field box that is open. The camera ought to be installed so that the entire box can be covered.

-Set the timer for three minutes and let the rat explore the test area.

- To reduce tension, the operator ought to vacate the behavioral testing chamber.

- After the session, take the rat out of the box and return it to its home cage.

4) Before the maze was cleaned, the number of fecal boli pellets inside was manually counted and recorded.

5) Use 70% ethanol and paper towels to thoroughly clean the entire box area before proceeding to the next test animal.

Result analysis:

The following variables, which reflect behavior and motor activity associated with anxiety, were measured:

1) Latency: the rat's departure time from the corner square in seconds.

2) Line crossings: a measure of locomotor activity, which is the frequency with which a rodent crosses gridlines with all four paws.

3) Urination and defecation: The frequency of these activities is noted.

4) Rearing: The amount of time the rat spent in the maze standing on its hind legs (the quantity and latency of rears are noted).

3.Modified T-maze

Procedures:

- Habituation: Since the maze's novelty encourages impromptu exploration and variation, no habituation to it was utilized.
- The animals were taken inside the testing room and left for five to ten minutes to ensure they were in the best possible state of alertness for the tests. In an effort to prevent overexcitation and lack of focus, they were not examined right away.
- The goal arm, which was chosen prior to the procedure, had to contain the complete animal, including the tip of the tail. We continuously monitored these criteria to minimize experimenter bias.
- In order to minimize the impact of diurnal variation on the rats' performance, testing was done in the afternoon.
- After every trial day, the T-maze was cleaned with 75% ethanol and then distilled water.
- Approximately 10 mm of bedding was applied to cover the whole maze floor.
- Sample run: The central divider was in place and all guillotine doors were closed when the maze was built. All the guillotine doors were lifted when the maze was built, and the center partition was in place. The rat was started at the start region (bottom of the "T") and given the opportunity to choose a goal arm at the beginning of each run. The rat was confined for 30 seconds in the selected arm, then the door silently slid down, gently removing the animal and returning it to the cage for a 10-minute intertrial period.
- Choice runs: The sample arm's guillotine door was lifted once again after the central divider was removed. To ensure the animal maintained its curiosity and prevented smell bias, a fresh woodchip bedding was placed to alter the floor's odor. In the start area, the rat was swapped out and given the option to select between the two open goal arms while facing away from them. Every experiment took a minute or two. In contrast to the previous run, alternation occurs when the rat slips the door silently down and returns to the cage for a 10-minute intertrial period after being confined for 30 seconds in the selected arm.

Alternation rate and side preference rate calculations

Determine the side preference and alternation percentages, where L denotes the rats' preference for the left arm. R: The right arm is chosen by the rats.

Correct response: in a given set (which consists of two runs), the second run differs from the first.

The rats made the wrong decision by selecting the same arm as in the previous run. **[13].**

4. Blood sampling

The animal was made comfortable in a restraint, and the tail vein blood sample collection procedure was used to obtain the blood. Rubbing the tail from the base to the tip will cause leukocytosis. The tail is submerged in 40°C warm water if the vein is not visible. A localized cosmetic cream was applied half an hour prior to the trial. Using a capillary tube or a syringe with a needle, blood is drawn from the blood vessel using a 23G needle. After the blood collection was finished, pressure was used to halt the bleeding **[14].**

A blood sample was taken and left to coagulate for half an hour in sterile plastic centrifuge tubes. Blood was centrifuged for 15 minutes at 3000 rpm in order to separate the serum. Using fine-tipped automated pipettes, the supernatant serum was pipetted off and frozen at -20 ºC until analysis. Frequent freezing and thawing were avoided **[15].**

5. Serum analysis

Serum levels of interleukin-6 (IL-6), tumor necrosis factor (TNF-α), lipid peroxide, malondialdehyde (MDA) enzyme, total antioxidant capacity (TAC), serum corticosterone, serum CRP, and serum vitamin D were examined. Additionally, immunoassay was used to determine the levels of serum and hippocampus Brain Derived Neurotropic Factor (BDNF). Following the trials, the rats were put to death while heavily sedated, and the blood was extracted to separate the serum. Left

hippocampi were likewise rapidly separated. Hippocampi were kept cold until additional examination. Hippocampal and serum BDNF levels

Statistical analysis:

The statistical analysis was conducted using IBM©, Chicago, IL, USA's SPSS v28. To assess if the data distribution was normally distributed, the Shapiro-Wilks test and histograms were employed. The means of two or more independent groups were compared using the one-way ANOVA test to see if there was statistical support for a significant difference in the related population means.

RESULTS

Estimation of vitamin D showed that its level was reduced in group 2 (depression induced) compared to other groups, **Table 1**.

Regarding behavioral parameters, rats in group 2 (depression induced) exhibited more depressive-like behavior in forced swim test than the other groups. Including increased immobility time and decreased period of swimming in FST. However, group 3 and 4 (vitamin d received) performed better in the forced swim test, **Table 2**.

Group 2 (restraint induced depression group) showed a significant decrease in gastric emptying when compared to that of group 1. Group 3 (preventive vitamin D group) showed a significant increase in gastric emptying when compared to group 2 and no significance compared to group 1. Group 4 (curative vitamin D group) showed a significant increase in gastric emptying if compared to group 2 and no significance ($P>0.05$) compared to group 1, **Table 3**.

Estimation of hippocampal and serum BDNF showed that their levels were significantly reduced in group 2 (depression induced) in comparison to other groups. However, there was no significant difference in hippocampal and serum BDNF between other groups, **Table 4**.

Serum corticosterone increased significantly in group 2 (depression induced) However, this increase was significantly less marked in group 3 and 4 (vitamin d received), **Table 5**.

Regarding oxidative stress markers, serum MDA in group 2 (depression induced) showed significant elevation. On the other hand, serum SOD and TAC were significantly reduced in group 2 compared to other groups. Group 2 (restraint induced depression group) showed a significant increase in serum IL6, TNF alpha and CRP if compared to group 1. Group 3 (preventive vitamin D group) showed a significant decrease in serum IL6 compared to group 2 and no significance when compared to that of group 1. Group 4 (curative vitamin D group) showed a significant decrease in serum IL6 when compared to that of group 2 and no significance compared to group 1, **Table 6**.

In group 1 (control group): cut section of the hippocampus (CA3) revealed normal neuronal cells with normal vesicular nuclei, **Figure s1.**

In group 2 (Depression induced group): cut section of the hippocampus (CA3) revealed many degenerated cells with pyknotic dark stained nuclei (black arrow) with scattered normal cells with normal vesicular nuclei (red arrows), **Figure s2**.

In group 3 (preventive vitamin D group) it had remarkable improvement with decrease in the number of degenerated cells with pyknotic dark stained nuclei) with increase in normal cells with normal vesicular nuclei showing a significant improvement when compared to that of group 2, **Figure 1**.

In group 4 (curative vitamin D group): cut section of the hippocampus (CA3) revealed moderate improvement with moderate decrease in the number of degenerated cells with pyknotic dark stained nuclei (black arrow) with increase in normal cells with normal vesicular nuclei (red arrows), **Figure s3**.

In group 1 (Control group): normal gastric mucosa (blue arrow), normal gastric submucosa (red arrow) and normal intermyenteric nerve plexus (red circle), **Figure s4**. While group 2 (Depression induced group) revealed marked lymphocytic infiltration in the intermyenteric nerve plexus (red circle), **Figure s5**.

Group 3 (preventive vitamin D group) had noticeable improvement with mild lymphocytic filtration in the intermyenteric nerve plexus showing a significant improvement unlike group 2, **Figure 2**. Regarding group 4 (curative VIT D group), it revealed moderate lymphocytic infiltration in

the intermyenteric nerve plexus (red circle), **Figure s6**.

Table 1: Vitamin D in all studied groups

	Group 1	Group 2	Group 3	Group 4
Vitamin D level (ng/ml)				
Mean	67.13	39.4	68.02	65.68
±SD	1.31	1.85	2.14	1.8
Range	64.88-68.92	36.98-42.51	64.87-71.22	63.43-68.87
F	527.04			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				0.01

Table 2: Behavioral parameters in all studied groups

	Group 1	Group 2	Group 3	Group 4
Swimming sec				
Mean	131	83.44	144.55	129.22
±SD	22.16	7.26	13.39	15.11
Range	102-161	73-94	121-169	108-151
F	26.82			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				<0.05
Immobility sec				
Mean	127.11	188.66	133.33	139
±SD	21.33	11.76	17.92	10.09
Range	100-157	172-206	105-161	125-154
F	28.1			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05
Climbing sec				
Mean	43.77	22.11	45.22	40.00
±SD	10.43	5.92	11.82	6.94
Range	31-60	12-32	30-64	28-50
F	12.34			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05

Table 3: Gastric emptying percentage in all studied groups

	Group 1	Group 2	Group 3	Group 4
Gastric emptying percentage %				
Mean	76.11	28.88	73.8	70.00
±SD	4.16	7.81	4.16	6.61
Range	70-80	20-40	70-80	60-80
F	129			
P value of LSD vs. Group 1		<0.001	>0.05	<0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05

Table 4: BDNF and hippocampal BDNF in all studied groups

	Group 1	Group 2	Group 3	Group 4
Serum BDNF (ng/ml)				
Mean	0.67	0.46	0.70	0.67
±SD	0.10	0.04	0.11	0.06
Range	0.49-0.81	0.42-0.55	0.49-0.83	0.58-81
F	14.83			
P value of LSD vs. Group 1		<0.01	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05
Hippocampal BDNF (ng/mg)				
Mean	2.25	0.75	2.35	2.34
±SD	0.49	0.004	0.54	0.496
Range	1.83-2.94	.75-.77	1.87-2.97	1.78-2.82
F	27.89			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05

BDNF, Brain Derived Neurotropic Factor

Table 5: Cortisone in all studied groups

	Group 1	Group 2	Group 3	Group 4
Corticosterone (ng /ml)				
Mean	25.27	119.68	28.55	33.57
±SD	0.76	11.67	1.34	1.79
Range	24.31-26.65	98.69-130.58	26.35-30.75	31.22-35.75
F	522.54			
P value of LSD vs. Group 1		<0.001	>0.05	<0.05
P value of LSD vs. Group 2			<0.001	< 0.001
P value of LSD vs. Group 3				<0.05

Table 6: Other biochemicals in all studied groups

	Group 1	Group 2	Group 3	Group 4
MDA (nmol/ml)				
Mean	0.56	8.44	0.59	0.81
±SD	0.09	0.53	0.099	0.06
Range	0.44-0.71	7.71-9.11	0.49-0.74	0.71-0.89
F	1797.42			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05
SOD (u/ml)				
Mean	190.61	52.11	194.4	187.72
±SD	4.07	7.7	3.44	3.93
Range	184.82-196.34	42.92-64.03	189.23-198.98	181.97-193.63
F	1680.6			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				<0.05

TAC (ng/ml)				
Mean	9.22	0.826	8.49	7.98
±SD	1.02	0.09	0.92	0.69
Range	7.63-10.83	0.69-0.99	7.11-9.98	7.05-10.83
F	227.28			
P value of LSD vs. Group 1		<0.001	>0.05	<0.01
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05
Serum IL-6 (pg/ml)				
Mean	72.1	184.15	75.92	71.86
±SD	2.15	10.19	1.28	2.47
Range	68.9-74.8	166.9-198.8	68.4-75.6	68.4-75.6
F	952.03			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05
TNF alpha (pg/ml)				
Mean	121.9	504.1	125.3	122.3
±SD	1.9	10.9	1.1	2.3
Range	119.3-124.8	488.3-520.3	123.3-126.9	119.5-126.1
F	9973.9			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05
CRP (ng/dl)				
Mean	10.26	82.28	13.53	11.31
±SD	1.57	10.83	1.51	1.48
Range	7.55-12.25	67.87-97.95	11.11-15.62	9.05-12.98
F	360.98			
P value of LSD vs. Group 1		<0.001	>0.05	>0.05
P value of LSD vs. Group 2			<0.001	<0.001
P value of LSD vs. Group 3				>0.05

MDA, malondialdehyde; TAC, total antioxidant capacity; IL-6, interleukin-6; TNF, tumor necrosis factor.

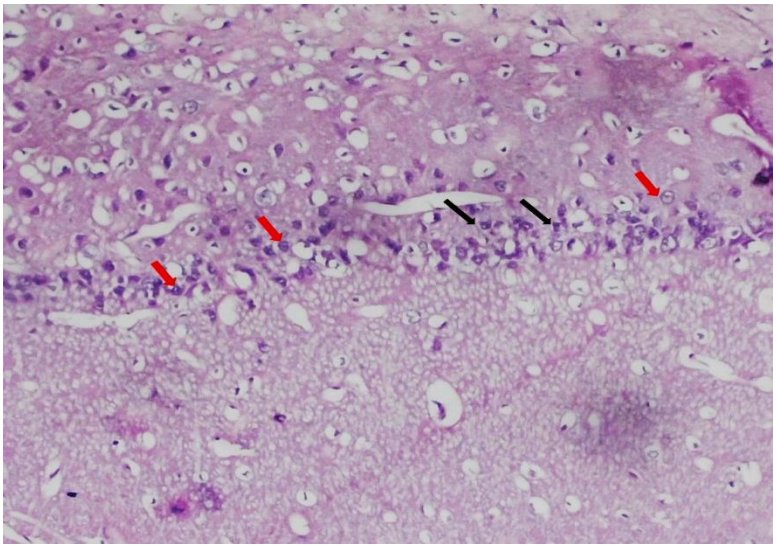

Figure 1: Group 3 (preventive vitamin D group): cut section of the hippocampus (CA3) revealed remarkable improvement with decrease in the number of degenerated cells with pyknotic dark stained nuclei (black arrow) with increase in normal cells with normal vesicular nuclei (red arrows). H&E staining X200 HPF.

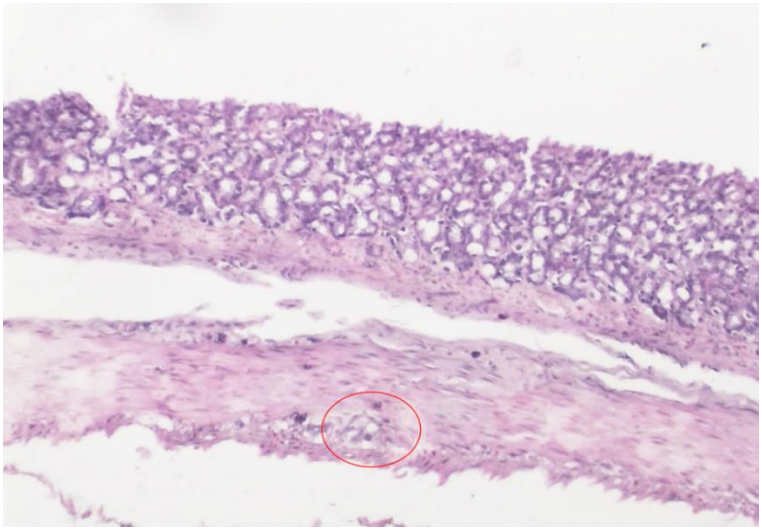

Figure 2: Group 3 (Depression induced +preventive VIT D group): revealed noticeable improvement with mild lymphocytic infiltration in the intermyenteric nerve plexus (red circle) H&E staining X200 HPF.

DISCUSSION

Regarding behavioral and cognitive tests, the present study showed that rats in group 2 exhibited depressive-like behavior while group 3 and 4 achieved better scores to come in line with group 1.

For modified forced test, rats in group 2 exhibited more depressive-like behavior with a substantial increase in immobility time and a significant decrease in swimming and climbing time in comparison to other groups.

Concerning another aspect, the present results showed that group 3 and 4 outperformed group 2 in behavioral tests (such as the forced swim test). Including a decrease in immobility time, and an increase in swimming time in forced swimming test (FST). This came in agreement with **Bakhtiari-Dovvombaygi et al. [16]** stated that depressive behavior as measured by the FST and OFT tests is reduced by vitamin D. On the contrary, **Koshkina et al. [17]** found that the behavioral and neuroendocrine traits of the long-term ovariectomized rats exposed to chronic unpredictable mild stress were unaffected by varying dosages of vitamin D.

Also, **Xie et al. [18]** discovered that vitamin D improves the prognosis for depression as well as the reduced incidence of depression. On contrary, **Gowda et al. [19]** stated that taking supplements of vitamin D did not significantly lower the incidence of depression.

In the present study, the behavioral and cognitive aberrations observed with chronic restraint stress (CRS) were paralleled by biochemical alterations, including increased corticosterone, inflammatory markers, and oxidative stress levels, as well as reduced hippocampal and serum brain derived neurotrophic factor (BDNF) levels. vitamin D was effective in reducing such deficits.

One of the most studied biological systems in Major Depressive Disorder (MDD) is the Hypothalamic-Pituitary-Adrenal Axis (HPA) axis. Research has shown that patients with MDD had elevated cortisol levels. Significantly, changes in HPA are associated with a decline in cognitive performance **[20].**

Prolonged stress disrupts the HPA axis' feedback loop and generates an excessive rise in corticosterone, which desensitizes lymphocytes' glucocorticoid receptors and results in dysfunctional lymphocytes **[21].** **Sedaghat et al. [22]** found that while animals given a higher concentration of vitamin D during the stress treatment avoided the rise in serum corticosterone (CORT) levels, the rats given 3 hours per day of restraint stress for 28 days saw an increase in CORT levels. This came in accordance with **Bakhtiari-Dovvombaygi et al. [16]** They demonstrated that the group receiving treatment for unexpected chronic moderate stress (UCMS) had higher corticosterone levels. Analysis of the current data obtained from vitamin D treated groups in this study showed that it was able to lower basal corticosterone high levels. These results were in agreement with **Al-Ramadhan et al. [6]** They discovered that

taking vitamin D daily through IP stopped corticosterone levels from rising.

On the contrary, **Fitzgerald et al. [23]** showed that the relationship between cortisol concentration and dichotomous 25(OH)D status was inversely correlated and statistically significant. Furthermore, **Sedaghat et al. [22]** did not find that the rats receiving vitamin D had significantly lower CORT levels.

Inconsistencies in the findings of several investigations may be due to the differences in the time of sampling and modifications in the diurnal cortisol rhythm, which are commonly observed in patients with MDD **[24].**

The brain is highly susceptible to the generation of reactive oxygen species (ROS) because it has a modest antioxidant capacity compared to 20% of the metabolized oxygen [25]. In contrast, UCMS boosts metabolic rate and ROS formation. Elevated ROS can cause harm to cells and tissues, ultimately culminating in oxidative stress. This damage modifies the antioxidant defense mechanism and modifies the equilibrium between the oxidant-antioxidative systems **[26].**

In line with these finding, **Pal et al. [27]** documented changes in immunological function brought on by stress According to oxidative stress markers, acute stress raised MDA levels, changed GSH levels, lowered SOD and catalase levels, and lowered the body's antioxidant system's capacity.

In support of this finding, the present study showed that vitamin D can prevent anxiety and depression in rats by preventing oxidative stress damage and neuroinflammation.

These findings were in agreement with **Bakhtiari-Dovvombaygi et al. [16]** They found that vitamin D treatment raised the concentration of total thiol, boosted SOD and CAT activity, and lowered MDA levels in cortical tissue. Conversely, there were no notable alterations in the indicators linked to antioxidant status in response to vitamin D as **Scholten et al. [28]** study found.

The current study shows that group 2 experienced a significant reduction in both hippocampal and serum BDNF following a 28-day period of CRS. This finding pertains to the effects of CRS on hippocampal BDNF levels.

It is generally known that there is a negative relationship between BDNF levels and depressive symptoms **Chakrapani et al. [29].** Lower levels of BDNF have been seen in the serum of MDD patients. By controlling neurogenesis, BDNF and other neuroplasticity regulators may influence behavior. MDD patients' leukocytes also had lower levels of BDNF mRNA **[20].**

Numerous lines of evidence connect mood disorders and the antidepressant effects of BDNF and its receptor TrkB (neurotrophic receptor tyrosine kinase 2) **[30].**

According to one study, supplementing depression-like behavior groups with vitamin D3 led to a noteworthy rise in BDNF levels in brain tissue **[31].** In accordance with these findings, the present study showed that vitamin d was able to upregulate serum and hippocampal BDNF.

Additionally, via interfering with the HPA axis, Vitamin D restored the changes in serum and hippocampus BDNF caused by CRS. However, it may also control the level of BDNF expression in the hippocampal cells through a variety of mechanisms under chronic stress, as documented by **Sedaghat et al. [22].**

Antidepressants have been shown in biochemical experiments to boost BDNF concentrations because many of them upregulate BDNF expression through cyclic AMP-responsive element-binding protein (CREB) signaling. It is reasonable to suppose that in long-term OVX rats with CUMS, vitamin D plays a role in BDNF–CREB signaling regulation **[17].**

Nonetheless, an investigation revealed that either at baseline or following voluntary wheel running, adult vitamin D insufficiency had no effect on the proliferation or survival of adult hippocampus neurons within the dentate gyrus **[32].**

As regards the effects of chronic restraint stress on inflammatory markers, our data showed increased concentrations of serum TNF-α, CRP and IL-6 in the (CRS) group compared to other groups.

Supporting this finding, **Haapakoski et al. [33]** revealed that patients with MDD had

increased levels of the plasma acute phase reactants IL-6, TNF-a, and CRP.

This came in accordance with **Zhong et al. [26]** who noted that patients' brains and peripheral areas had higher concentrations of these cytokines in cases of serious depressive illnesses. A growing body of research suggests that inflammation contributes to the development of MDD.

Furthermore, it appears that people with higher CRP levels had worse treatment outcomes, a particular pattern of depressed symptoms, and more severe symptoms **[34].**

The present study demonstrated that the anti-inflammatory properties of vitamin D resulted in a decrease in serum levels of IL-6, TNF-α, and CRP in stressed mice. Furthermore, **Bakhtiari-Dovvombaygi et al. [16]** demonstrated that vitamin D alleviated oxidative stress in depression brought on by UCMS in male rats and decreased the inflammatory cytokine interleukin-6. **Emam and Abu-Raia [31]** revealed that depression-like behavior groups treated with vitamin D had a significant reduction in TNF-α levels and brain cell death.

There has also been an increase in study on the effect of vitamin D on depression. At least three lines of evidence exist at present time to support this association: First, there is a higher expression of vitamin D receptors (VDRs) in specific brain regions, such as the prefrontal and cingulate cortices, which are important for mood regulation. Second, the potential immune-modulatory mechanism by which vitamin D may regulate the relationship between depression and inflammation. Lastly, new knowledge on vitamin D's neuroprotective qualities (due to its anti-inflammatory effects) **[35].**

According to the current study, the (CRS) group has lower vitamin D levels, suggesting that depression may be the cause of vitamin D insufficiency.

In accordance with these findings, **Mulugeta et al. [36]** found little evidence for a significant impact of vitamin D status on the risk of depression, but did find genetic evidence linking depression to low 25(OH)D concentrations.

Patients with MDD frequently complain of physical complaints. However, poorer results are often linked to stomach symptoms. Those patients have much reduced gastrointestinal motility when compared to healthy controls **[3].**

The current work revealed that depression group shows delayed gastric emptying while groups treated with vitamin D show improvement of gastric motility.

Liu et al. [37] revealed that the start and progression of gastrointestinal symptoms may be linked to aberrant brain anatomy and function. Studies examining alterations in brain anatomy in MDD individuals exhibiting git symptoms have not yet been conducted, though.

Rats under CUMS had substantial relief from depression-like symptoms when given medication. By reestablishing the gastric electrical rhythm and encouraging gastrointestinal propulsion, gastrointestinal motility was also enhanced **[4].**

More severe depression symptoms are often the result of gastrointestinal problems in MDD patients. Moreover, MDD individuals with gastrointestinal problems have anatomical changes in their brains. There is evidence that the neuroendocrine system, intestinal microbiota, brain plasticity, and the inflammatory response cascade may all play a role in a common pathophysiological mechanism that may be responsible for both gastrointestinal and depressive symptoms in MDD patients **[38].**

Kwon et al. [39] imply that stomach dysmotility in Parkinson's disease may be caused independently by a vitamin D deficit, yet it is still unclear what the underlying mechanism is.

Numerous studies highlight the connection between depression and weight fluctuations in terms of chronic inflammatory states, the hypothalamic-pituitary-adrenal axis (HPA axis), and dysregulation of the stress system **[40].**

In a similar vein, a histological analysis of the hippocampal tissues was conducted in order to investigate other tissue-level mechanisms. The findings demonstrated evidence of neuronal damage in the hippocampal regions of the depression-induced group, including many vacuolations, irregularly degenerating neurons with eosinophilic cytoplasm, and

darkly stained pyknotic nuclei. These results validated those of **Chandrasekhar et al. [41]**, who noticed hippocampal neuronal death after CRS.

Elevated levels of glucocorticoids that may be linked to depression-related hippocampal damage **[42].**

Furthermore, an analysis of the stomach wall's histopathology revealed inflammatory infiltrates and degradation. Confirming these conclusions **Singh et al. [43]** shown that functional dyspepsia is associated with elevated eosinophil and especially mast cell density in the stomach.

Furthermore, as noted by, Gastric Precancerous Lesion and CUMS may exacerbate an inflammatory response and affect the gastric mucosa's ability to secrete **Zheng et al. [44].**

There are a few potential explanations for the association between vitamin D and gastric emptying time since vitamin D status can alter the hormonal regulation of stomach emptying. The regulation of gastric emptying time is aided by a number of GI-related peptides, such as ghrelin, CCK, motilin, GLP-1, and peptide YY. Additionally, vitamin D is recognized as a critical immune response regulator that may improve the intracellular removal of replicating bacteria because its receptor significantly affected gastric mucosa homeostasis and protected the host from H. pylori infection, which lowers serum ghrelin levels and impairs stomach motility **[39].**

Conclusions

In conclusion, our results revealed that exposure to chronic stress such as chronic restraint stress induces depressive behaviors. Vitamin D supplementation, in turn, has a good effect in mitigating these damages. We can conclude that vitamin D has a preventative and curative impact against chronic restraint stress-induced behavioral and biochemical abnormalities and relieving somatic symptoms as improvement of gastric emptying. Therefore, we recommended vitamin D as a widely available useful supplement for everyone to improve their resistance against daily life stressors also it can be used as an adjuvant therapy for patients with MDD.

Conflict of interest

The authors declared that they have no conflicts of interest with respect to the authorship and/ or publication of this article.

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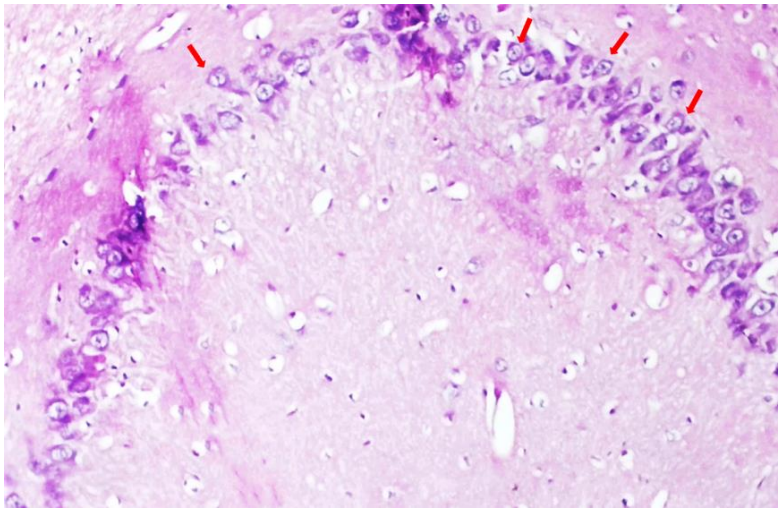

Figure s1: Group 1 (control group): cut section of the hippocampus (CA3) revealed normal neuronal cells with normal vesicular nuclei (red arrows) H&E staining X200 HPF.

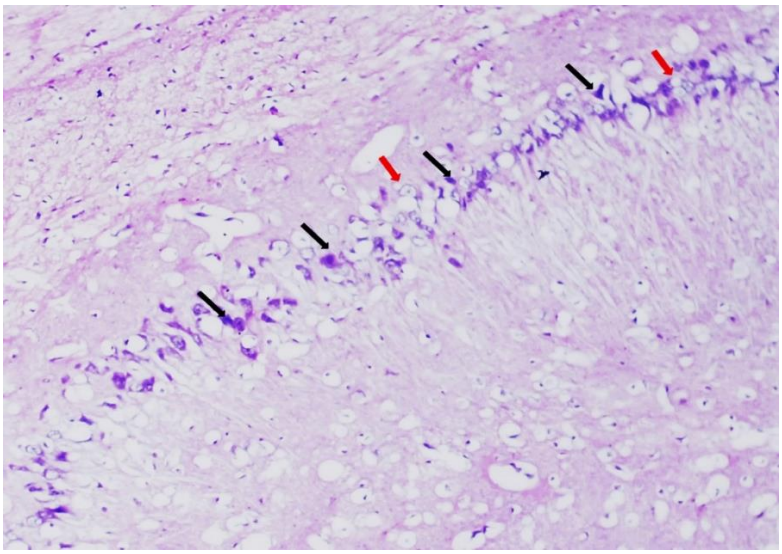

Figure s2: Group 2 (Depression induced group): cut section of the hippocampus (CA3) revealed many degenerated cells with pyknotic dark stained nuclei (black arrow) with scattered normal cells with normal vesicular nuclei (red arrows). H&E staining X200 HPF.

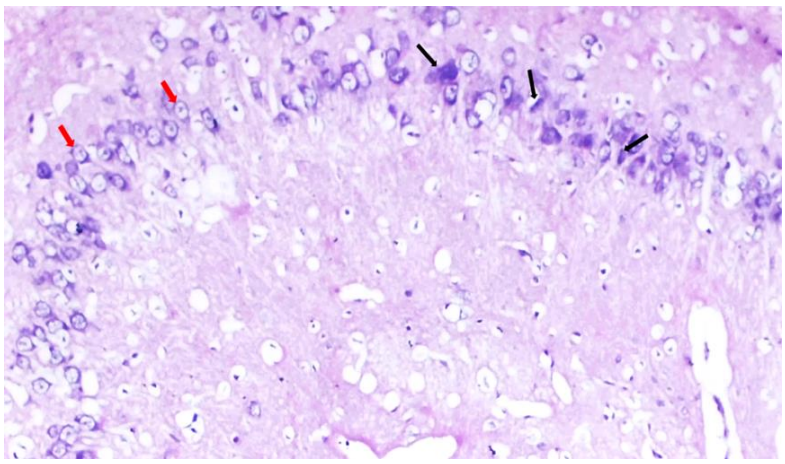

Figure s3: Group 4 (curative Vitamin D group): cut section of the hippocampus (CA3) revealed moderate improvement with moderate decrease in the number of degenerated cells with pyknotic dark stained nuclei (black arrow) with increase in normal cells with normal vesicular nuclei (red arrows). H&E staining X200 HPF.

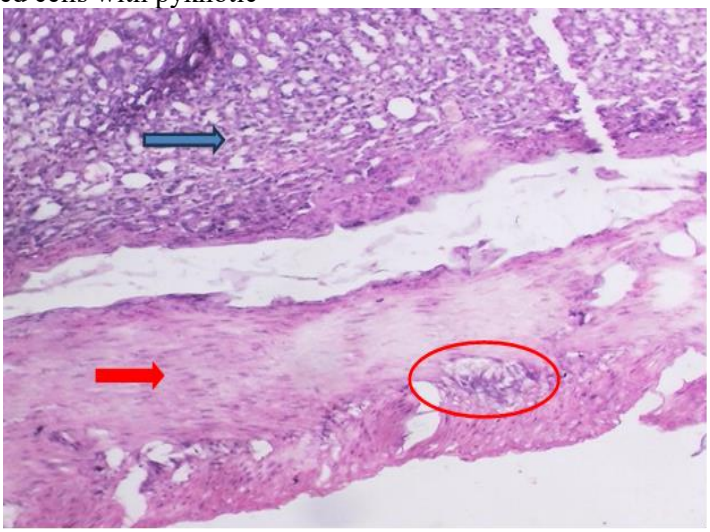

Figure s4: Group 1 (Control group): normal gastric mucosa (blue arrow), normal gastric submucosa (red arrow) and normal intermyenteric nerve plexus (red circle) H&E staining X200 HPF.

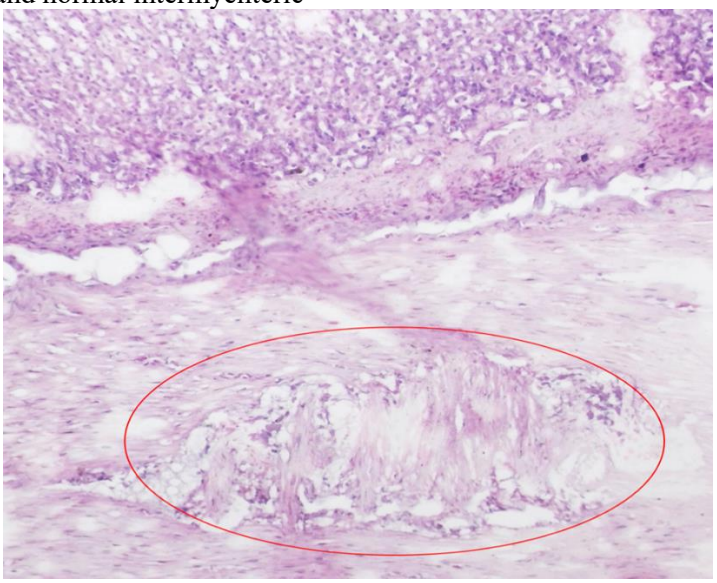

Figure s5: Group 2 (Depression induced group): revealed marked lymphocytic infiltration in the intermyenteric nerve plexus (red circle) H&E staining X200 HPF.

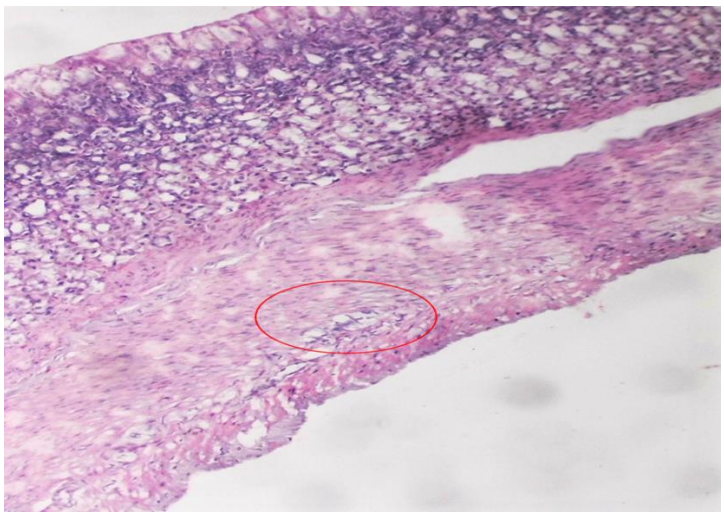

Figure s6: Group 4 (curative VIT D group): revealed moderate lymphocytic infiltration in the intermyenteric nerve plexus (red circle) H&E staining X200 HPF

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