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Research Article

Effect of Experimentally Induced Depression on the Adrenal Cortex of Adult Male Albino Rats and the Possible Ameliorative Role of Voluntary Exercise

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Abstract | This study was designed to investigate the changes that occur in adrenal cortex in an animal model of depression and the effect of voluntary exercise on these changes. Thirty-two adults male Wistar rats were included based on their forced swimming test behavior. The rats were divided into 4 groups. Group 1 was the control group, group II was the control exercise group, group III was the depression group, and group IV was the depression exercise group. The forced swimming protocol was used to induce depression, while the rat voluntary wheel was used for voluntary exercise. After scarification, estimation of corticosterone level was conducted, and samples of adrenal gland were examined for structural changes by light and Electron microscope, and for immunohistochemical expression. Rats from group III showed statistically increased corticosterone level and increased cortical thickness compared to other groups. Voluntary exercise improved these measures in rats of group IV. Histological disorganization of cortical cells was observed in group III with manifest large cytoplasmic vacuoles. Enormous increase in the number of both apoptotic and proliferative cells was seen with significantly higher mean number of the proliferative cells. By EM, the nuclei were shrunken, secretory vesicles were depleted, and the smooth endoplasmic reticulum showed massive dilatation. These findings were partially restored in rats of group IV. This study concluded that voluntary exercise enhanced recovery from the injurious effect of depression on adrenal cortical cells that partially showed regain of their normal structure and corticosterone secretion.

Keywords | Adrenal cortex, Cortisol, Depression, Rats, Voluntary exercise

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INTRODUCTION

Depression is one of the most common psychiatric disorders, affecting millions of people all over the world. It ranks among the leading causes of disability worldwide (Murray and Lopez, 1997) that results in an enormous social and financial burden on modern society (Greenberg et al., 2003; Kessler et al., 2005). The link between endocrine dysfunction and mood disorders has attracted researchers for many decades. Subsequent

researches in the field of neuroendocrinology have approved the role of endocrine systems in the etiology and pathogenesis of mood disorders (Rubin et al., 1995; Kessing et al., 2011).

Some previous researches postulated that major depression is accompanied with hyperactivity of the hypothalamo-pituitary-adrenocortical axis (HPA). These researches found a statistically significant increase in adrenal volume in patients with major depression compared to controls, which was state-related that returned to normal on recovery from the depressive episode (Rubin et al., 1995). However, other researches showed a negative correlation between depression and adrenal gland volume. To explore this controversy, it was suggested that further studies have to be done (Kessing et al., 2011). Cortisol is released from the adrenal cortex as the end product of the HPA axis. Corticotropin-releasing hormone (CRH) secreted from the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary which in turn stimulates the release of cortisol from the adrenal cortex (Burgese and Bassitt, 2015).

Neuroendocrine studies have found variations in cortisol levels and changes in the HPA axis of patients with depression (Shapira et al., 2000; Castro and Moreira, 2003; Schatzberg and Lindley, 2008; Grønli et al., 2009). These studies have shown that increased cortisol levels in plasma, urine and cerebrospinal fluid exaggerated cortisol responses to ACTH. In addition, enlarged pituitary and adrenal glands were observed in patients with severe mood disorders (Webster et al., 2002). Increased plasma cortisol level, observed in patients with major depression, has been associated with multiple behavioral changes as sleep disorders, lassitude, decreased attention and libido, psychomotor disorders, anxiety and suicide ideation (Wolkowitz and Reus, 1999).

Exercise training has proved to induce beneficial effects on stress-related mental disorders for several decades (Mul, 2018). Many studies have examined the effect of physical exercise on rodent behaviors, but the results remain controversial. Some studies on rodents reported that voluntary exercise improved depression-like behaviors (Bjørnebekk et al., 2005; Eldomiaty et al., 2017).

This research investigated the effect of depression induced in experimental animals by using forced swimming protocol on the histological structure of adrenal cortex and verified the ameliorating role of exercise on these effects, with an emphasis on the role of cortisol in relation to depression.

MATERIALS AND METHODS

Animals

Forty-six-adult male Wistar rats aged 8-10 weeks with an average weight 200-250 g were used in this study. All animal handling and experimental procedures were conducted in the animal house and the lab. of the college of medicine at Taibah university according to the EC Directive 86/609/EEC for animal experiments (http://ec.europa.eu/environment/chemicals/labanimals/legislation_en.htm) and the guidelines of the National Committee of Bio-Ethics in Saudi Arabia (Alahmad, 2017) and all experiments complied with the guidelines of the national institute of health guide for the care and use of laboratory animals. The animals were housed in well ventilated cages with food and water ad libitum under normal room temperature and it was adjusted at 12/12 h light/dark cycle. Before the beginning of the experiment, the rats were put under observation for 7 days. Four rats showed diminished movement and diminished access to food than ordinary, and so they were excluded from the study. The remaining 42 rats were exposed to a 6 min forced swimming test FST and only 32 rats were selected to be included in this experiment based on their FST behavior (Andrus et al., 2012).

Experimental design

Animal groups

The selected rats were divided into 4 groups (8 rats each). Rats of group 1 (control group) were maintained on a standard chow diet for 5 weeks and left without any intervention. Rats of group II (exercise group) were maintained without intervention for 2 weeks, and then they were allowed to perform voluntary exercise for 3 weeks. Rats of Group III (depression group) were subjected to forced swimming protocol for 2 weeks, and then they were maintained without any intervention for further 3 weeks. While rats of Group IV (depression-exercise group) were subjected to forced swimming protocol for 2 weeks, and then they were allowed to perform voluntary exercise for the next 3 weeks. The duration of the experiment lasted for 35 days. Just before scarification, all rats were weighed, and assessment of their locomotor activity was done using a 6-min FST.

Forced swimming protocol for induction of depression (Porsolt et al., 1978; Sun and Alkon, 2003; Eisch et al., 2003).

Rats were subjected individually to forced swimming inside vertical glass cylinders (height 60 cm, diameter 22 cm) and containing water (height 45 cm) at 23-24 °C for 15 min. Then after, the rats were removed and dried for 15 min in a heated enclosure at 32 °C before being returned to their cages. This procedure was done once daily for successive 14 days. To assess development of depression, the locomotor activity of the rats were evaluated on the last day of the forced swimming protocol using the 6-min FST. Automatic tracing was done using computer software

(Ethovision XT version: 8.0) to assess the distance moved by the rat in centimeters and the immobility duration (time spent passively floating) in seconds.

Protocol of voluntary exercise (Jonsson, 2012; Eldomiaty et al., 2017)

Voluntary exercise was allowed for the exercise groups (group II and group IV) by allowing voluntary wheel running. Rats in these groups were put in separate cages equipped with rat running wheels with activity wheel counters (Lafayette Instrument Company, Inc. 3700 Sagamore Parkway North Lafayette, IN 47904 USA) (Figure 1) for 3 weeks.

Figure 1: A cage equipped with voluntary rat running wheel with activity wheel counter.

Methods of the study

Assessment of physical measures

The weight of each animal was determined at the beginning of the experiment and immediately before death and weight gain was estimated. The movement of animals and their access to food were observed.

Serological study

Immediately after sacrification, blood was collected from the left ventricle of each rat, placed in test tubes and the serum was obtained and frozen for assessment of serum cortisol level. Serum cortisol was measured using a radioimmunoassay kit (Biochemicals, Costa Mesa, CA, USA) and the values expressed as ng cortisol/ ml serum.

Light microscopic study

The right adrenal glands were collected, fixed in 10% formal saline, dehydrated, cleared and embedded in paraffin wax. Sections of 5 µm thick were cut and stained with haematoxylin and eosin (H and E) to verify histological details of adrenal cortex. Sections were examined by a bright field automated microscope (Olympus BX 36 provided with a true-colour image analysis software package) and the images were digitised by a DP27 digital video camera (2448×1920-pixel matrix).

Immunohistochemical study

Sections of 5 µm thick were blocked and incubated with the primary antibodies; activated caspase-3 (ab2302; Abcam, USA) for detection of apoptosis and PCNA (sc-56, Santa Cruz Biotech, USA) that detect the proliferating cell nuclear antigen for detection of cell division and proliferation. Then the slides were incubated with the corresponding biotinylated IgG for 60 min at room temperature followed with streptavidin biotin-horseradish peroxidase complex for another 60 min. The immunoreactivity was visualized by using 3,3′-diaminobenzidine (DAB) hydrogen peroxide. Mayer's hematoxylin was used as a Counterstain. Negative controls were done by excluding the primary antibodies (Buchwalow and Böcker, 2010). Sections were examined by light microscope and the images were analyzed as before.

Morphometric study

The mean thickness of the adrenal cortex from under the capsule to the corticomedullary junction was measured. Ten measurements were obtained from each slide at x200 (Gannouni et al., 2014). The mean number percentage of caspase-3 and PCNA immunohistochemical positive cells were calculated in ten randomly selected, non-overlapping illustrative fields from each slide at a magnification x400 with a microscopic field area of 786.432_m2. Using ImageJ (NIH) software, version 2.0.0-beta 4 (National Institutes of Health, Bethesda, MD, USA).

Electron microscopic study

The left adrenal glands were prepared for electron microscopic (EM) examination. Four to five small pieces were taken from the cortex of each gland and fixed in glutaraldehyde 3% and osmium tetroxide. The fixed parts were dehydrated and embedded in Epon 812. Semithin sections, 1 µm thick were cut and stained with toluidine blue and examined by LM to choose the selected areas for proper orientation. Ultrathin sections were stained by uranyl acetate and lead citrate. The EM study was performed with a Jeol 1010 Transmission Electron Microscope.

Statistical analysis

Data of body weight gain of all rats from different groups, plasma cortisol level from blood samples collected just after sacrification, the mean thickness of the adrenal cortex in all groups, and the percentage number of caspase-3 and PCNA immunohistochemical positive cells were expressed in mean±SD. For multiple comparisons, the statistical significance was assessed using one-way analysis of variance followed by the Scheffe test to compare pairs of

groups. P values ≤ 0.05 were considered significant while P values ≤ 0.001 were considered highly significant (Dawson et al., 1994).

RESULTS AND DISCUSSION

Assessment of physical measures

All rats gained weight at the end of the experiment (Figure 2A). Although the rats of the control group tended to gain weight more than that of the other groups, the differences in weight gain between different groups were statistically insignificant. Depressed rats showed retarded movement and diminished access to food, while rats of exercise groups (II and IV) showed increased activity and appetite.

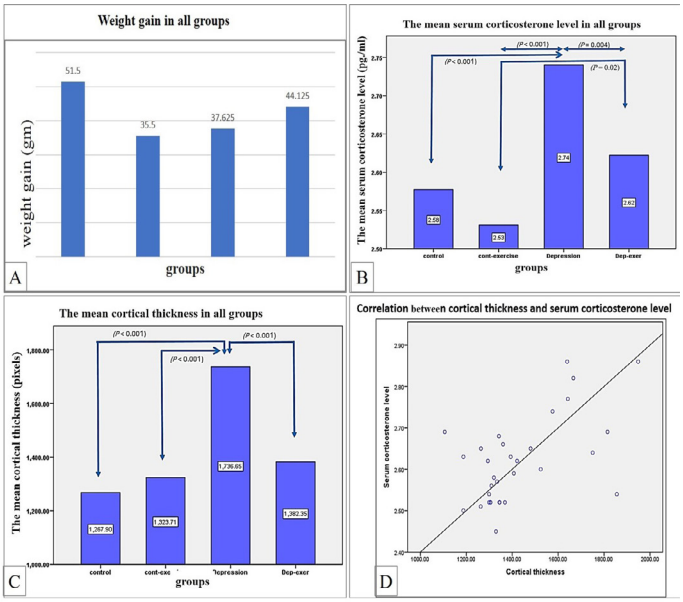

Figure 2: (A) Weight gain in all groups. (B) Differences in serum corticosterone levels in all groups. (C) Cortical thickness in all groups. (D) Correlation between cortical thickness and serum cortisol level.
Note: p ≤ 0.001 is highly significant, p≤0.05 is significant.

Serological results

Serological study revealed highly significant elevation of serum corticosterone level in rats with depression (group III) compared to other groups. Significant difference was also found between depression- exercise group (group IV) and control-exercise group (group II) (Figure 2B).

Morphometric results

Measurements of cortical thickness revealed highly significant increase in depression group compared to other groups (Figure 2C). However, non-significant increase of cortical thickness was found in both exercise groups (control- exercise and depression-exercise) compared to that of the control. Positive correlation was found between increased cortical thickness and serum cortisol level (Figure 2D).

Light microscopic results

Light microscopic examination of sections of the adrenal gland of the control group revealed the usual architecture. The gland was formed of outer cortex and inner medulla and covered with a thin layer of connective tissue capsule. The cortex was divided into three zones; glomerulosa, fasciculata and reticularis. The zona glomerulosa (ZG) was formed of clusters of cells arranged in arches beneath the capsule. The zona fasciculata (ZF) cells were arranged into cords. In the zona reticularis (ZR), the cells formed a network of irregular anastomosing cords. The cortical cells were polyhedral acidophilic cells with central rounded nucleus. Tiny cytoplasmic vacuoles were observed in the zona fasciculata cells. The cortical cells in the three zones were separated by blood sinusoids (Figure 3A, B).

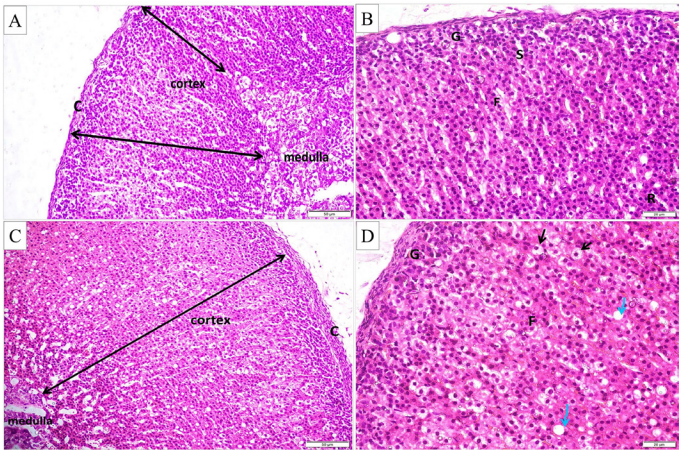

Figure 3: (A and B) Histological structure of adrenal gland in control group (H and E stain X200 and X400 respectively), showing the capsule (c), cortex and medulla. The cortex is formed of three zones; zona glomerulosa (G), fasciculata (F) and reticularis (R). Notice the blood sinusoids (s). (C and D) Histological structure of adrenal gland in the control exercise group (H and E stain X200 and X400, respectively), showing apparent increase of cortical thickness with hypertrophied cells (black arrows) and large cytoplasmic vacuoles (green arrows) in zona fasciculata.

Sections obtained from group II (exercise group) showed apparent increase of cortical thickness compared to that of the control. The cortex was formed of the three zones which appeared with the same histological pattern as that of the control. However, the zona fasciculata (ZF) cells were hypertrophied with large cytoplasmic vacuoles (Figure 3C, D).

In group III (depression group), the cortical thickness was increased compared to that of the control. The capsule showed intracapsular cavities and deposition of fat cells (Figure 4A, B). Some sections showed disorganization of the cortical cells and loss of the normal architecture (Figure 4B). Hypertrophied ZF cells with large cytoplasmic

vacuoles were manifest. Some cells showed absent nuclei (Figure 4C).

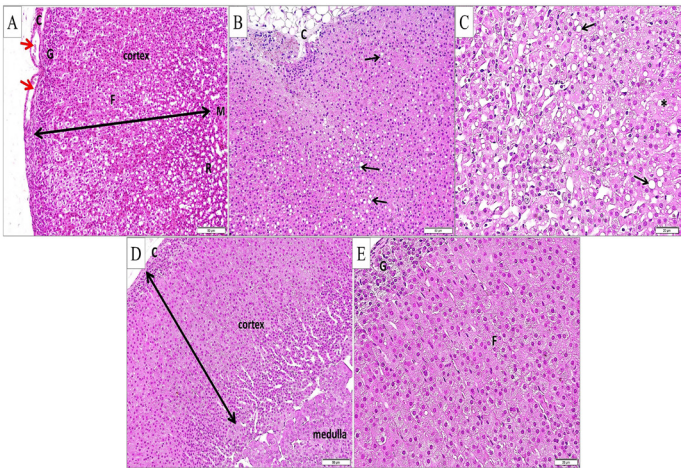

Figure 4: (A, B and C) Adrenal gland from depression group (H and E stain A and B X200 and C X400, respectively), showing. (A) Cortical zones; glomerulosa (G), fasciculata (F) and reticularis (R) and the medulla (M), with apparent increase of cortical thickness and apparent intracapsular cavities (red arrows). B) Disorganization of cortical zones and fat deposition in the capsule (c). (C) Hypertrophied cells in zona fasciculata with large cytoplasmic vacuoles (black arrows). Some cells show absent nuclei (*). (D and E) Adrenal gland from the depression exercise group (H and E stain X200and X400 respectively) with restoration of the normal adrenal architecture and normal cellular organization in zona glomerulosa (G) and fasciculata (F).

Sections obtained from the depression-exercise group (group IV) showed reduction of the thickness of adrenal cortex compared to that of the depressed rats (group III). Apparent restoration of the normal architecture was manifest. The cells in ZG arranged in arches beneath the capsule and that of ZF were found in cords. The cells appeared polyhedral acidophilic with tiny cytoplasmic granules as that of the control group (Figure 4E).

Immunohistochemical study

Caspase 3 immunoreactivity

Examination of the immunohistochemical reaction of caspase 3 stained sections of control group revealed immune-positive cells with its cytoplasmic reaction located mainly in ZG and dispersed throughout ZF (Figure 5A). Sections obtained from the control exercise group showed mild increase of the caspase 3 positive cells in both ZG and ZF (Figure 5B).

In the depression group there was extensive increase in the caspase 3 positive cells with intense cytoplasmic reaction. Many immune-positive cells were seen in the ZG, whereas aggregates of the immune-positive cells appeared throughout the ZF (Figure 5C).

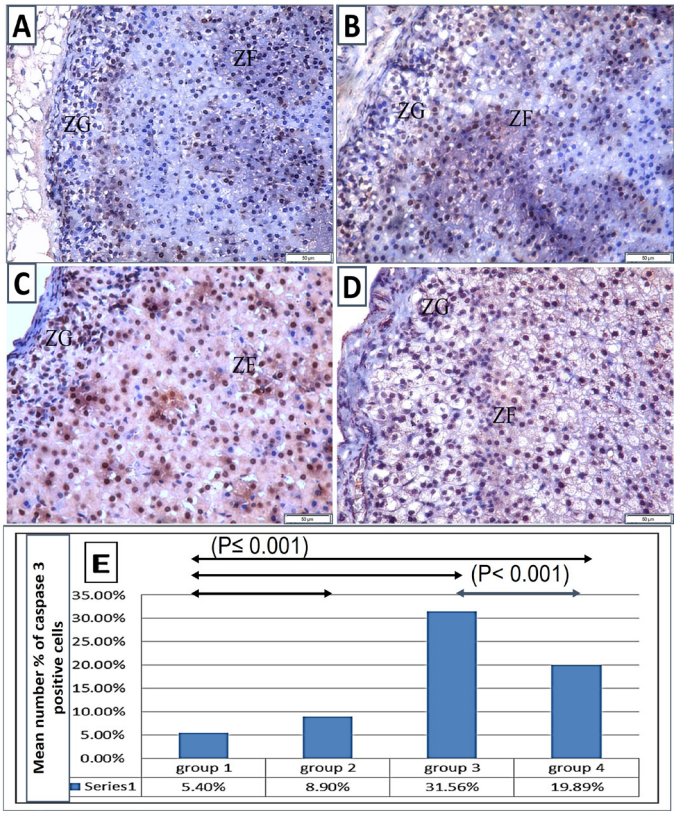

Figure 5: The immunohistochemical reaction of caspase 3 stained sections (X 400): (A) control group showing immune-positive cells with cytoplasmic reaction located mainly in zona granulosa and dispersed throughout zona fasciculata. (B) Control exercise group showing mild increase of the caspase3 positive cells in both zona glomerulosa and zona fasciculata. (C) Depression group showing extensive increase in the caspase3 positive cells in the zona granulosa, and aggregates of the immune-positive cells throughout the zona fasciculata (thick arrows). (D) Depression exercise group showing obvious reduction of immunoreactive cells in comparison to the depression group. The cells showed intense reaction and are dispersed in the zona glomerulosa and zona fasciculata (E) The mean numbers % of caspase 3 positive cells in different groups. Note: ZG, zona granulosa; and ZF, zona fasciculata.

Examination of sections of depression exercise group showed obvious reduction of immunoreactive cells in comparison to the depression group. The positive cells showed intense reaction and were dispersed in the ZG and ZF (Figure 5D).

There was significant difference in the mean number % of caspase 3 positive cells between all experimental groups in comparison to the control (P≤0.001) and between the depression and depression exercise group (P<0.001) (Figure 5E).

PCNA immunoreactivity

Examination of control sections showed few sporadic

immunoreactive PCNA positive cells with nuclear reaction in the ZG and ZF (Figure 6A). In control exercise group, apparent increase of the PCNA positive cells was obvious. The cells were dispersed in ZG and ZF (Figure 6B).

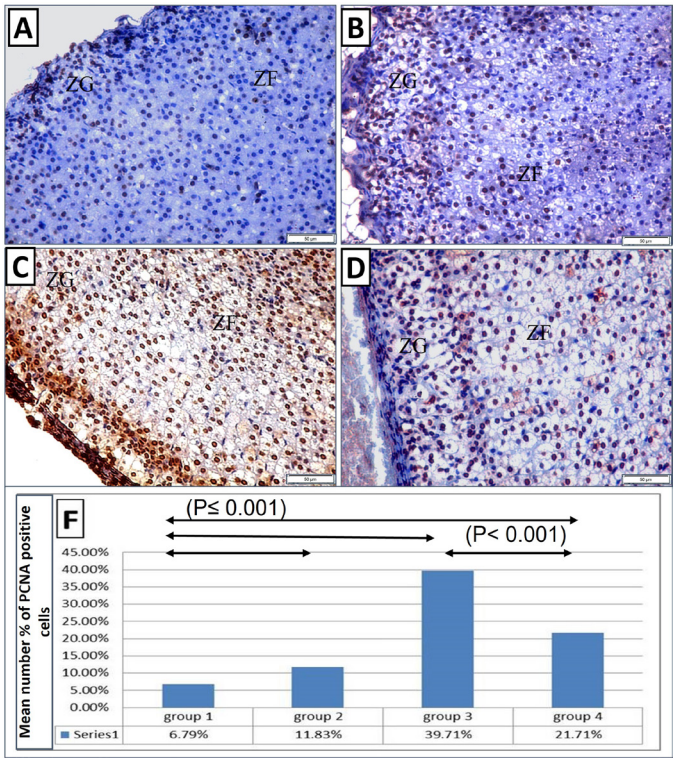

Figure 6: The immunohistochemical reaction of PCNA stained sections (X 400): (A) control group showing few sporadic immunoreactive PCNA positive cells with nuclear reaction in zona glomerulosa and zona fasciculata. (B) Control exercise group showing obvious increase of the PCNA positive cells in zona glomerulosa and zona fasciculata. (C) Depression group showing enormous increase of the PCNA positive cells with intense nuclear reaction in zona glomerulosa, at the junction between in zona glomerulosa and zona fasciculata and dispersed throughout zona fasciculata. (D) Depression exercise group showing less PCNA positive cells than those in the depression group, with the stained cells dispersed in zona glomerulosa and zona fasciculata. (E) The mean numbers % of PCNA positive cells in different groups.

Sections of the depression group revealed enormous increase of the PCNA positive cells with intense nuclear reaction in the ZG, at the junction between ZG and ZF, and dispersed throughout the ZF (Figure 6C, D).

The PCNA positive cells in the depression exercise group appeared less than those in the depression group and were dispersed in ZG and ZF (Figure 6E).

The differences in mean number % of PCNA positive cells were significant between all experimental groups in comparison to the control (P ≤ 0.001), and between the depression group and depression exercise group (P < 0.001) (Figure 6F).

It is conspicuous that, there was significantly higher mean number of the proliferative cells than that of apoptotic cells in the depression group (P= 0.003).

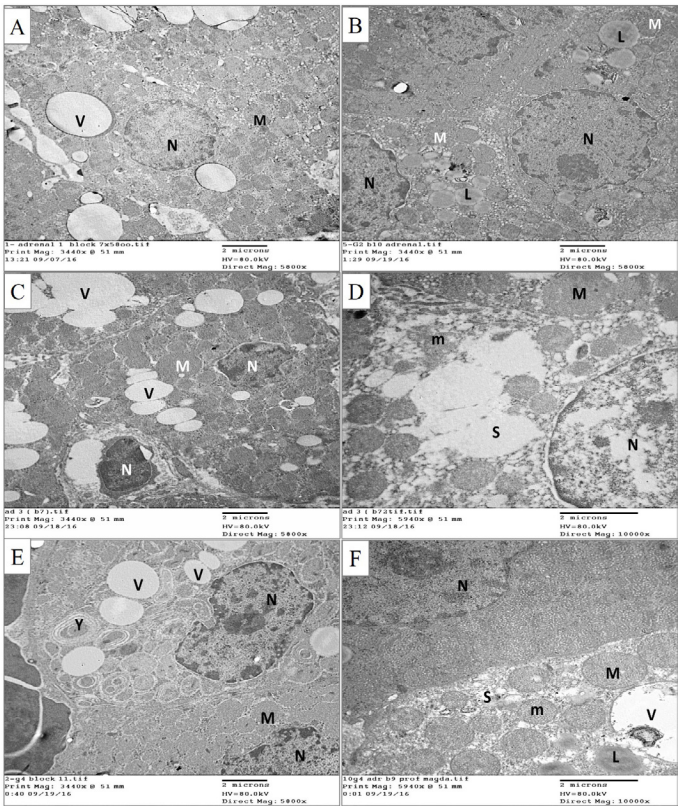

Figure 7: Electron microscopic pictures for the zona fasciculata cells of the adrenal cortex from different groups. (**A**) Cells of the control group showing rounded regular nucleus (N) with dispersed chromatin, multiple vesicular mitochondria (M) and vacuoles (V). (**B**) Cells of the control exercise group, showing rounded nuclei (N) with dispersed chromatin, multiple vesicular mitochondria (M). Notice the multiple vacuoles with lipid content (L). (**C**) Cells of the depression group showing shrunken irregular nuclei (N), swollen mitochondria (M) and multiple vacuoles of different sizes (V). (**D**) Cells of the depression group showing some swollen mitochondria (M), mitochondria with destructed cristae (m) and dilated vesicular smooth endoplasmic reticulum (S). (**E**) Cells of the depression exercise group showing normal sized nuclei (N) with irregular contour and multiple normal vesicular mitochondria (M). Few vacuoles (V) and lysosomes containing substance (Y) appear in the cytoplasm. (**F**) Cell from the depression exercise group showing normal nucleus (N), mitochondria with normal size and cristae (m) and nearly normal smooth endoplasmic reticulum (S). Few mitochondria appear dilated (M). Few vacuoles (V) appear with some showing lipid content (L). A, B, C and E x5800, and D and F x10000 - scale bar 2µ.

Electron microscopic study

The cells from zona fasciculata of adrenal cortex of the control group had central rounded regular nuclei with dispersed chromatin. The cytoplasm was full of multiple vesicular mitochondria. Vacuoles with lipid content were seen (Figure 7A). Cells of the adrenal cortex of group II (exercise group) showed multiple vesicular mitochondria throughout the cytoplasm and multiple vacuoles of different sizes full of lipid material (Figure 7B).

The cortical cells from depressed rats of group III showed shrunken irregular nuclei which possessed areas of clumped chromatin. The cytoplasm was filled of abundant vacuoles of different sizes with apparent depletion of lipid material. Mitochondria were either swollen or destructed and smooth endoplasmic reticulum was extremely dilated (Figure 7C, D).

Cells from the adrenal cortex of group IV showed normal nuclei with irregular contour and prominent nucleoli. Multiple normal vesicular mitochondria were seen in the cytoplasm, while others appeared swollen with disrupted cristae. Decreased cytoplasmic vacuoles with different lipid content were observed and smooth endoplasmic reticulum appeared less dilated than that of the depression group. The cytoplasm showed also 2ry lysosomes (Figure 7E, F).

The current study used the forced swimming protocol for producing an animal model of depression. We studied the plasma corticosterone level and investigated the structure of adrenal cortex. The effect of exercise on adrenal cortex activity was also evaluated in both normal and depressed rats. Previous evidences suggested that regular exercise has a positive impact on the brain and has an antidepressant and anxiolytic effects (Salmon, 2001; Binder et al., 2004; Algaidi et al., 2019). It has been suggested that exercise training revealed behavioural changes in both healthy subjects and patients. Exercise increases mood in normal subjects and has antidepressant effect in depressed patients (Dimeo et al., 2001).

Neuroendocrine studies have found variations in cortisol levels with depression (Shapira et al., 2000; Schatzberg and Lindley, 2008). However, although these biological changes are among the well-established researches in psychiatry, their implications on the structure of adrenal cortex are still unclear. Our results showed significant increase in serum corticosterone level in depressed group compared to other groups. Also, corticosterone level was significantly higher in depression-exercise group compared to control-exercise group. Recent studies have reported a significant increase of corticosterone level in depressed patients compared to controls (Burgese and Bassitt, 2015). Others found an overall effect of exercise on baseline plasma corticosterone levels and added that, corticosterone level was lowest in combined exercise and antidepressant treatment (Droste et al., 2006).

The current study revealed that all rats gained weight at the end of the experiment but there were no significant differences between different groups. Similar results were found by other studies (Droste et al., 2006). Increased food intake in exercise groups and decreased appetite in depressed rats, observed in the current study, may have a role in modulating the effect of corticosterone level on weight. Saraiva et al. (2005) explained that chronically stressed animals had high levels of circulating corticosteroids and predominance of depressive behaviors, such as decreased locomotion and reduced exploratory attitudes that lead to anorexia.

The present work showed highly significant increase of thickness of adrenal cortex in the depressed group compared to other groups. Positive correlation was detected between corticosterone level and cortical thickness. A large literature has revealed elevated plasma corticosterone levels and adrenal enlargement in depressed patients (Amsterdam et al., 1987; Rubin et al., 1995; Kessing et al., 2011). Moreover, many studies demonstrated increases in adrenal weight in individuals who have committed suicide (Korovini-Zis and Zis, 1987; Dumser et al., 1998; Arpita and Manjari, 2014). Previous researches found a positive correlation between adrenal weight and total cortical thickness providing direct evidence that increased adrenal weight in suicide victims is due to cortical hypertrophy (Szigethy et al., 1994; Willenberg et al., 1998).

Previous studies postulated that increase in adrenal weight is a well-known finding after exercise (Kjaer, 1998) and that exercise increased the size of both cortex and medulla producing an overall increase in adrenal size (Droste et al., 2006). Our study showed insignificant increase of cortical thickness in exercise groups (control-exercise and depression-exercise groups) which might be attributed to the size of the sample. Droste et al. (2006) observed a contradiction between decreased baseline levels of circulating corticosterone with increased adrenal size in exercising mice and explained this finding as the two parameters are incomparable. They attributed the changed hormone level to the adrenal gland's secretory activity while the size of the gland provides only an indication to its secretory potential (Droste et al., 2006). The decreased glucocorticoid responses after voluntary running could be attributed to the evident changes at different levels of the hypothalamic-pituitary-adrenal (HPA) axis and the decreased anxiety related behavior with long term voluntary exercise in both mice and humans (Salmon, 2001; Droste et al., 2003; Reul and Droste, 2005).

The histological and ultrastructural effects of both depression and exercise on the adrenal cortex were hardly found in the literature. This work may be the first study that focused on these effects in details. Many studies found clear evidence that the elevated glucocorticoid levels resulted in mood and cognition disorders, neurophysiological alterations, variations in the activity of neurotransmitters and neuroanatomic changes (Sapolsky et al., 1985, 1986). Light microscopic studies done on adrenal cortical cells showed that even in the normal unstressed mice, adrenocortical cell cytoplasm may appear vacuolated to varying degrees as part of the diurnal functional activity of the cortical cells but can increase secondary to disruption of normal steroidogenesis (Rosol et al., 2001).

Structural examination of the adrenal cortex in this study revealed that exercise has a stated effect even on control rats. The zona fasciculata cells appeared hypertrophied with vacuolated cytoplasm. The vacuoles were of different sizes and containing lipid material denoting increased secretory activity of the gland secondary to exercise. In depressed rats, there were increase of the cortical thickness and hypertrophy of zona fasciculata cells compared to control rats which resembled the initial cortical response to stress. The capsule showed intracapsular spaces and fat deposition. Some disorganization of the cortical cells appeared with some shrunken nuclei and multiple cytoplasmic vacuoles with depletion of lipid material denoting hypersecretion of glucocorticoids. Dilated smooth endoplasmic reticulum was also observed denoting hyperactivity of the adrenal cortex.

In a line with the previous structural changes, immunohistochemical study in the current work demonstrated enormous increase of caspase 3 positive cells in the depression group in comparison to other groups. Sadek et al. (2021) who observed similar results in rats exposed to chronic unpredictable mild stress protocol explained that caspases are a family of cysteine protease enzymes that act as death effector molecules in apoptosis, and proposed that chronic stress might lead to activation of certain processes that would end in cell apoptosis through activation of caspases.

In the present study and through counting of the proliferative cells (PCNA stained cells) and the apoptotic cells (caspase 3 cells), It was noticeable that the mean number of proliferative cells in depressed rats was significantly higher than that of the apoptotic cells. This finding might explain the hyperplasia and increased cortical thickness found in depression group in this study. In accordance with our results, Ulrich-Lai et al. (2006) demonstrated increased positive Ki67 cells in the outer ZF as a marker for increased cell division and hyperplasia in an animal model of chronic variable stress which possess many behavioral and biochemical characteristics similar to human depression. Also, Zaki et al. (2018) explained the increase in the proliferating cells in the adrenal cortex of adult and senile stressed rats as the cellular turnover in the adrenal cortex occurs only to accommodate the physiological needs.

Some researchers considered that the increase in adrenal weight is a better general indicator of stress than circulating corticosteroids (Creasy et al., 2013). The hypertrophy and hyperplasia of adrenal cortex are evident causes of increased adrenal weight and thickness (Harvey and Sutcliffe, 2010; Patra et al., 2015) that indicate activation of the HPA axis and reflect the overproduction of glucocorticoids (Hayashi et al., 2014).

Depressed rats performing exercise showed apparent improvement of the histological and ultrastructural manifestations compared to the depressed rats. The cortical cells appeared organized, the cytoplasmic vacuoles decreased, and the dilatation of smooth endoplasmic reticulum were less than in depression group.

The dual action of exercise on both normal and depressed rats was assessed immunohistochemically. This study showed significant increase of caspase 3 immunoreactive cells in group II. Phaneuf and Leeuwen (2001) postulated that exercise induces apoptotic cell death in tissues exposed to specific stresses as calcium, glucocorticoids and radicals, and this may be a normal process used to remove partially damaged cells. In addition, exercise group showed significant increase of PCNA positive cells. Steiner et al. (2021) stated that exercise is considered as an acute physiological stress that initiates a multiple processes aimed to restore physiological homeostasis and subsequent adaptation. The most important component of the stress response to exercise is the rapid release of hormones from the adrenal gland, including glucocorticoids. The secretion of adrenal stress hormones during exercise is short-lived, and the circulating levels rapidly return to baseline values within 1-3 hours post-exercise in the post-exercise recovery period (Steiner et al., 2021).

On the other hand, the depressed rats performing exercise in group IV showed significant reduction of both apoptotic and proliferative cells in comparison to group III. Seo et al. (2016) suggested that voluntary exercise could suppress apoptosis in chronic restraint stressed mice as indicated by suppression of caspase-3 protein expression. One of the theories which may explain this finding is that voluntary exercise could increase the level of the anti-apoptosis Bcl-2 protein expression (Mokhtari-Zaer et al., 2014). However according to Seo et al. (2016) the increase of Bcl-2 is not a fixed result in different studies, so its role must be evaluated in future researches.

One of the distinctive findings observed in this study is the allocation of the proliferative cells at the junction of ZG and ZF and in the outer ZF. This finding may be explained according to one of the theories mentioned by (Bozzo et al., 2011) who reported that the cellular replacement in different adrenal cortical zones could be assigned to the transformation theory which proposed that the cells proliferate in an intermediate zone between ZG and ZF then the cells migrate towards both the capsule and the medulla.

A balance between apoptotic and proliferative cells were noticed in both exercise groups (group II and IV). According to (Bozzo et al., 2011) the adrenal gland shows dynamic histological changes in the form of cellular death and cellular proliferation, and the balance between those two processes must be achieved to ensure normal structure and function of adrenal gland.

Thus, our results indicated the ameliorating effect of voluntary exercise on the adrenal cortical structure, the integrity of the adrenal cells, and the regulation of apoptotic and proliferative processes. These results explain the decreased corticosterone level in the exercise group.

CONCLUSIONS AND RECOMMENDATIONS

We concluded that depression has manifest structural changes on the adrenal cortex which is in line with the elevated plasma corticosterone level. Exercise has structural implications on both control and depressed rats. Although it increased the activity of the gland, it did not cause hypersecretion of corticosterone into circulation thus adjusting its plasma level. Allowing patients with depression to practice voluntary exercise can help improving their condition through structural alleviation of adrenal cortex and reduction of corticosterone secretion into the plasma.

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NOVELTY STATEMENT

The authors confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

AUTHOR'S CONTRIBUTION

All authors contributed to the study notion, design, material preparation, investigations, data collection and analysis. All authors read, and approved the final manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

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