Significance of vitamin D in combination with calcium in modulation of depression in the experimental model

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ABSTRACT

Depression is considered as a mood disorder that is characterized by change in mood, lack of confidence and lack of interest in surroundings. The present study was planned to investigate the antidepressant activity of vitamin D plus calcium in combinationagainst depression induced in the experimental model in attempt to clarify its mode of action.This study was conducted on 40 adult male albino rats (3 months old) divided into 4 groups n=10 Gp.(1) negative control group, Gp.(2) reserpinized group (positive control group) received reserpine in a dose of 0.1 mg/kg b.wt., Gp.(3) reserpinized group treated with low dose of vitamin D(6 mg/kg b.wt) plus calcium (13.5 mg /rat/day) and Gp.(4) reserpinized group treated with high dose of vitamin D (12 mg/kg b.wt) plus calcium (27 mg /rat/day) . Brain neurotransmitters (serotonin and dopamine) contents, the value of brain proinflammatory cytokine (TNF-α), the content of brain derived neutrophic factor (BDNF) and the level of the antiapoptic mediator (Bcl-2) as well as survivin expression and the histological examination in the brain tissue were carried out. In comparison with the negative control group, the reserpinized group recorded significant decrease in brain serotonin and dopamine contents and significant increase in the brain content of TNF-α . Moreover, significant decrease in brain BDNF and Bcl-2 contents were detected in the reserpinized group compared with the negative control group. Histological examination of brain tissue sections of rats in the reserpinized group showed neuronal damage and shrinkage. The basophilic neurons showed core pyknosis in cerebrum. Also, dark neurons of the hippocampus and marked necrosis of the pyramidal neurons were noticed. Immunohistochemical examination of brain tissue using antibody against survivin showed weak positive reaction indicating low expression level of survivin in the brain of rats in the reserpinized group with respect to the negative control group. On the other hand, treatment of the reserpinized groups with either low or high dose of vitamin D plus calcium resulted in remarkable improvement in the biochemical parameters, immunohistoccchemical and histological examination in a dose dependent manner. In conclusion, the present work provides a clear evidence for the antidepressant effect of vitamin D plus calcium through activation of serotoninergic and dopaminergic system, reduced levels of ACTH and corticosterone, inhibition of proinflammatory cytokine, promotion of neurotrophic factor and upregulation of antiapoptic markers in the brain.

Key words: Depression, vitamin D, neurotransmitters, apoptosis, Histopathology.
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INTRODUCTION

Depression is defined as mood disorder that leads to the alteration of mood, lack of interest in surroundings, decreased energy level,lack of confidence, poor concentration, sleep disturbance and the arousal of negative thoughts[1].

Depression is the most commonly occurring life-threatening neuropsychiatric disorder with a lifetime prevalence approaching 15–25%. Globally, it ranks fourth among the leading causes of disability [2, 3]. Moreover, according to World Health Organization, it will be the second largest contributor to global burden of disease by the year 2020[4]. The increasing burden and prevalence of depression make the search for an extended understanding of the aetiology and pathophysiology of depression highly significant [5, 6], yet despite this its exact aetiology and neurobiology still remain unclear.

Depressive disorders are currently split into two major categories:major depressive disorder (MDD, aka. unipolar depression) and bipolar disorder (BD). Depression itself is characterised by feelings of hopelessness, low self-esteem, inability to experience pleasure(anhedonia), and cognitive deficits. Depression is also frequentlypreceded by and comorbid with numerous other symptoms and conditions such as anxiety [7,8,9], tinnitus [10]pain [11], stroke (Poststroke Depression(PSD) [12], migraines [13,14], epilepsy [15], irritable bowel syndrome(IBS)[16], cardiovascular disease[17], chronic fatigue syndrome(CFS/ME)[18], and neurodegenerative conditions [19,20,21]. Thefrequent comorbidity of depression, taken together with itsincreasingassociation with biochemical changes throughout the body [22, 23, 24], challenges the orthodox conceptualisation of depression as adiscreteentity responsible for various somatic symptoms, and insteadsuggests that it may often be associated with broader dysfunctionin the body, and could result from aetiological factors commonto other associated disorders. In particular, whilst most aetiologicalmodels of depression typically emphasise genetic, neurodevelopmentaland psychological stressors, relatively little attention hasbe given to other environmental factors and specifically a neurometabolicunderstanding of the illness[25].

Reserpine, the most important Rauwolfia indole alkaloid, is awell known sympatholytic, antihypertensive and sedative agent.It was isolated from the roots of the plant Rauwolfia serpentina. Its biological action is to inhibit the storage of dopamine in the synaptic vesicles, thereupon generating evacuation of catecholamines of the sympathetic and central nervous system. It is used as a sedative and hypnotic as well as for reducing blood pressure. It works by decreasing the heart rate andrelaxing the blood vessels so that blood can flow more easily through the body. It is also used to treat severe agitationin patients with mental disorders. The side effects of reserpine include sleepiness, depression, galactorrhoea, ulcer, diarrhoea andbreast cancer in women over 50 years old. Therefore, determination of reserpine is of great importance and interest. Chemically reserpine is (Methyl(3β,16β,17α,18β,20α)-11,17dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]yohimban-16-carboxylate) [26]**.**

Vitamin D is a steroid with a broken ring and, as such, is named a seco-steroid. Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are the two major forms of vitamin D. Vitamin D2 is derived from plants while vitamin D3 is produced photochemically in the animal epidermis. The action of UVB radiation (295—310 nM) on 7-dehydrocholesterol results in the production of pre-vitamin D which, after thermo-conversion and two separate hydroxylations (performed by the P450 enzymes 25-hydroxylase and 1a-hydroxylase, respectively), gives rise to the active 1,25-(OH)2D. Vitamin D synthesis peaks at wavelengths between 295 and 297 nm (UV index greater than 3) and satisfactory amounts of vitamin D are produced after 15 min of sun exposure, at least twice a week. When exposed to UVB rays during a longer period, the body degrades pre-vitamin D as fast as it generates it and equilibrium is achieved.

1,25-(OH)2D can be considered as an hormone which is released into the circulation and, with the assistance of vitamin D binding protein (VDBP), is transported to various target organs [27].Vitamin D3 is traditionally recognized as a potent regulator of calcium and phosphorus metabolism [28].

The focus of our interest was to evaluate the antidepressant efficacy of a combination of vitamin D plus calcium against depression induced in the animal model with special concern on its possible mechanism of action.

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MATERIALS AND METHODS

Experimental Animals

Adult male albino rats (120-150g), 12 weeks old were obtained from the Animal House Colony of the National Research Centre. They were kept in plastic cages at room temperature (25^oC) and humidity (55%) under 12h dark-light cycle. All animals were accommodated with laboratory conditions for at least two weeks before the experiment and maintained under the same of conditions all over the experiment. Diet and water were allowed *adlibitum*. All animals received human care in compliance with the guidelines of the Ethical committee of Medical Research of the National Research Centre, Cairo, Egypt.

Experimental Design

Animals were randomly divided into four groups (10 rats for each). The first group received saline solution orally and served as negative control group. The second group received reserpine intraperitoneal (i.p.) in a dose 0.1 mg / kg b.wt[29]for 45 days to develop a model of depression. These animals remained to receive reserpine for 30 days more (reserpinized group or positive control group).The third group was a reserpinized group that received low dose of vitamin D(6 mg/kg b.wt) plus calcium (13.5 mg /rat/day)orally in the last 30 days. The fourth group was a reserpinized group that received high dose of vitamin D (12 mg/kg b.wt) plus calcium (27 mg /rat/day) orally in the last 30 days.

Sample Collection

At the end of the experimental period (75 days), the animals were scarified and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, weighed and sagitally divided into two halves. One half of each brain was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-Hcl and 300 mM sucrose, pH 7.4 [30]. The homogenate was centrifuged at 1800xg for 10 min in cooling centrifuge at 4^oC. The supernatant (10%) was separated for the biochemical analyses. The other half of each brain was fixed in formalin buffer(10%)for immunohistochemical investigation of survivin and histopathological examination.

Biochemical analyses

Quantitative estimation of total protein level in the brain homogenate was carried out according to the method of Lowry et al [31]. Serotonin (5-HT) content of the brain was determined by enzyme linked immunosorbent assay (ELISA) technique using a serotonin assay kit purchased from Wkea Med Supplies Corp.,New York, USA, according to the manufacturer's instructions provided with the serotonin assay kit. Brain dopamin content was estimated by fluorometric method as described by Ciarlone [32]. ACTH was determined in brain using Biosource Immunoassay kit,(ELISA) purchased from DRG International Inc.,Co.,USA according to the method describe by Odell et al. [33].The "DS-EIA-Steroid-Corticosterone" kit is intended for the quantitative determination of Corticosterone concentration by a microplate enzyme immunoassay (ELISA) purchased from DSI S.r.I., Co., Saronno,Volonterio,Italy, according to the method of check et al. [34].Brain tumor necrosis factor-α (TNF-α) content was determined by ELISA technique using TNF-α assay kit purchased from Assay Pro.,Co., USA according to the method described by Taylor [35] Brain derived neurotrophic factor (BDNF) content in the brain was estimated by ELISA technique using BDNF assay kit purchased from Wkea Med Supplies Corp., New York, USA, according to the manufacturer's instructions provided with BDNF assay kit. Brain Bcl-2 content was assayed by ELISA technique using Bcl-2 assay kit purchased from Wkea Med Supplies Corp., New York, USA, according to the manufacturer's instructions provided with Bcl-2 assay kit.

After blood sampling animals were dissected and the brains of each group were removed carefully. The brains were divided into two equal halves. One of it was used foe biochemical analysis and the other was used for immunohistochemical and histopathological studies.

Immunohistochemical method

Survivin expression in the brain was determined according to the method described by Bancroft and Gamble,(2008) [36]using immunohistochemical technique. In brief, samples were taken from brain of rats of the different groups and fixed in 10% formalin buffer for 24 hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56^oC in hot air oven for 24 hours. Sections were cut into 4μ thick by slidge microtome then fixed on positive slides in a 65^oC oven for 1 hr. Slides were placed in a coplin jar filled with 200 mL of triology working

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solution (Cell Marque, CA-USA) which combines the three pretreatment steps: deparaffinization, rehydration and antigen unmasking. Then, the jar is securely positioned in the autoclave which was adjusted so that temperature reached 120°C and maintained stable for 15 min after which pressure is released.Thereafter, the coplin jar is removed to allow slides to cool for 30 min. Sections were then washed and immersed in Tris-buffer saline (TBS) to adjust the pH and these were repeated between each step of the IHC procedure. Quenching endogenous peroxidase activity was performed by immersing slides in 3% hydrogen peroxide for 10 min. Broad spectrum LAB-SA detection system (Invitrogen, USA) was used to visualize any antigen-antibody reaction in the tissue. Background staining was blocked by putting 2-3 drops of 10% goat non immune serum blocker on each slide and incubating them in a humidity chamber for 10 min. Without washing, excess serum was drained and two-three drops of the ready to use primary antibody of survivin (Thermo Scientific, USA) were applied. Then, slides were incubated in the humidity chamber overnight at 4 °C. Henceforward, biotinylated secondary antibody was applied on each slide for 20 min followed by 20 min incubation with the streptavidin horse reddish peroxidase (HRP) enzyme conjugate. 3,3´-diaminobenzidine (DAB) chromogen was prepared and 2-3 drops were applied on each slide for 2 min. DAB was rinsed, after which counterstaining with Mayer hematoxylin and cover slipping were performed as the final steps before slides were examined under the light microscope (Olympus Cx21 with attached digital camera). Image analysis was performed using the image J, 1.41a NIH, (USA) analyzer.

Histological examination
While for histopathologicalexamination the brains were fixed in buffer formalin for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax (melting point 55-60°C). Sections of 6μm thickness were prepared and stained with haematoxylin and eosin [37]. In this method the paraffin sections were stained in Harris`s haematoxylin for 5 minutes. Sections were washed in running water for bluing and then stained in 1% watery eosin for 2 minutes, washed in water, dehydrated, cleared and mounted in Canada balsam.

Statistical analysis
In the current study, the results were expressed as mean ± S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 14. The difference was considered significant when $P < 0.05$. Percentage of differene representing the percent of variation with respect to the corresponding control group was calculated using the following formula

$$\% \text{ difference} = \frac{\text{Treated value- Control value}}{\text{Control value}} \times 100$$

<div align="center">

RESULTS

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I-Biochemical Results
- **Neurotransmitters**

1-*Serotonin* **(5-HT)**
The data presented in Table (1) revealed significant decline ($P<0.05$) in brain serotonin content of the reserpinized group after 75 days of reserpine administration (positive control group) when compared with that of the negative control group (182.96 pg/mg protein vs 309.99 pg/mg protein) with the percent of difference -40.98%. In contrast, treatment of the reserpinized groups with the different doses of vitamin D plus Ca led to significant elevation ($P<0.05$) in brain serotonin content as compared to that of the positive control group. Brain serotonin content of the reserpinized group treated with low dose ofvitamin D plus Ca was 249.99 pg/mg protein vs 182.96 pg/mg protein for the positive control groupwith the percent of difference 36.63%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 254.99 pg/mg protein vs 182.96 pg/mg protein for the positive control groupwith the percent of difference 39.37%.

2-*Dopamine* **(DA)**
The results of brain dopamine content in the negative control and other studied groups are illustrated in Table (1). The recorded value of brain dopamine content in the reserpinized group (positive control group) after 75 days of reserpine administration revealed significant decrease ($P<0.05$) as compared to that in the negative control group (171.75 µg/mg protein vs 277.20 µg/mg protein with the percent of difference-38.04%).However, significant increase ($P<0.05$) in brain dopamine content was detected in the reserpinized groups treated with vitamin D plus Ca as compared to that in the positive control group.Brain dopamine content of the reserpinized group treated with low

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dose ofvitamin D plus Ca was 225.25 g/mg protein vs 171.75 µg/mg protein for the positive control groupwith the percent of difference 31.15%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 229.74 µg/mg protein vs 171.75 µg/mg protein for the positive control groupwith the percent of difference 33.76%.

Table (1): Effect of treatment with different doses of vitD plus calcium on brain neurotransmitters (serotonin and dopamine) contents of the reserpinized rats (Data are represented as Mean ± S.E for 10 rats /group)

Parameters Groups	Serotonin (pg/mg protein)	Dopamine (µg/mg protein)
Negative control group	309.99±23.83	277.20±11.95
Reserpinized group (positive control)	182.96±16.69 [a] (-40.98%)	171.75±12.73 [a] (-38.04%)
Reserpinized group treated with low dose of Vitamin D+Ca	249.99± 16.60 [b] (36.63%)	225.25±16.78 [b] (31.15%)
Reserpinized group treated with high dose of Vitamin D+Ca	254.99± 13.93 [b] (39.37%)	229.74±9.63 [b] (33.76%)

a: Significant change at P <0.05 in comparison with the negative control group.
b: Significant change at P <0.05 in comparison with the positive control group.
(%): percent of difference with respect to the corresponding control value.

- **Hormones**
1-*ACTH*
The results of serum ACTH level are depicted in Table (2) .The present findings showed that serum ACTH level exhibits significant elevation ($P<0.05$) in the reserpinized group for 75 days(positive control group) when compared to that in the negative control group (60.95 pg/ml vs 26.49 pg/ml with the percent of difference 130.09%). However, significant decrease ($P<0.05$) in serum ACTH level was detected in the reserpinized groups treated with vitamin D plus Ca as compared to that in the positive control group. Serum ACTH level of the reserpinized group treated with low dose of vitamin D plus Ca was 45.97 pg/ml vs 60.95 pg/ml for the positive control groupwith the percent of difference -24.58%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 44.58 pg/ml vs 60.95 pg/ml for the positive control groupwith the percent of difference -26.86%.

2-*Corticosterone*
The data of serum corticosterone level of the different groups under investigation are recorded in Table (2) .Serum corticosterone level displayed significant increase ($P<0.05$) in the reserpinized group for 75 days (positive control group) as compared to that in the negative control group 85.04 nmol/l vs 35.41nmol/l representing a percent of difference 140.16%. In contrast, treatment of the reserpinized groups with the different doses of vitamin D plus Ca led to significant decline ($P<0.05$) in serum corticosterone level as compared to that of the positive control group.Serum corticosterone level of the reserpinized group treated with low dose of vitamin D plus Ca was 58.37 nmol/l vs 85.04 nmol/l for the positive control groupwith the percent of difference -31.36%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 54.99 nmol/l vs 85.04 nmol/l for the positive control groupwith the percent of difference -35.34%

Table (2): Effect of treatment with vitD plus calcium on serum ACTH and coticosterone levels of the reserprinized rats (Data are represented as Mean ± S.E for 10 rats/group)

Parameters Groups	ACTH (pg/ml)	Corticosterone (nmol/l)
Negative control group	26.49±1.52	35.41±0.86
Reserpinized group (positive control)	60.95±3.44 [a] (130.09%)	85.04±4.73 [a] (140.16%)
Reserpinized group treated with low dose of Vitamin D+Ca	45.97±4.09 [b] (-24.58%)	58.37±4.05 [b] (-31.36%)
Reserpinized group treated with high dose of Vitamin D+Ca	44.58±4.02 [b] (-26.86%)	54.99±3.41 [b] (-35.34%)

a: Significant change at P <0.05 in comparison with the negative control group
b: Significant change at P < 0.05 in comparison with the positive control group.
(%): percent of difference with respect to the corresponding control value.

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- **Proinflammatory Cytokine**

Tumor necrosis factor-α **(TNF-α)**

The results in Table (3) represented the values of brain TNF-α in the different studied groups. Significant increase ($P<0.05$) in brain TNF-α content was detected in the reserpinized group (positive control group) after 75 days of reserpine administration when compared to that in the negative control group (0.45 ng/mg protein vs 0.13 ng/mg protein) with the percent of difference 246.15%.Whereas, the treated groups with vitamin D plus Ca showed significant decrease ($P<0.05$) in brain TNF-α content as compared to that in the positive control group. Brain content of TNF-α in the reserpinized group treated with low dose of vitamin D plus Ca was 0.33 ng/mg protein vs 0.45 ng/mg protein for the positive control groupwith the percent of difference -26.67%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 0.30 ng/mg protein vs 0.45 ng/mg protein for the positive control groupwith the percent of difference-33.33%.

Table (3): Effect of treatment with different doses of vitD plus ca on brain TNF-α of the reserpinized rats (Data are represented as Mean ± S.E for 10 rats /group)

Parameters Groups	TNF-α (ng/mg protein)
Negative control group	0.13±0.005
Reserpinized group (positive control)	0.45± 0.035 [a] (246.15%)
Reserpinized group treated with low dose of Vitamin D+Ca	0.33± 0.014 [b] (-26.67%)
Reserpinized group treated with high dose of Vitamin D+Ca	0.30± 0.014 [b] (-33.33%)

a: Significant change at P <0.05 in comparison with the negative control group.
b: Significant change at P <0.05 in comparison with the positive control group.
(%): percent of difference with respect to the corresponding control value

- **Neurotrophic factor**

Brain derivedneurotrophic factor **(BDNF)**

On measuring of the value of brain derived neurotrophic factor (BDNF) in the brain of the different studied groups, the data revealed that brain BDNF content exhibits significant reduction ($P<0.05$) in the reserpinized group (positive control group) after 75 days of reserpine administration when compared to that in the negative control group (0.095 ng/mg protein vs 0.220 ng/mg protein) with the percent of difference -56.82%. In contrast, the treatment of the reserpinized groups with vitamin D plus ca resulted in significant elevation ($P<0.05$) in brain BDNF content with respect to that in the positive control group.Brain BDNF content of the reserpinized group treated with low dose of BDNF content of the reserpinized group treated with low dose of vitamin D plus Ca was 0.159 ng/mg protein vs 0.095 ng/mg protein for the positive control groupwith the percent of difference 67.37%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 0.165 ng/mg protein vs 0.095 ng/mg protein for the positive control groupwith the percent of difference 73.68 % (Table 4).

- **Antiapoptic mediator**

B-cell lymphoma-2 **(Bcl-2)**

Table (4) represented the results of brain of Bcl-2 content in the negative control group and the other studied groups. Significant reduction ($P<0.05$) in brain Bcl-2 content was detected in the reserpinized group (positive control group) after 75 days of reserpine administration when compared with that in the negative control group (0.052 ng/mg protein vs 0.115 ng/mg protein) with the percent of difference -54.78%. On the other hand, significant increase ($P<0.05$) in brain Bcl-2 content was recorded in the groups treated with vitamin D plus ca as compared to that in the positive control group. Brain Bcl-2 content of the reserpinized group treated with low dose ofvitamin D plus Ca was 0.082 ng/mg protein vs 0.052 ng/mg protein for the positive control group with the percent of difference 57.69%, and that for the reserpinized group treated with high dose of vitamin D plus Ca was 0.085 ng/mg protein vs 0.052 ng/mg protein for the positive control group with the percent of difference 63.46 %.

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Table (4): Effect of treatment with different doses of vit D plus caon brain neurotrophic factor (BDNF) and antiapoptotic mediator (Bcl-2) of the reserpinized rats (Data are represented as Mean ± S.E for 10 rats/group)

Parameters Groups	BDNF (ng/mg protein)	Bcl-2 (ng/mg protein)
Negative control group	0.220±0.013	0.115±0.006
Reserpinized group (positive control)	0.095±0.007 [a] (-56.82%)	0.052±0.005 [a] (-54.78%)
Reserpinized group treated with low dose of Vitamin D+Ca	0.159±0.013 [b] (67.37%)	0.082± 0.007 [b] (57.69%)
Reserpinized group treated with high dose of Vitamin D+Ca	0.165±0.014 [b] (73.68%)	0.085±0.005 [b] (63.46%)

a: Significant change at P <0.05 in comparison with the negative control group.
b: Significant change at P <0.05 in comparison with the positive control group.
(%): percent of difference with respect to the corresponding control value.
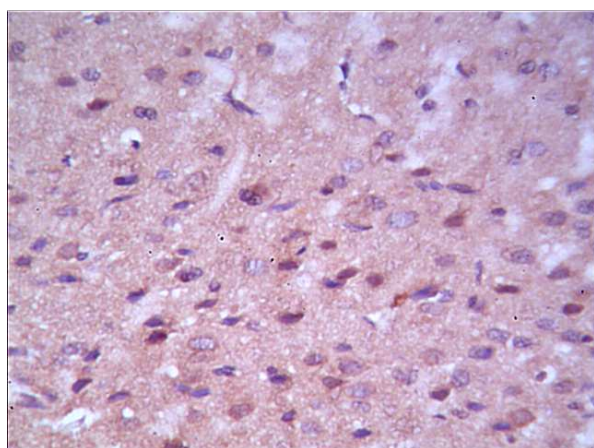

Fig.(1):Photomicrograph of immunohistochemical staining of brain tissue section of rat in the negative control group using antibody against survivin showed sever postive reaction
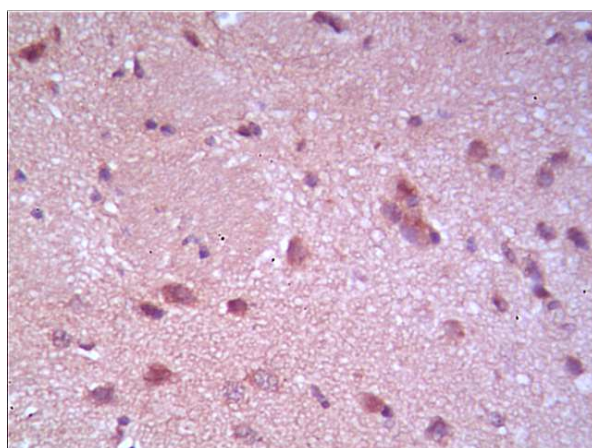

Fig.(2):Photomicrograph of immunohistochemical staining of brain tissue section of rat in the reserpinized group (positive control group) after 75 days of reserpine administration using antibody against survivin showed weak positive reaction.
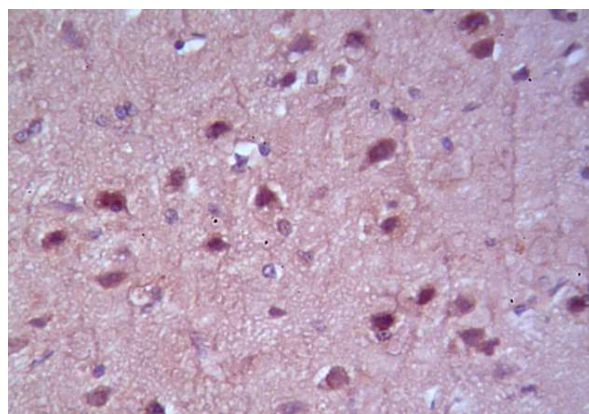

Fig.(3):Photomicrographofimmunohistochemical staining of brain tissue section of rat in the reserpinized group treated with low dose of vitamin D and Ca using antibody against survivin showed mild positive reaction.
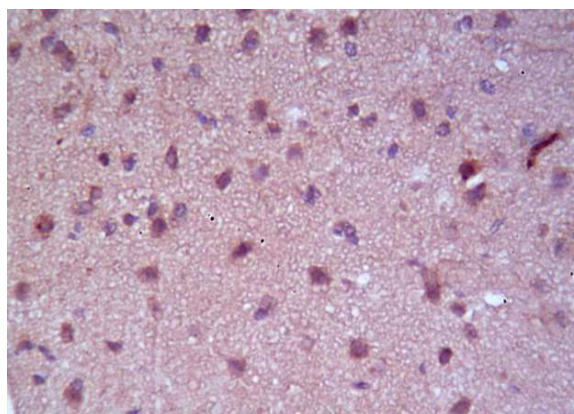

Fig.(4):Photomicrographof immunohistochemical staining of brain tissue section of rat in the reserpinized group treated with high dose of vitamin D and Ca using antibody against survivin showed mild positive reaction.

III-Immunohistochemical Results
Survivin
Immunohistochemical investigation of brain tissue section of the negative control group using antibody against survivin showed sever positive reaction (Fig.1). Whereas, the immunohistochemical examination of brain tissue

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section of the reserpinized group (positive control group) after 75 days of reserpine administration using antibody against survivin showed weak positive reaction (Fig.2).Figs(3,4) showed the results of The immunohistochemical investigation of reserpinized group treated with low and high doses of vitD and Ca using antibody against survivin showed mild positive reactions with respect to reserpinized positive control group.

II -Histological Results

Histological investigation of brain tissue sections of rats in the negative control group showed the highly active nerve cells (neurons) that having huge nuclei with relatively pale stain.The nuclear chromatin and the prominent nucleoli of these cells are disappeared. The surrounding supporting cells (glial cells) appeared with dense stain small nuclei and the condensed chromatin with no visible nucleoli. The background substances (neuropil) are shown in the cortex (**Fig. 1**).

Histopathological investigation of brain tissue section of a control rats showed the normal structure of hippocampus. The hippocampal neurons and vessels exhibited a regular arrangement with distinct edges, and clear nuclei and nucleoli. The glial cells and neuropil were appeared. No necrosis of pyramidal neurons was found (**Fig. 2).**Microscopic examinationof brain tissue section of rats in the reserpinized group after 75 days showed neuronal damage, and shrinkage. The basophilic neurons showed core pyknosis (**Fig. 3**).

Microscopic examinationof brain tissue section of rats in the reserpinized group after 75 days showed dark neurons of the hippocampus and significant necrosis of pyramidal neurons(**Fig. 4).**

Microscopic examination of brain tissue sections of reserpinized rats treated with low or high dose of vitamin D and Ca showed the normal structure of cerebrum (**Figs. 5,7)** respectively. Some dark neurons have been noticed in case of treatment with low dose of vitamin D and Ca (**Fig. 5).** Microscopic examination of brain tissue section of reserpinized rats treated with low dose of vitamin D and Ca showed the dark neurons and some normal structure of the neurons of the hippocampus (**Fig. 6).**Histopathological examination of brain tissue section of reserpinized rats treated with high dose of vitamin D and Ca showed few dark neurons and normal structure of the neurons of the hippocampus (**Fig. 8).**
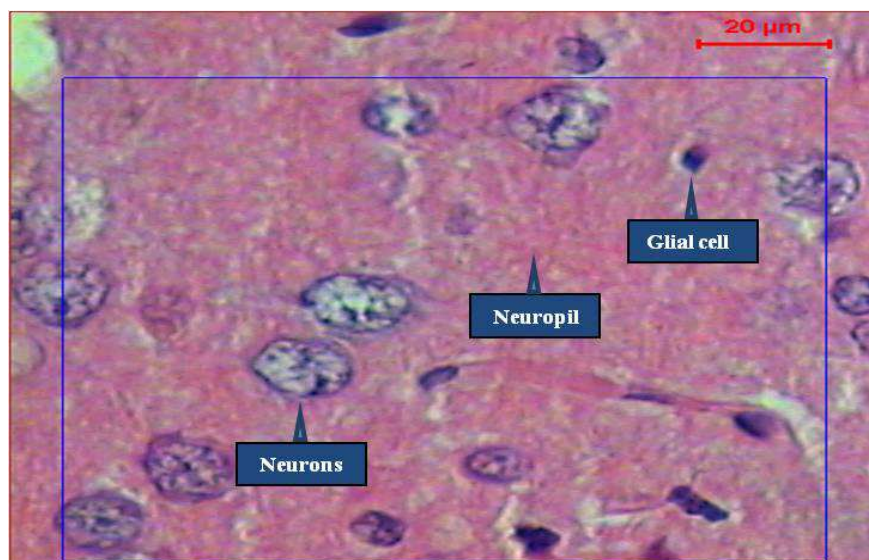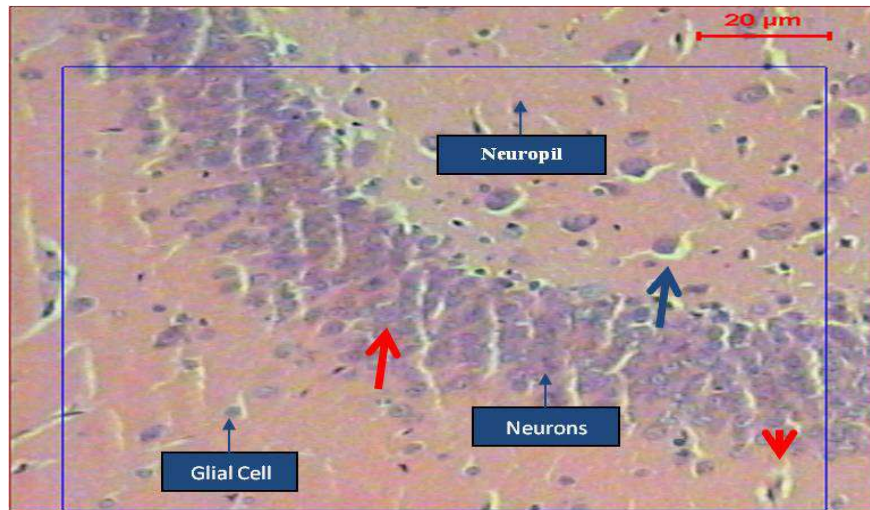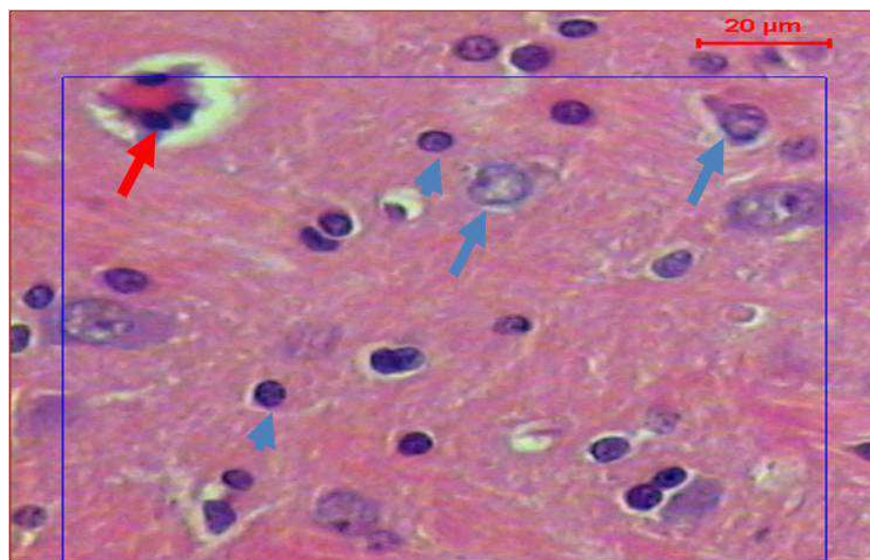

Fig. (1): Micrograph of brain tissue section of control rat showing the highly active nerve cells (neurons) that having huge nuclei with relatively pale stain. The nuclear chromatin and prominent nucleoli are disappeared.The surrounding supporting cells (glial cells) have small nuclei with dense stain, and the condensed chromatin with no visible nucleoli.The background substances (neuropil) are shown in the cortex (H & E, Scale bar 20 μm)

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Fig. (2): Micrograph of brain tissue section of control rat showing the normal structure of hippocampus. The hippocampal neurons (Neurons) and vessels exhibited a regular arrangement with distinct edges (arrowhead), and clear nuclei and nucleolui (arrow). Glial cells and neuropil are appear. No necrosis of pyramidal neurons is found (blue arrow) (H & E, Scale bar 20 μm)

Fig. (3): Micrograph of brain tissue section from rat administered reserpine for 75 daysshowingneuronal damage (red arrow), and shrinkage (blue arrow) of cerebrum. The basophilic neurons show core pyknosis (arrowhead) (H & E X 400, Scale bar 20 μm).
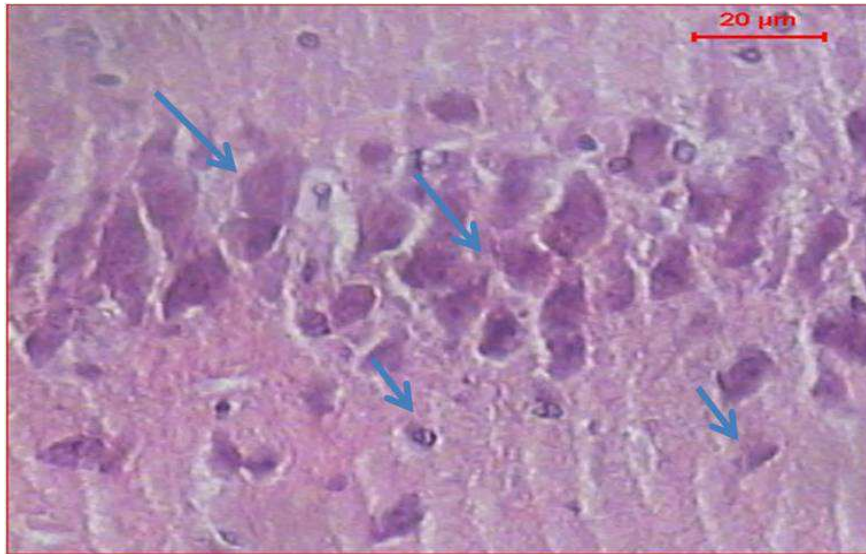
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Fig. (4): Micrograph of brain tissue section of rat in the reserpinized group after 75 days showing dark neurons of the hippocampus (arrows) and significant necrosis of pyramidal neurons (arrowhead) (H & E, Scale bar 20 μm).

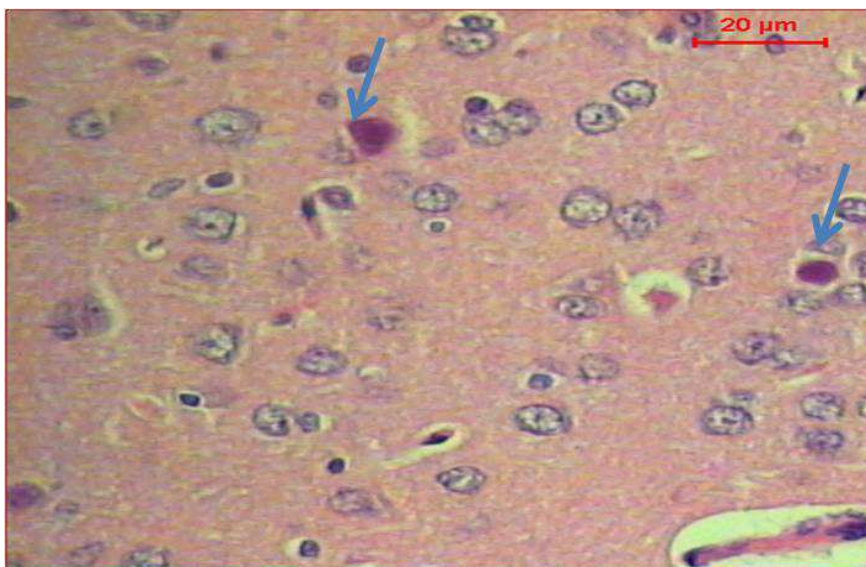**Fig. (5): Micrograph of brain tissue section of reserpinized rats treated with low dose of vitamin D and Ca showing the normal structure of cerebrum with the appearance of some dark neurons (H & E, Scale bar 20 μm).**
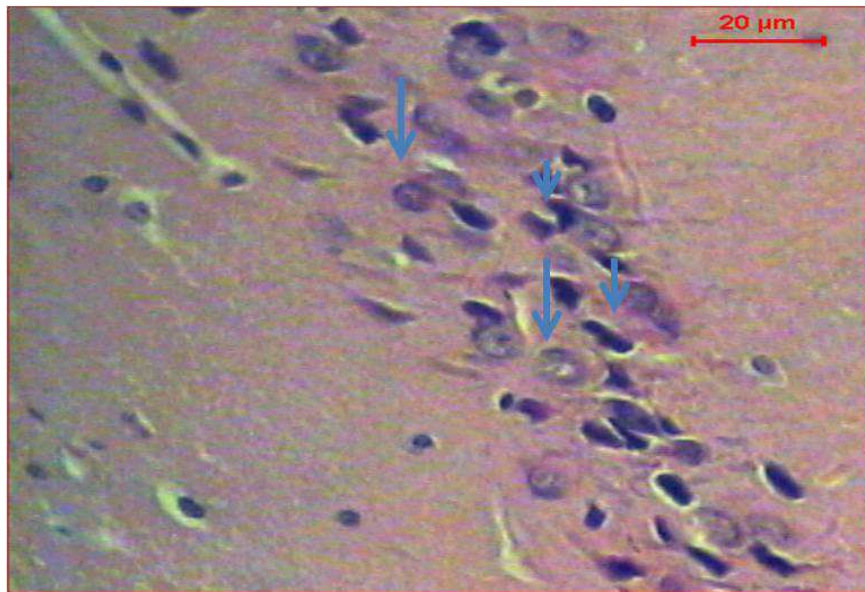
Fig. (6): Micrograph of brain tissue section of reserpinized rats treated with low dose of vitamin D and Ca showing the dark neurons (arrowhead) and some normal structure of the neurons of the hippocampus(arrows) (H & E, Scale bar 20 μm).
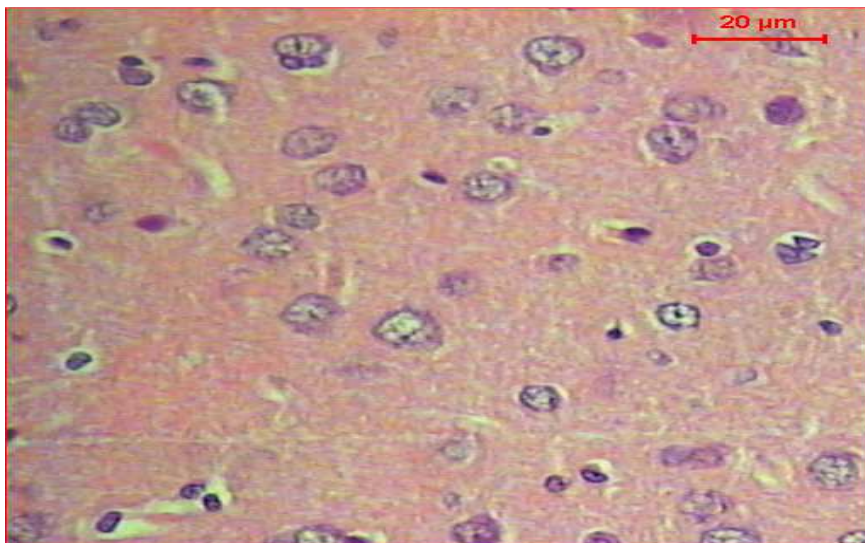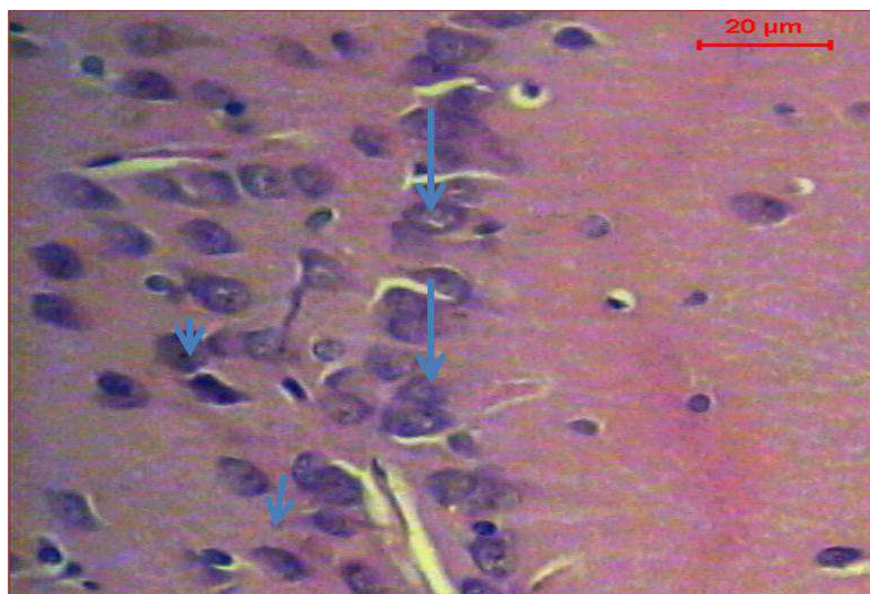

Fig. (7): Micrograph of brain tissue section of reserpinized rats treated with high dose of vitamin D and Ca showing the normal structure of cerebrum (H & E, Scale bar 20 μm).

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Fig. (8): Micrograph of brain tissue section of reserpinized rats treated with high dose of vitamin D and Ca showing few dark neurons (arrowhead) and normal structure of the neurons of the hippocampus (arrows) (H & E, Scale bar 20 µm).

DISCUSSION

In the view of the present results, reserpine administration resulted in significant reduction in brain serotonin and dopamine contents in comparison with the negative control group. According to the monoamine theory, the most important neurochemical process in depression is the impairment of monoaminergic neurotransmission with the concomitant decrease in the extracellular concentration of norepinephrine and serotonin[38].It has been postulated that the debilitating and often chronic symptoms of depression result from a perturbation in serotonin (5HT), norepinephrine and/or dopamine transmission. This hypothesis stems from the work done in the late 1950s showing that monoamine oxidase inhibitors and tricyclic antidepressants that elevate the levels of monoamines through preventing their metabolism or blocking their reuptake were effective antidepressants [39]. Reserpine as an antihypertensive agent has been found to decrease brain monoamines with consequent detrimental effects on mood [40]. Reserpine is known to induce hypothermia, hypomotility, ptosis and catalepsy as well as to slow the frequency and increase the amplitude of electroencephalogram (EEG) waves by deleting intracranial monoamines such as norepinephrine, serotonin and dopamine [41,42]. Because these actions of reserpine are antagonized by tricyclic antidepressive agents, the reserpine-induced neurochemical changes is considered as a model of depression and is used frequently for the evaluation of the antidepressive agents [43].

For classical neurotransmitters such as monoamines, vesicular storage involves transport from the cytoplasm where transmitters accumulate after synthesis or removal from the synapse. Reserpine has been found interact with the vesicular amine transporter as reserpine can bind at the site of amine recognition and inhibit the vesicular uptake of monoamine neurotransmitters (serotonin, norepinephrine and dopamine) which are subsequently metabolized by monoamine oxidase enzyme. As a result, reserpine could deplete amine stores in the brain[44].

A growing number of evidence has demonstrated indoleamine 2,3-dioxygenase (IDO) as an enzyme involved in depressogenesis, not only because of its effect on serotonin biosynthesis[45] but also because of its putative contributions to excitotoxicity and oxidative stress. IDO is highly inducible by proinflammatory cytokines (IFN-γ and TNF-α) and is secreted by activated macrophages and other immunoregulatory cells, which catalyzes the degradation of tryptophan (serotonin precursor) to kynurenine [3].As kynurenine degradation leads to the formation of 3-hydroxykynurenine (3-HK, generates free radical species that can cause oxidative stress) and quinolinic acid (QA, a glutamate receptor agonist), the increased glutamate receptor activity in depression has a major role due to that IDO mediated imbalance of kynurenine pathway metabolites as a result of cytokine production [46]. Thus, cytokine- and IDO-mediated degradation of tryptophan through the kynurenine pathway is hypothesized to influence

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serotonergic biosynthesis and neurotransmission in the brain resulting in significant neuropsychiatric consequences including depression.

Thus, the concomitant increase in the immobility time in the FST and the significant decrease in the monoamine contents of the brain support the usefulness of the reserpinized animal model of depression to test new antidepressant agents.

Stress is characterized by physiological changes that occur in response to novel or threatening stimuli. These changes comprise a cascade of neuroendocrine events mediated by stress systems such as the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the HPA axis results in the release of hypothalamic corticotropin-releasing hormone (CRH), which in turn stimulates pituitary adrenocorticotropic hormone (ACTH) release, culminating in the secretion of adrenal glucocorticoids (cortisol in humans and corticosterone in rodents) into the circulatory system [47].

Glucocorticoids then act at target tissues throughout the body to confer physiological changes that enable an organism to deal with an acute stressor and then return to a pre-stress level of functioning. Normal HPA axis functioning is thus essential for survival because it acts to maintain bodily equilibrium*(deKloet et al., 1998* [48, 49]. However, repeated HPA axis activation can produce damaging physiological effects and exert a profound impact on brain function [48, 49,50]. For example, repeated exposure to high levels of glucocorticoids leads to a downregulation of hippocampal glucocorticoid receptors (GR), which impairs the ability of the hippocampus to control glucocorticoid negative feedback [51]. This leads to a further hypersecretion of glucocorticoids, and this is thought to produce neuronal changes in several brain regions, including the hippocampus [51, 52] and amygdala [53, 54]. Within the hippocampus, persistently elevated glucocorticoids levels lead to dendritic remodeling of CA3 neurons, decreased neurogenesis, and finally cell death [50, 55-57].

In the current study, reserpine administration produced significant increase in serum levels of ACTH and corticosterone when compared with the negative control group. This is another document for establishing reserpinized animal as experimental model of depression. Depression is often associated with HPA axis hyperactivity, which is characterised by hypercortisolaemia in human [55, 59]. Whereas hyperactivity of the HPA axis may be prevented by means of an inhibitory feedback mechanism as the dysregulation of this feedback mechanism appears to occur in depressive disorders[60].

The current results demonstrated that brain tumor necrosis factor-α (TNF-α) significantly increased in reserpinized group as compared to the negative control group. The observed increase in the brain content of these inflammatory cytokines is associated with the significant decrease in the brain content of brain derived neurotophic factor (BDNF) as shown in the present study. It has been demonstrated that the amount of peripheral cytokine production largely depends on the state of immune activation. In pathological conditions, such as acute or chronic inflammation and tissue damage, the immune system is activated and macrophage activity is increased which accounts for the increased production and release of cytokines, such as IL-1β, IL-6 and TNF-α. Moreover,it has been demonstrated that IL-1β may stimulate the production of other cytokines such as IL-6 and TNF-α by astrocytes and microglia and hence promoting inflammatory processes in the brain [61].The activation of microglia and the production of proinflammatory cytokines as well as oxidative stress, all together contributed in dopaminergic and serotonergic neurons and terminals damage [62].

Changes in the amount of cytokine production may also be due to neuroendocrine influences on the immune system [63]. In this respect, the effects of corticosteroids, which are produced by the adrenal cortex as the final component of the HPA axis, are most important. These hormones, particularly cortisol and corticosterone have been demonstrated to be involved in the regulation of immune responses and thereby the production of cytokines. As it has been shown that even low concentrations of corticosteroids stimulate proinflammatory cytokine production [64, 65].On the other hand, the potential mechanisms leading to cytokine-induced depression are numerous and were reviewed by Dantzer *et al.* [66]*,* Dantzer[67]*,* Maes *et al.* [3]*,* Miller *et al.* [68], and others. Several lines of evidence demonstrating how cytokines can contribute to HPA axis hyperactivity [69] and affect the serotonergic and dopaminergic systems [70,71].Proinflammatory cytokines are potent activators of the HPA axis*,* and therefore play a critical role in activating the HPA axis in major depression. Furthermore, there is evidence that cytokines counteract the negative feedback action of corticosteroids on the HPA axis, leading to HPA axis dysregulation [72]. The mechanism by which cytokines may disturb inhibitory feedback of corticosteroids on the HPA axis, may involve the

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induction of corticosteroid receptor resistance in the hypothalamus and pituitary gland, i.e., brain areas that normally mediate HPA axis downregulation. Alterations in the functioning of these central corticosteroid receptors could then lead to decreased sensitivity of hypothalamus and pituitary to elevated corticosteroids, thereby resulting in lack of the negative feedback[72].

The increased inflammatory process and the reduced neurogenesis has been reported to associated with depression. Even though the direct effect of neuroinflammation on neurotrophic system and neurogenesis is unknown, increasing evidence suggested that proinflammatory cytokines and neuroinflammation may contribute in the reduction of neurogenesis through three ways 1) stimulating the HPA axis to release glucocorticoids that suppress neurogenesis [73],2) changing glial cell functions, in which the changes in astrocyte-produced neurotrophins could make significant contribution, and 3) overproduction of oxygen radicals that can directly damage neurons *via* the activation of microglia [74]. As a consequence of microglial activation, the functions of the other glial cells mainly astrocytes are changed and the production of neurotrophins may be altered. Thus, it has been stated that depression likely contributes to impairment of cellular resilience by a variety of mechanisms, including reductions in the levels of BDNF, facitating glutamatergic transmission *via* N-Methyl-D-aspartate (NMDA) and non-NMDA receptors, and reducing energy capacity of the cells. Neurotrophins and their receptors compose a major neuroprotective system in the brain because they stabilize and maintain homeostasis (protection and repair), control and clean neurotoxins, regulate neurotransmission and modulate neuronal regenesis and degenesis [75,76].

The neurotrophin hypothesis postulates that loss of neurotrophins, particularly brain-derived neurotrophic factor (BDNF), plays an important role in the pathogenesis of major depression, and hence neurotrophin restoration may represent an important mechanism for antidepressant efficacy[77]. This hypothesis is supported by postmortem studies of brain samples from depressed patients, which exhibit lower BDNF levels than those obtained from patients undergoing antidepressant treatment[78,79].

In view of the present results, rats administered reserpine displayed significant decrease in brain antiapoptic marker (Bcl-2) compared with the negative control ones. Similarly, reserpine caused significant reduction in the number of positive cells for survivin expression as indicated by the weak reaction of antibody against survivin in the brain tissue as shown in the current immunohistochemical results. Apoptosis is regulated by different classes of proteins[80,81]including caspase-3, which can induce the cleavage of other proteins and alter cell integrity. Activation of caspase-3 is considered to be a hallmark of apoptosis [81,82]. Upstream caspase-3 are antiapoptotic proteins such as Bcl-2 and proapoptotic proteins (such as Bax),which regulate the release of cytochrome c from mitochondria, activating caspase to induce apoptosis. It has been shown that Bcl-2 shuts off the apoptotic signal transduction pathway upstream of caspase activation [83].The Bax: Bcl-2 ratio is used as an index of vulnerability for apoptosis [81,84]. A shift in the ratio of these proteins in favor of the proapoptotic members triggers the release of mitochondrial cytochrome c(an electron carrier) which binds to apoptosis protease activator factor-1 (Apaf-1) and triggers the cleavage (activation) of caspase-3.

Proinflammatory cytokines have been found to display proapoptotic properties in limbic areas such as the hippocampus, further supporting their role in the pathophysiology of depression[85,86].Another factor contributes in decreasing brain Bcl-2 in reserpine administered rats as an experimental model of depression is the reduction of BDNF in the brain of the reserpinized rats.

One of the major mechanisms by which BDNF promotes cell survival is through increasing the expression of the major cytoprotective protein, Bcl-2. Bcl-2 attenuates cell death *via* a variety of mechanisms, including impairing the release of calcium and cytochrome c, sequestering proforms of death-inducing caspase enzymes, and enhancing mitochondrial calcium uptake. The actions of BDNF are mediated by the TrkB receptor which in turn phosphorylates and activates the transcription factor cAMP response element-binding protein (CREB). Activated CREB enhances the transcription of many genes, including BDNF itself. Both BDNF and CREB, through the mediation of Bcl-2 family members, contribute to neuronal survival [87].

Survivin localizes in mitochondria and its ability to associate with caspase-9 and second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI (Smac/DIABLO) indicates that it may inhibit the intrinsic pathway of apoptosis by interfering with postmitochondrial events [88,89].Several hypotheses have been existed to explain the mechanisms of antiapoptotic activity of survivin. Survivin might directly bind and inhibit caspases [88]. Indirectly, survivin has affinity to Smac/ DIABLO so it may inhibit apoptosis through antagonizing

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the proapoptotic ability of Smac/DIABLO [90].Disruption of the survivin induction pathway results in an increase of apoptosis.

As mentioned before, depression is associated with the induction of glucocorticoids with a consequent activation of glucocorticoid receptors(GR).The transcriptional effects of GR include the activation of the tumor suppressor protein p53 which, in turn, represses the expression of the antiapoptotic molecules [91]. P53 may induce apoptosis by antagonizing the antiapoptotic activity of survivin.The negative regulation of survivin by p53 is poorly understood. Survivin promoter has a p53 binding element. A transcriptional activator (E2F) may also bind survivin promoter [92]. P53 has an affinity to E2F and thus, it is possible that p53-E2F complex can represse survivin gene expression [93,94].Additionally, P53 interacts with transcriptional regulator (sin3) andhistone deacetylase enzyme (HDAC) that together can form a p53-sin3-HDAC complex and bind survivin promoter to repress it [93,95]. It may be possible that p53 directly binds survivin promoter alone or in combination with other protein(s) to repress survivin.

Vitamin D_3 is synthesized in the skin from 7-dehydrocholesterol *via* nonenzymatic photochemical reactions driven by ultraviolet B (UVB); a crucial rate-limiting step. Under conditions of inadequate photosynthesis, vit D_3 is an essential nutrient [96]. A growing body of evidence suggested that vit D_3 likely has important functions in the human brain. Thus, vit D_3 has been considered as a neurosteroid [97,98]. Eyles *et al*.[98]identified vitamin D receptor(VDR) in multiple areas of the brain, including the prefrontal cortex, hippocampus, cingulate gyrus, thalamus, hypothalamus, and substantia nigra.Many of these areas have been implicated in the pathophysiology of depression [99].

 The physical and mechanistic evidences of vit D_3 in the brain underscored its potential for biological functions. Studies have shown that vitamin D_3 may protect the structure and integrity of neurons through detoxification pathways and neurotrophin synthesis [100].

The current study revealed that vit D_3 plus Ca supplementation significantly reduces the immobility time of the reserpinized rats as compared to the reserpinized group (positive control group). The behavioural effect of vit D3 could be done through enhancement of serotonin release.Espejo and Minano [101] stated that the behavioural profile of low immobility and enhanced swimming is correlated with a selectively high serotonergic activity. In addition,the decrease of immobility time may be also due to the induction of brain dopamine content by vit D_3 supplementation. Dopamine in the central nervous system is involved in the control of both motor and emotional behaviour [102]. This finding is evidenced by many behavioural studies that have shown the important role mesolimbic dopamine system in regulating exploratory and locomotor behavior[103].

The present study revealed significant increase in brain serotonin content in the reserpinized rats supplemented with vit D_3 plus Ca as compared to the positive control rats. In accordance with this result, Cass *et al*. [104]stated that vit D_3 preserves serotonin content in the brains of animals repeatedly administered with neurotoxic doses of methamphetamine. The physiologic pathways between vit D_3 and depressive symptoms have been identified and vit D_3 in its active form in the body has been shown to stimulate serotonin release [105].

Experimental evidence demonstrated that dietary manipulation of vit D_3 alters cholinergic, dopaminergic and noradrenergic neurotransmitters systems. Abnormalities in these neurotransmitters have been implicated in various neuropsychiatric disorders such as depression [106].

The current data revealed that supplementation with vit D_3 and Ca significantly elevates brain content of dopamine in the reserpinized group as compared to the positive control group. A number of studies described how vit D_3 leads to alteration in some aspects of dopamine neurotransmission. When vit D_3 was administered in newborn rats and the dopamine and noradrenalin contents were measured in the adult offspring, these neurotransmitters were shown to be elevated in brain stem [107].Moreover, adult animals treated with high doses of vit D_3 in a long-run also showed increased basal striatal dopamine content as well as increased dopamine release[108].A recent study has shown that subcutaneous administration of vit D_3 in adult rats increases sub cortical dopamine release and dopamine storage in striatal terminals when these animals are challenged three weeks later with amphetamine [109].This is could be due to that vit D_3 is able to increase dopamine synthesis itself *via* increasing its synthetic enzyme tyrosine hydroxlase[110]. In accordance with this report, Wang *et al*. [111];Chen *et al*. [112]and Sanchez *et al*. [113]stated that the systemic administration with vit D_3 ameliorates neurological insults initiated dopaminergic toxicity possibly

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due to the elevation of the rate-limiting synthetic enzyme for dopamine which is tyrosine hydroxylase (TH). Moreovere, pretreating animals with vit D_3 for one week preserves dopaminergic function when selective dopamine toxins, such as 6OHDA, are later administered [108,111].

The data in the present study revealed that serum ACTH and corticosterone levels are significantly decreased after supplementation with vit D_3 plus Ca in the reserpinized rats as compared to the positive control ones. Barton *et al.* [114] demonstrated that vit D in its active form in the body has been shown to be associated with downregulation of glucocorticoid receptor gene activation which is found to be upregulated in depression.

The active metabolite of vitD,1,25-dihydroxyvitamin D_3 (1,25(OH)2D3) can be produced not only by the kidney, but also by macrophages, including peritoneal macrophages, especially during inflammation[115].Furthermore, it has been well established that the physiological role of 1,25(OH)2D3 is not limited to mineral and skeletal homeostasis, but it also plays a role as an important immune response regulator at inflammatory sites [116].

The results of the present work showed significant decrease in the inflammatory markers including TNF-α contents in the brain the reserpinized rats supplementated with vit D_3 plus Ca as compared to the positive control rats. Oral administration with vit D_3 has been found to reduce IL-1β production in rat hippocampus [117]. Lemire and Archer[118]demonstrated that vit D_3 inhibits Th1 cells activity and in turn the productionof Th1 cytokines like IL-6 and TNF-α. The possible mechanism for the inhibition of TNF-α by vit D_3 is mediated through the inhibition of NFκB, the major transcription factor for TNF-α [119].Therefore, the suppressive effect of vit D_3 on NFκB, appeared to be a general effect that occured in various cell types and affected various inflammatory and immune processes [119].There is considerable direct evidence for a modulatory effect of vit D_3 treatment on proinflammatory cytokines both *in vitro* and *in vivo*. vit D_3 could 1) decrease the production of proinflammatory cytokines in a variety of cell types including monocytes [120,121,122], microglia ,an important source of proinflammatory cytokines in the brain [123] and 2) decrease TNF-α levels and suppresse disease symptoms in a rodent model of inflammatory bowel disease (IBD) [124].

Neurotrophins are proteins necessary for neuronal survival in aging and neuropathological conditions [125].

The current study showed that vit D_3 plus Ca supplementation in reserpinized rats resulted in elevation in the brain BDNF content as compared to the positive control rats. Various studies have demonstrated that vit D_3 can act on cells of the nervous system by modulating the production of neurotrophins [126]. Vit D_3 could upregulate neurotrophin factors, such as neurotrophin-3 (NT-3)[127] and glial cell line derived neurotrophic factor (GDNF) [128].It has been reported that the stimulation of neurotrophin production by vit D_3 is correlated with its neuroprotective effect [129,130].Vit D_3 was found to be a potent inducer of BDNF expression and it is contributed to the regulation of BDNF *in vivo* [128,131]. In this regard, it is noteworthy that vit D_3 has been shown to attenuate the hypokinesia and neurotoxicity induced by 6-hydroxydopamine in rats [111]. This indicated that vit D_3, has a valuable role in the treatment of neurodegenerative diseases.

The data in the current study revealed remarkable elevation in the brain content of Bcl-2 in the reserpinized rats supplemented with vit D_3 plus Ca as compared to the reserpinized group (positive control group). This result is in agreement with that of *Wang et al.* [132]who stated that vit D_3 treatment increases Bcl-2 messenger RNA and protein levels, elevates the Bcl-2/Bax ratio, and protects thyrocytes from apoptosis. Also, in keratinocytes, the increase in Bcl-2 level has been shown to mediate the antiapoptotic actions of vit D_3[133].

Manggau *et al.* [134] demonstrated that the antiapoptotic action of vit D_3 is associated with an acceleration of Bcl-2 protein expression.

Vit D_3 plays an important role in the regulation of proliferating cells in the brain and various aspects of their differentiation. Additionally, the ability of vit D_3 to 1) regulate calcium transients *via* its ability to downregulate voltage sensitive L-type calcium channels, 2) provide trophic support for developing and mature neurons and 3) protect neurons from the reactive oxygen species, could all contribute to the viability and connectivity of individual neurons. These mechanisms may be central to neuropathological conditions that have been closely linked with vit D_3 status such as multiple sclerosis [135].

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The present result demonstrated that vitD$_3$ plus Ca supplementation produces mild positive reaction by using antibody against survivin in the immunohistochemical investigation of the brain tissue of the reserpinized rats as compared to the positive control rats. Masoumi *et al.* [136]. demonstrated that vit D$_3$ protects neural cells against apoptosis. Moreover, it is possible that vit D$_3$ could modulate neuronal survival and differentiation during development [137].

Prolonged and repeated depression is associated with atrophy in cortex and hippocampus and the decrease in hippocampal volume is associated with the repeated depression and stress [138,139,140].

Microscopic examination of brain tissue sections of rats given reserpine for 75 days showed neurnal damage, shrinkage of neurons, and basophilic neurons with core pyknosis in the cerebrum as well as a significant necrosis of pyramidal neurons of the hippocampus. These findings are in agreement with those of Gould and Tanapat[141]and McEwen[142] who stated that stress, a risk factor for depression evokes a dendritic shrinkage and neurnal loss within the hippocampus in animal models that mimic human depression [141]. The hippocampus appears to be particularly sensitive to stress stimuli in both animals and humans as this brain area undergoes selective volume reduction and dendritic retraction. Thus, it has been suggested that depression may be associated with decreased hippocampal plasticity [143].Additionally, it has been reported that the repeated restraint stress or a combination of daily stressors in rats induces atrophy of hippocampal CA3 pyramidal neurons [144]. This atrophy is mimicked by daily treatment with corticosterone [145].indicating that elevated circulating adrenal steroids secreted during stress may be involved in triggering these morphological alterations.

Microscopic investigation of brain tissue sections of the reserpinized rats treated with vit D$_3$ plus calcium showed an improvement of the morphological feature of brain tissue represented by normal structure of cerebrum and normal structure of hippocampus neurons with the apperence of few dark neurons in low dose of vit D$_3$ plus Ca. It has been shown that chronic peripheral treatment of rats with vit D$_3$ retards the age-related decrease in neuronal density seen in rodent hippocampus [146] and protects against damage in a rodent model of stroke[130]. Nevertheless, it is unclear whether these apparent neuroprotective effects of vit D$_3$ are attributable to indirect actions on peripheral Ca 21 and PO4 regulation) or to direct actions on brain neurons. The rat hippocampal cultures treated for several days with vit D$_3$, revealed neuroprotection against excitotoxic insults. In that study, low concentrations of vit D$_3$ (1–100 nM) were protective, but higher, nonphysiological concentrations (500–1000 nM) were not [147].

CONCLUSION

The current datashed light on the antidepressant effect of a combination of vitamin D plus calcium. This effect was evidenced by the activation of serotoninergic and dopaminergic system, inhibition of ACTH and corticosterone levels, suppression of proinflammatory cytokine, promotion of neurotrophic factor and upregulation of antiapoptic markers in the brain.

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