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Research Article

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Evolving concepts of depression provide new therapeutic options

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ABSTRACT

Depression is a highly prevalent and disabling psychiatric disease that often requires long-term treatment. This study was planned to investigate the antidepressant activity of DHEA in the experimental model. This study was conducted on 40 adult male albino rats assigned into 4 groups; Gp. (1) control group, Gp. (2) reserpinized group, Gp.(3) reserpinized group treated with low dose of DHEA and Gp.(4) reserpinized group treated with high dose of DHEA. Brain neurotransmitters, serum ACTH and corticosterone, brain proinflammatory cytokines, brain derived neutrophic factor and brain antiapoptic mediator as well as the histological examination of the brain tissue sections were carried out. In comparison with the control group, the reserpinized group recorded significant decrease in serotonin, dopamine, BDNF and Bcl-2, significant increase in ACTH, corticosterone were detected in the reserpinized group. Histological examination of brain tissue sections of rats in the reserpinized group showed neuronal damage and shrinkage. On the other hand, treatment of the reserpinized groups with either low or high dose of DHEA resulted in remarkable improvement in the biochemical parameters associated with restoration in the structure organization of the brain in a dose dependant manner. The present work provides a clear evidence for the antidepressant potential of DHEA. This was evidenced by activation of serotoninergic and dopaminergic system, reduced levels of ACTH and corticosterone, inhibition of proinflammatory cytokines, promotion of neurotrophic factor and improvement the morphological structure of the brain tissue sections.

Key words: Depression, DHEA, ACTH, corticosterone, neurotransmitters, Inflammation, Pathology.

INTRODUCTION

Depression is an important public-health issue with a high incidence; it has also been recognized as a major cause of disability and death, both by suicide and from high rates of physical disorders [1]. The prevalence of depression that is associated with chronic disease is increasing, and depression is predicted to be the second leading cause of disease burden by the year 2020 [2].

The increasing burden and prevalence of depression make the search for an extended understanding of the aetiology and pathophysiology of depression highly significant [3]. The pathophysiology of depression is hypothesised to be associated with various neurobiological changes [4]. An emphasis has been given to neuroimmune processes for directly and indirectly affecting other important neurobiological processes of depression [5].

Clinical depression refers to a state of intense sadness, melancholia or despair, accompanied by anhedonia (loss of interest or pleasure), disturbed sleep, appetite and sexual libido, low self-estimation, feelings of guilt, and cognitive disturbances. Comorbidity with other conditions such as anxiety and cardiovascular disease is common, and depression is causally linked to type II diabetes. The disorder has a heritability rate of 40–70%, but the interaction of genes with environmental events, including psychological factors and life experiences (commonly grouped under the title 'stress'), is important in the initiation of, and recovery from the disease [5].

Several risk factors appear to be contributed in depression, including the increased translocation of lipopolysaccharides (LPS) from gram-bacteria (leaky gut hypothesis) [6] immunotherapy (IFN-α,IFN-β,IL-2), bacterial and viral infection , other medical conditions (cardiovascular disease), exposure to chronic or early life stressors, genetics factors (polymorphisms in IL-10 gene) and glucocorticoids resistance [7] Also, the increased level of soluble intercellular adhesion molecule (sICAM) ,C-reactive protein (CRP) and interleukin-6 (IL-6) was predictive of the development of depressive symptoms [8].

Reserpine, the most important Rauwolfia indole alkaloid, is a well 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 and relaxing 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 and breast 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) [9]**.**

More recently, it has been hypothesized that DHEA may have a role in the pathogenesis of depression.

DHEA is primarily secreted from the adrenal gland specifically by the reticularis zone of the adrenal cortex [10]**.** As,its sulfated form, DHEA-S, which is the most abundant steroid secreted by the human adrenal gland and which circulates in up to 30-fold concentration greater than cortisol. DHEA-S undergoes conversion to DHEA by steroid sulfatases in many peripheral tissues [11]**.**

DHEA is cosecreted with cortisol in response to corticotropin releasing hormone (CRH) and corticotrophin (adrenal corticotropic hormone (ACTH)).

Dissociation of DHEA from cortisol release is present during acute stress, such as may occur following burns or acute trauma, and psychological stress as encountered prior to surgery [12]**.**

Under normal circumstances, DHEA is converted to more potent androgens including androstenedione and testosterone as well as estrogens, 17β_-estradiol and estrone, by the enzymes 3β_-hydroxysteroid dehydrogenase (3β_-HSD) and 17β _-hydroxysteroid dehydrogenase (17β-HSD) which are found not only in classic steroidogenic tissues (placenta, adrenal cortex, ovary, and testis) but also in a number of peripheral tissues [13]**.** Its conversion to androstenedione and subsequent aromatization to estradiol and estrone is thought to account for the majority of estrogen biosynthesis in postmenopausal women [14]**.**

DHEA is intermediate in the production of both androgens and estrogens and it exists in equilibrium with its sulfated form, DHEAS [15]**.** Since DHEA and DHEAS exist in the serum at much higher concentrations than other steroid hormones.

There is some sparse evidence suggesting that DHEA is also synthesized in the brain; it is thought that DHEA acts on neurotransmitter receptors [16]**.**

Neurosteroids are known to be involved in conditioned behavioral processes which are regulated by psychological processes, such as response to stressful events, cognition, anxiety, aggression, depression, and in other regulatory behaviors, such as sleep, ingestion and reinforcement [17]. DHEA appears to have a wide range of beneficial effects when administered to humans and rodents. Several examples include anticarcinogenic, antidiabetic and immunomodulatory activities.

EXPERIMENTAL SECTION

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^{o}C) and humidity (55%) under 12 h 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 assigned 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 intraperitoneally (i.p.) in a dose of 0.1 mg / kg b.wt [18]. 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 DHEA (30 mg/kg b.wt) orally in the last 30 days. The fourth group was a reserpinized group that received high dose of DHEA (60 mg/kg b.wt) 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 [19]. The homogenate was centrifuged at 1800xg for 10 min in cooling centrifuge at 4^{o}C. The supernatant (10%) was separated for the biochemical analyses. The other half of each brain was fixed in formalin buffer for histological investigation.

Biochemical analyses

Quantitative estimation of total protein level in the brain homogenate was carried out according to the method of Lowry et al [20]. 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 [21]. 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 [22]. 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. [23]. Brain interlukin-1β (IL-1β) content was determined by ELISA technique using IL-1β assay kit purchased from Assay Pro.,Co., USA , according to the method described by Fan et al. [24].The content of IL-6 in the brain was estimated by ELISA technique using IL-6 assay kit purchased from Koma Biotech Inc.,Co. Seoul, Korea according to the manufacturer's instructions provided with the IL-6 assay kit. 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 histopathological studies. 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 oC). Sections of 6μm thickness were prepared and stained with haematoxylin and eosin [25]. 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

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

RESULTS

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 DHEA 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 of DHEA was 258.99 pg/mg protein vs 182.96 pg/mg protein for the positive control group with the percent of difference 41.56%. Brain serotonin content of the reserpinized group treated with high dose of DHEA was 263.99 pg/mg protein vs 182.96 pg/mg protein for the positive control group with the percent of difference 44.29%.

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 differenc -38.04%. However, significant increase ($P<0.05$) in brain dopamine content was detected in the reserpinized groups treated with DHEA as compared to that in the positive control group. Brain dopamine content of the reserpinized group treated with low dose of DHEA was 235.20 µg/mg protein vs 171.75 µg/mg protein for the positive control group with the percent of difference 36.94%. Brain dopamine content of the reserpinized group treated with high dose of DHEA was 239.72 µg/mg protein vs 171.75 µg/mg protein for the positive control group with the percent of difference 39.57%.

Table (1): Effect of treatment with different doses of DHEA on brain neurotransmitters (serotonin and dopamine) content of the reserpinized rats (Experimental model of depression)

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 DHEA	258.99± 12.83 [b] (41.56%)	235.20±16.70 [b] (36.94%)
Reserpinized group treated with high dose of DHEA	263.99± 22.39 [b] (44.29%)	239.72±6.38 [b] (39.57%)

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%). In the contrary,Serum ACTH level of the reserpinized group treated with low dose of DHEA was 43.38 pg/ml vs 60.95 pg/ml for the positive control group with the percent of difference -28.83%, and that for the reserpinized group treated with high dose of DHEA was 39.60 pg/ml vs 60.95 pg/ml for the positive control group with the percent of difference -35.03%.

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%. Whereas,Serum corticosterone level of the reserpinized group treated with low dose of DHEA was 50.65 nmol/l vs 85.04 nmol/l for the positive control group with the percent of difference -40.44%, and that for the reserpinized group treated with high dose of DHEA was 46.95 nmol/l vs 85.04 nmol/l for the positive control group with the percent of difference -44.79%.

- **Proinflammatory Cytokines**

1-*Interleukin-1 beta* (IL-1β)

The data given in Table (3) illustrated the effect of treatment with DHA on brain IL-1β content of the reserpinized rats as an experimental model of depression. The results showed significant increase ($P<0.05$) in brain IL-1β content of the reserpinized group (positive control group) after 75 days of reserpine administration as compared to that in the negative control group (5.39 pg /mg protein vs 1.75 pg /mg protein with the percent of difference 208% . On the other hand, treatment of the reserpinized groups with DHEA resulted in significant decrease ($P<0.05$) in brain IL-1β content as compared to that in the positive control group.Brain IL-1β content of the reserpinized group treated with low dose of DHEA was 2.68 pg/mg protein vs 5.39 pg/mg protein for the positive control group with the percent of difference -50.28%, and that for the reserpinized group treated with high dose of DHEA was 2.54 pg/mg protein vs 5.39 pg/mg protein for the positive control group with the percent of difference -52.88%.

Table (2): Effect of treatment with different doses of DHEA on hormones 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 DHEA	43.38±4.26 [b] (-28.83%)	50.65± 1.19 [b] (-40.44%)
Reserpinized group treated with high dose of DHEA	39.60±2.87 [b] (-35.03%)	46.95±1.13 [b] (-44.79%)

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.

Table (3): Effect of treatment with different doses of DHEA on brain proinflammatory cytokines of the reserpinized rats (Experimental model of depression)

Data are represented as Mean ± S.E for 10 rats /group

Parameters / Groups	IL-1β (pg/mg protein)	IL-6 (pg/mg protein)
Negative control group	1.75 ± 0.12	26.04±1.74
Reserpinized group (positive control)	5.39 ± 0.18[a] (208%)	70.17±5.47 [a] (169.47%)
Reserpinized group treated with low dose of DHEA	2.68 ± 0.15[b] (-50.28%)	39.36±1.89 [b] (-43.91%)
Reserpinized group treated with high dose of DHEA	2.54 ± 0.17[b] (-52.88%)	36.92±3.40 [b] (-47.38%)

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.

2-*Interleukin-6* (IL-6)

Data recorded for brain IL-6 content in the negative control group, and other studied groups are depicted in Table(3). In comparison with the negative control group,the reserpinized group (positive control group) after 75 days of reserpine administration revealed significant elevation ($P<0.05$) in brain IL-6 content (26.04 pg/mg protein vs 70.17 pg/mg protein) with the percent of difference 169.47%. However, as compared to the positive control group, the treated groups with DHEA recorded significant reduction ($P<0.05$) in brain IL-6 content.The brain IL-6 content of the reserpinized group treated with low dose of DHEA was 39.36 pg/mg protein vs 70.17 pg/mg protein

for the positive control group with the percent of difference -43.91%, and that for the reserpinized group treated with high dose of DHEA was 36.92 pg/mg protein vs 70.17 pg/mg protein for the positive control group with the percent of difference -47.38 %.

- **Neurotrophic factor**

***Brain derived neurotrophic 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 DHEA 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 DHEA was 0.167 ng/mg protein vs 0.095 ng/mg protein for the positive control group with the percent of difference 75.79%, and that for the reserpinized group treated with high dose of DHEA was 0.172 ng/mg protein vs 0.095 ng/mg protein for the positive control group with the percent of difference 81.05 %.

- **Antiapoptic marker**

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

Table (4) represented the results of the effect of treatment with DHEA on brain Bcl-2 content of the reserpinized rats. 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 DHEA as compared to that in the positive control group.Brain content of Bcl-2 in the reserpinized group treated with low dose of DHEA was 0.086 ng/mg protein vs 0.052 ng/mg protein for the positive control group with the percent of difference 65.38%, and that for the reserpinized group treated with high dose of DHEA was 0.089 ng/mg protein vs 0.052 ng/mg protein for the positive control group with the percent of difference 71.15 %.

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).**

Table (4): Effect of treatment with different doses of DHEA on brain neurotrophic factor (BDNF) and antiapoptotic marker (Bcl-2) of the reserpinized rats (Experimental model of depression)

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 DHEA	0.167±0.010 [b] (75.79%)	0.086±0.009 [b] (65.38%)
Reserpinized group treated with high dose of DHEA	0.172±0.012 [b] (81.05%)	0.089±0.007 [b] (71.15%)

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.

Microscopic examination of 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 examination of 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).** Histopathological investigation of brain tissue sections of reserpinized rats treated with low or high dose of DHEA showed the normal structure of cerebrum **(Figs. 5,7)** respectively. Noteworthy, some dark neurons are appeared in case of treatment with low dose of DHEA **(Fig. 5).** Histopathological investigation of brain tissue sections of reserpinized rats treated with low or high dose of DHEA showed the normal structure of hippocampus. Noteworthy, few dark neurons were found **(Figs 6, 8)** respectively.

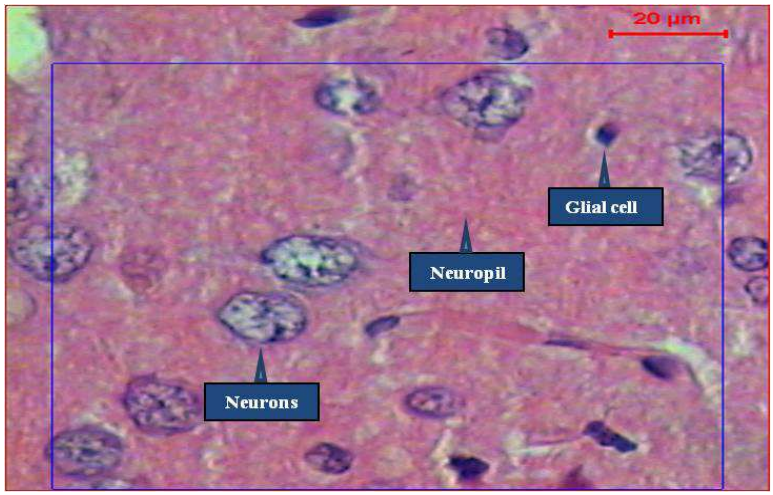

Fig. (1): Micrograph of brain tissue section of control rat showing the highly active nerve cells (neurons) that having huge nuclei with relatively pale stain. The nuclear chromatin and prominent nucleoli are disappeared. The surrounding supporting cells (glial cells) have small nuclei with dense stain,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

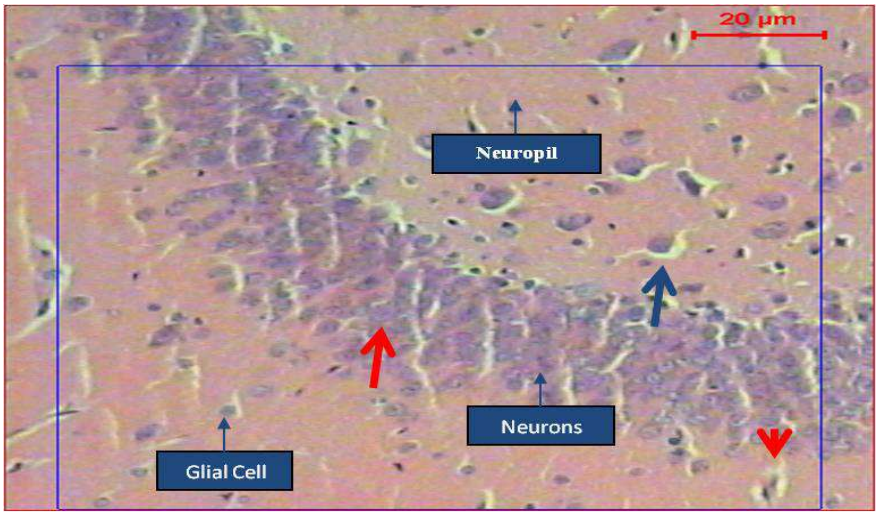

Fig. (2): Micrograph of brain tissue section of control rat showing the normal structure of hippocampus. The hippocampal neurons (Neurons) and vessels exhibited a regular arrangement with distinct edges (arrowhead), and clear nuclei and nucleolui (arrow). Glial cells and neuropil are appeared. No necrosis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

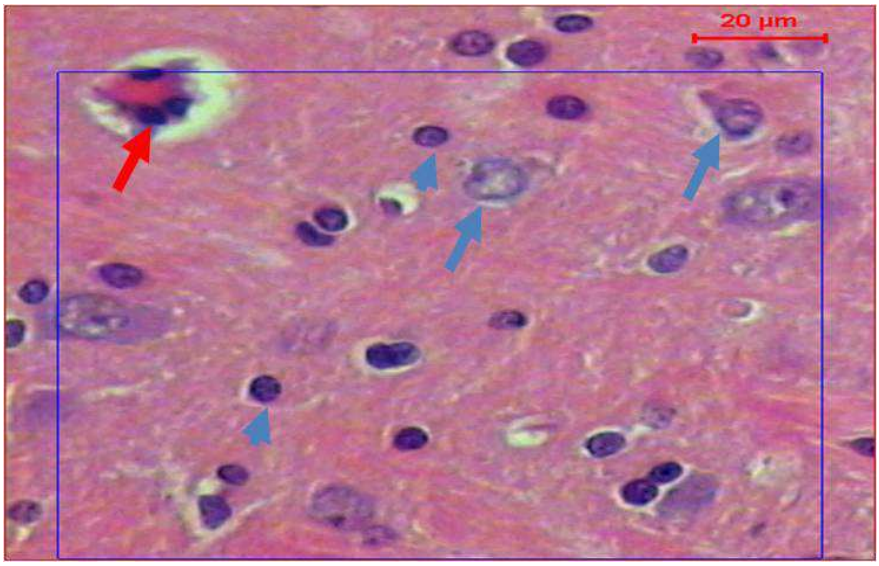

Fig. (3): Micrograph of brain tissue section from rat administered reserpine for 75 days showing neuronal damage (red arrow), and shrinkage (blue arrow) of cerebrum. The basophilic neurons show core pyknosis (arrowhead) (H & E X 400, Scale bar 20 µm)

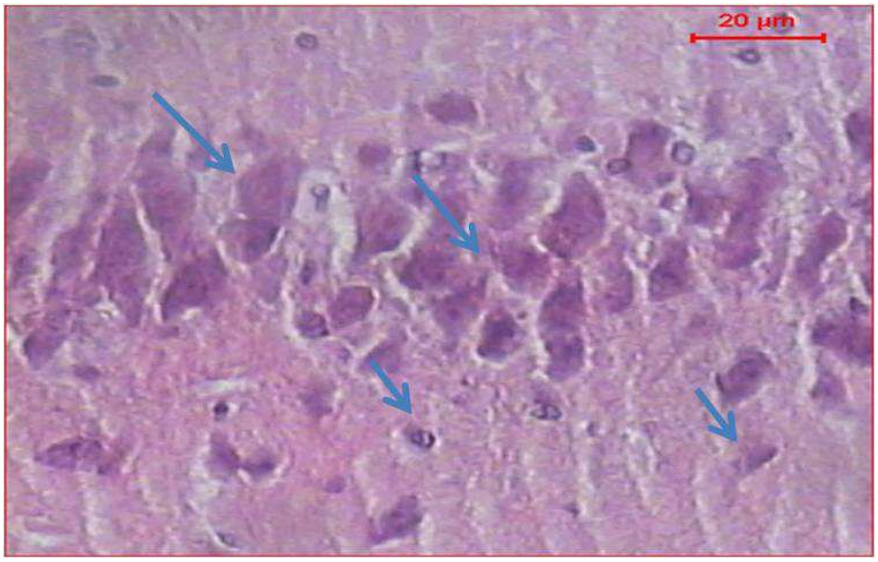

Fig. (4): Micrograph of brain tissue section of rat in the reserpinized group after 75 days showing dark neurons of the hippocampus (arrows) and significant necrosis of pyramidal neurons (arrowhead) (H & E, Scale bar 20 µm)

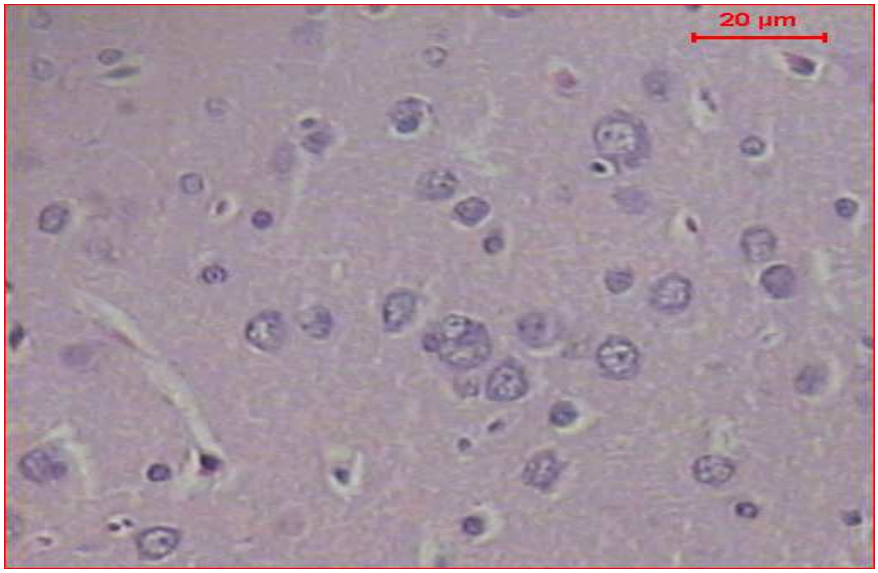

Fig. (5): Micrograph of brain tissue section of reserpinized rats treated with low dose of DHEA showing the normal structure of cerebrum with the appearance of some dark neurons (H & E, Scale bar 20 µm)

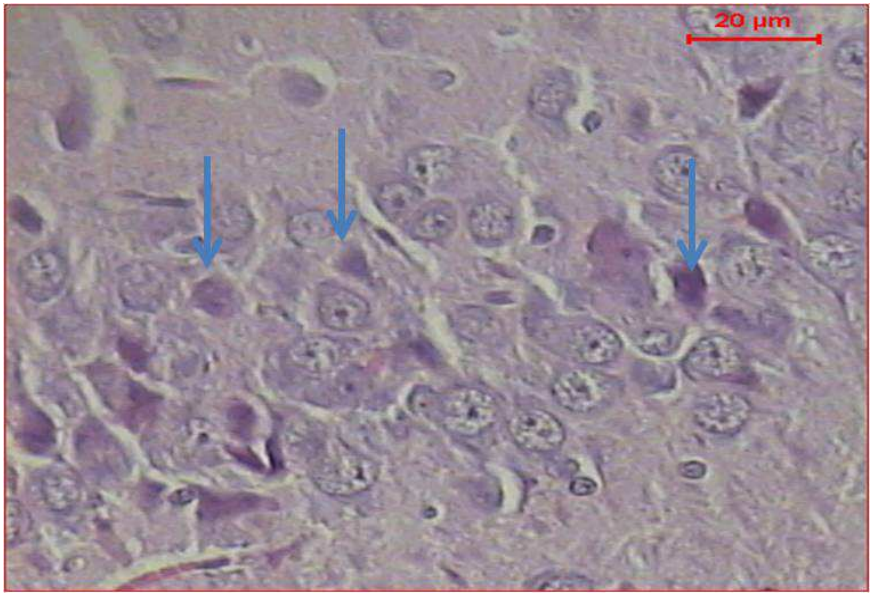

Fig. (6): Micrograph of brain tissue section of reserpinized rats treated with low dose of DHEA showing the normal structure of hippocampus. Few dark neurons (arrows) are noticed (H & E, Scale bar 20 µm)

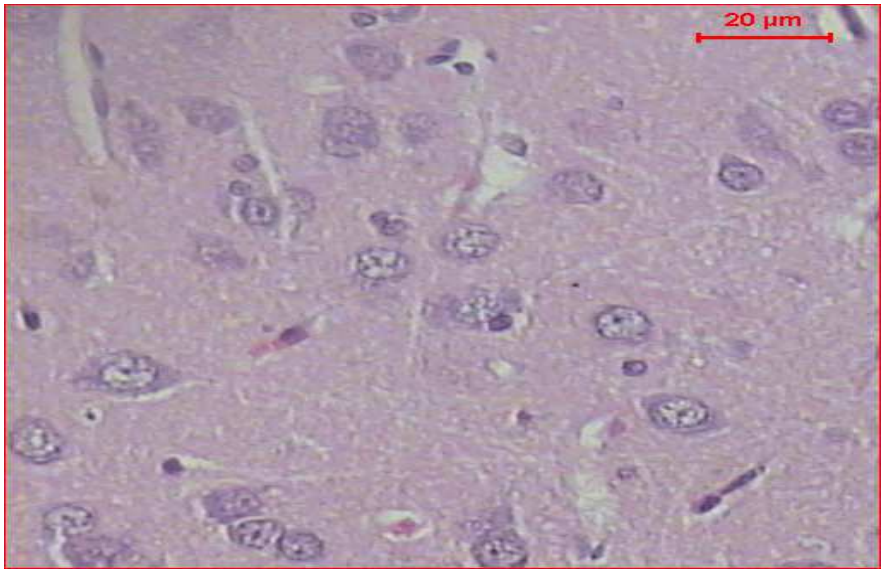

Fig. (7): Micrograph of brain tissue section of reserpinized rats treated with high dose of DHEA showing the normal structure of cerebrum (H & E, Scale bar 20 µm)

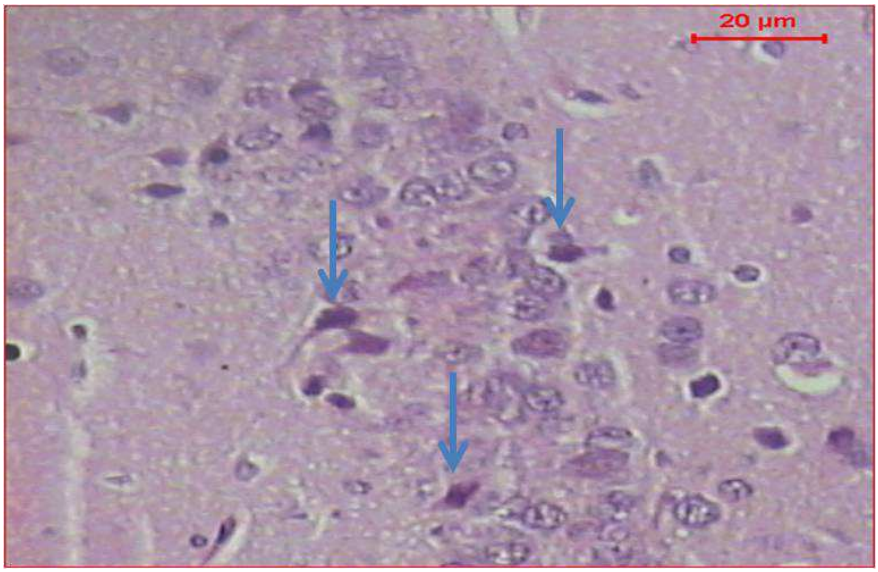

Fig. (8): Micrograph of brain tissue section of reserpinized rats treated with high dose of DHEA showing the normal structure of hippocampus. Few dark neurons are observed (arrows) (H & E, Scale bar 20 µm)

DISCUSSION

The results of the present study revealed that 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 [26]**.** 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 [27]**.** Reserpine as an antihypertensive agent has been found to decrease brain monoamines with consequent detrimental

effects on mood [28]**.** 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 [29]**.** 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 [30]**.**

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 [31]**.**

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 [32] 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 [6]. 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 [33]**.** Thus, cytokine- and IDO-mediated degradation of tryptophan through the kynurenine pathway is hypothesized to influence 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 [34]**.**

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**.** However, repeated HPA axis activation can produce damaging physiological effects and exert a profound impact on brain function [35]. 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 [36]**.** This leads to a further hypersecretion of glucocorticoids, and this is thought to produce neuronal changes in several brain regions, including the hippocampus [37] and amygdala [38]**.** Within the hippocampus, persistently elevated glucocorticoids levels lead to dendritic remodeling of CA3 neurons, decreased neurogenesis, and finally cell death [39]**.**

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 [40]. 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 [41].

The current results demonstrated that brain interlukin-1β (IL-1β) and interlukin-6 (IL-6) are 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 [42]**.** 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 [43].

Changes in the amount of cytokine production may also be due to neuroendocrine influences on the immune system [44]. 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 [45]**.**On the other hand, the potential mechanisms leading to cytokine-induced depression are numerous and were reviewed by Miller et al. [46], and others. Several lines of evidence demonstrating how cytokines can contribute to HPA axis hyperactivity [47] and affect the serotonergic and dopaminergic systems [48]. Proinflammatory cytokines are potent activators of the HPA axis [49] 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 [50]**.** The mechanism by which cytokines may disturb inhibitory feedback of corticosteroids on the HPA axis, may involve the 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 [50]**.**

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 [51] **,**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 [52]. 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 digenesis [53]**.**

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 [54]**.** 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 [55].

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 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 [56]**.** 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 [57].The Bax: Bcl-2 ratio is used as an index of vulnerability for apoptosis [58]**.** 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 [59]**.** 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 [60]**.**

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) together defined as DHEA/S are the major secretary products of adrenal gland [61]**.** DHEA and in parallel, DHEAS, are also produced in the brain, thus termed neurosteroids [62]**.** Neurosteroids are known to be involved in conditioned behavioural processes which are regulated by psychological processes, such as response to stressful events, cognition, anxiety, aggression, depression, and in other regulatory behaviours, such as sleep, ingestion and reinforcement [17]**.** In paticular, Wolkowitz and Reus [63] reported that DHEA has a potential applications in the treatment of depression.

As mentioned before, serotonin, noradrenaline, and dopamine are believed to be involved in mental depression [64]. According to the monoamine theory, depletion of serotonin and/or dopamine is one of the causes of depression [65]**.** The present results demonstrated that DHEA administration induces significant increase in brain serotonin content of the reserpinized rats as compared to the reserpinized group (positive control group). This result is in agreement with the finding of Svec and Porter [66] which showed that administration of DHEA increases hypothalamic serotonin levels**.** This result supports the positive relationship between DHEA and brain serotonin and the possible positive effect of DHEA in depression.

The antidepressant-like effects of DHEA [67] could be explained by the interaction between the sigma 1 receptor agonist, DHEA and or /DHEAS [68] and norepinephrin, dopamine and serotonin (5-HT) neurotransmission [69]. Enhancement of norepinephrin [70] **,** serotonin [71] and dopamine [72] neurotransmission is considered to have an antidepressant effect.

Serotonergic drugs (such as 5-HT reuptake inhibitors and 5-HT2B/2C agonists) are anxiolytic [73]. Thus, it is possible that serotonergic stimulation by DHEA is involved in the anxiolytic effect of the steroid in on humans [74] and animal models [75].

The suggested mechanism for serotonergic stimulation by DHEA was the MAO inhibitory effect of DHEA which led to its beneficial effect on mood [76]. This hypothesis deserves further investigation but it is consistent to the effect of the steroid on behaviour associated to serotonergic neurotransmission system such as mood, aggression and anxiety.

It has been shown that DHEA modulates several neurotransmitter systems and this might be involved in its behavioural effects [76]. Some studies suggested that DHEA and its sulfated ester (DHEAS) may reduce dopamine (DA) D2 receptor activation [77]**,** while increase D1 receptor-dependent Protein kinase A (PKA) activity [69]**.** Moreover, both DHEA and DHEAS could increase DA release from dopaminergic neurons [78].

The data in the current study revealed a significant elevation in the brain content of dopamine after treatment with DHEA in the reserpinized rats as compared to the positive control ones. This finding might be attributed to the modulatory effects of DHEA on dopamine neurotransmission at both presynaptic and postsynaptic sites [78]**.**

It has been demonstrated that treatment with DHEA increased the dopamine content in the ventral tegmental area (VTA) and the nucleus accumbens (Nac) of the brain [75]**.** Moreover, DHEA administration at the dose of 25 mg/kg

has been found to increase dopamine content in the lateral hypothalamus [79]**.** Sagi et al. [80] reported that MAO inhibition is involved in the neuroprotective effect of DHEA. This property enables DHEA to stimulate the dopaminergic system. As the selective MAO inhibitors (MAOI's) such as selegiline and rasagiline should a neuprotective effect in experimental models of parkinsonism as well as in other paradigms of cell damage [81].

DHEA and DHEAS might increase dopamine release through σ1R since these steroids have been shown to act as agonists at this site [82]**.**In the reward system, there is the σ1 receptor (σ1R), which is an intracellular protein present on the endoplasmic reticulum membrane that can be translocated after activation [83,84] resulting in calcium mobilization and modulation of several neurotransmitter responses. It has been suggested that σ1R is a signal transduction activator that facilitates DA neurotransmission [85]**.**

Thuse, DHEA and its sulfate ester might stimulate dopamine release as sigma receptor agonists and D2 receptors antagonists [86]**.** These means that DHEA may increase dopamine release through presynaptic D2 receptors inhibition and/ or sigma receptor activation.

The current results revealed that serum ACTH and corticosterone levels are significantly reduced after administration of DHEA in the reserpinized group as compared to the positive control group. In accordance of our results, [87] found that DHEA administration, causes a decrease in both ACTH and corticosterone levels in animal model of depression. This result could be explained by that DHEA is a neuroactive steroid which suppresses HPA system activity [88].

In the view of the obtained results, DHEA has antidepressant effects *via* several plausible mechanisms, 1) counteracting glucocorticoid actions, 2) agonistic action on sigma1 receptors, and 3) enhancing of noradrenaline and serotonin neurotransmission [71]**.**

DHEA is the most abundant andrenal steroid with immunomodulatory activity [89]**.** This may be due to the ability of DHEA to inhibit nuclear factor kappa B (NFκB). *In vitro* studies showed that DHEA increases secretion of IL2 by activated T lymphocytes and decreases production of IL4, IL5, and IL6, thus it could stimulate Th1-type cytokines [90]**.** In particular, DHEAS has been found to repress the expression and activity of IL6 gene promoter, supporting the concept of the antiinflammatory effect exerted by androgenic steroids [91].

The present study demonstrated a decrease in the brain content of IL-1β and IL-6 after administration of DHEA in the reserpinized rats compared with the positive control group. This finding is in accordance with that of Murialdo et al. [92] who stated that mechanisms proposed for DHEA's putative neuroprotective effects include inhibition of the production of pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α. A growing body of evidence demonstrated that DHEA can reduce the release of the proinflammatory cytokines IL-1, IL-6, and TNF-α [93]**,** and the metabolites of DHEA, including androstenediol and androstenetriol may also regulate macrophage cytokine secretion [94]**.** These findings indicated that DHEA and DHEAS have anti-inflammatory and immunomodulatory effects [95]**.** The suggested mechanism for these properties is the ability of DHEA and DHEAS to inhibit NF-κB activation *via* the inhibition of hydrogen peroxide-induced NF-κB activity and the activator protein-1 (AP1; fos/jun)-mediated transcription, which is known to be a radical-sensitive transcription factor [96]**.** Thus, it has been proposed that DHEA and DHEAS act on NF-κB indirectly through a cytokine-induced signaling pathway involving reactive oxygen species [96].

Steroids are known to have genomic actions, but they can also have nongenomic effects by interacting with several types of neurotransmitter receptors and neuromodulatory proteins [97]**.**

On the basis of this evidence, the result of the current work revealed elevated level of brain BDNF as a result of DHEA administration in the reserpinized rats as compared to the positive control ones.

Our result is in agreement with that of Naert et al. [98] who investigated the possible mechanism by which DHEA(S) can promote neurogenesis and neuronal survival. These authors reported that single intraperitoneal injection of either DHEA (25mg/kg) or DHEAS (50 mg/kg) into rats change the regional brain concentrations of BDNF during the 300 minutes of the experiment. The rats received DHEA have increased BDNF in the hypothalamus compared to sham rats that received sesame oil [98]**.** It has been proposed that DHEA and DHEAS play a role in neurodevelopment, due to a transient expression of steroidogenic enzyme P450 17-alpha hydroxylase

(P450c17) [99]**.**As DHEA and DHEAS have been found to aid in neuronal pathway formation [100]. Thus, the role of DHEA in depression comes from its antidepressive effect and its role in neurogenesis.

Bcl-2 has been known to play a critical role in promoting cell survival. The data in the present study revealed that administration of DHEA in reserpinized rats produces significant increase in brain content of Bcl-2 as compared to the reserpinized group (positive control group). DHEA has been shown to have oxygen-free radical scavenger properties that may play a role in its neuronal protective activity [101]**.**

Some investigators suggested that the neuroprotective activity of DHEA against excitotoxicity-induced neuronal death is mediated by its conversion to testosterone and further to estradiol by aromatase [102]. It has also been hypothesized that 7-alpha-hydroxylation of DHEA mediates its neuroprotective activity [103].

DHEA has been found to regulate Bcl-2 at the transcriptional levels, because of the exposure of vascular endothelial cells to DHEA increased Bcl2 promoter activity and mRNA level [104]**.**The mechanism by which DHEA could stimulate Bcl-2 expression is explained as DHEA is able to bind and activate G-protein coupled membrane receptor alpha inhibitory subunit (Gαi) which, in turn, activates tyrosine kinase c (Src), protein kinase C (PKC) and MAPK/ERK pathway. These kinases activate the prosurvival transcription factors cAMP response element binding protein (CREB) which stimulate the expression of antiapoptotic proteins such as Bcl-2 and Bclxl [105]**.**Thus, DHEA could increase Bcl-2 level and stimulate Bcl-2 function.

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 McEwen, [106] 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 [107]. 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 [108].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 [109]**.** This atrophy is mimicked by daily treatment with corticosterone [110] indicating that elevated circulating adrenal steroids secreted during stress may be involved in triggering these morphological alterations.

CONCLUSION

The current study provides an experimental evidence for the antidepressant efficacy of DHEA. This was achieved through the activation of serotoninergic and dopaminergic system, reduced levels of ACTH and corticosterone, inhibition of proinflammatory cytokines, promotion of neurotrophic factor and improvement of the morphological structure of the brain.

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