Original Article

# Effects of Chronic Administration of Antipsychotic Drug (Olanzapine) on the Anterior Pituitary Gland of Adult Male Albino Rats and the Potential Protective Role of Hypericum Perforatum

*Eman S. El-Roghy and Reham Abdallah Elbarbary*

*Department of Histology, Faculty of Medicine, Menoufia University, Shebin el Kom, Egypt*

## ABSTRACT

**Introduction:** Olanzapine (OLZ) is an atypical antipsychotic drug, has been used as approved therapy for schizophrenia and bipolar disorder. Hypericum perforatum (HP) is a herbaceous plant, has been described as a neuroprotective agent with potent antioxidant and anti-inflammatory effects.

**Objectives:** To evaluate the effects of chronic use of olanzapine on the anterior pituitary gland and the possible protective role of hypericum perforatum

**Material and method:** Fifty six adult male albino rats were divided into 4 groups, received treatment orally for 8 weeks; (I) control group, (II) HP supplemented group (80 mg/kg/day), (III) OLZ treated group (10 mg/kg/day), (IV) OLZ and HP group (10 mg of OLZ and80 mg of HP/kg/day) . Finally, rats were weighted, anesthetized and sacrificed. Blood samples were collected for biochemical study. Pituitary gland were dissected out and processed for histological study.

**Results:** Olanzapine treated rats exhibited a high significant increase in rat`s body weight. Plasma levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were significantly decreased but thyroid stimulating hormone (TSH) and prolactin hormone were increased. OLZ induced degeneration of pars distalis cells. Where, cells had small dark nuclei and cytoplasmic vacuolization. Eosinophilic material deposition and congestion of blood sinusoid were noticed. Most chromophiles contained degenerated mitochondria and dilated rough endoplasmic reticulum. Furthermore, there was a high significant increase in the number of positive caspase-3 immune-stained cells. Hypericum perforatum co-administration induced improvement of these changes occurred by OLZ.

**Conclusion:** Olanzapine induced morphological and biochemical changes in the anterior pituitary gland. Hypericum perforatum could ameliorate these changes.

**Received:** 17 September 2022, **Accepted:** 17 November 2022

**Key Words:** Caspase-3; EM; hypericum perforatum; olanzapine; pituitary gland.

**Corresponding Author:** Eman S. El-Roghy, MD, Department of Histology, Faculty of Medicine, Menoufia University, Shebin el Kom, Egypt, **Tel.**: +20 10 0977 4722, **E-mail:** e.shehata27@yahoo.com

**ISSN:** 1110-0559, Vol. 47, No. 1

## INTRODUCTION

Antipsychotic medications have unique efficiency in the treatment of numerous mental illnesses such as schizophrenia and other chronic psychoses[1]. Olanzapine (OLZ) is a second-generation atypical antipsychotic drug that has been increasingly used as approved therapy for schizophrenia and bipolar disorder. Also, it is used as an adjunctive therapy for other disorders as obsessive–compulsive disorder (OCD), depression, mania, insomnia, and post-traumatic stress di=sorders[2]. OLZ efficiency is superior to that of the older antipsychotic drugs due to its minimal side effects on extra pyramidal tract[3]. OLZ is a serotonin-dopamine receptor antagonist. It has binding affinities to many neurotransmitter receptors, including dopamine receptors, serotonin receptors, histamine H1 receptor, muscarinic receptors, alpha α and beta β-adrenergic receptors[4]. These receptors are widely distributed throughout the body in different organs. Dopaminergic D2 receptors are most abundant in anterior pituitary and brain. Stimulation of these receptors modulates synthesis and secretion of hormones in the pituitary gland[5]. Long-term usage of atypical antipsychotics causes some adverse effects such as weight gain, insulin resistance, hyperlipidemia and cardiovascular diseases[6,7].

Hypericum perforatum (HP) is a herbaceous perennial plant that belongs to the Hypericaceae family and is commonly known as St. John's wort. HP has a worldwide distribution and has been used in the traditional medicine for treatment of wounds, hemorrhoids, menstrual problems and kidney stones. It is considered the only herbal alternative to synthetic antidepressants. So it is described as a neuroprotective agent used in the treatment of anxiety and mild to moderate depression[8]. HP is a highly effective antidepressant with potent antioxidant and anti-inflammatory effects; therefore it is effective for the treatment of the pathological changes induced by antipsychotic drugs[9].

The best of our knowledge is that the effect of OLZ on the structure of anterior pituitary is not studied before. Considering that olanzapine act on the dopaminergic receptor and this receptor has been detected in the anterior pituitary. So, we investigated the Olanzapine possibility

 *DOI: 10.21608/ejh.2022.163482.1776*

to induce structural changes in the anterior pituitary gland and if so, the possibility of HP to play a protective role, as regards its antioxidant activity and it is already used as an antidepressant treatment.

## MATERIALS AND METHODS

### *Chemicals*

Olanzapine (ZyprexaTM) was purchased from Eli Lilly pharmaceutical Company (Indianapolis, Indiana 46285 USA) and available in form of tablets each contains 10mg Olanzapine.

Hypericum perforatum (Safamood) was purchased from Atos for Production of Medicinal Herbs (Atos Pharma, El Salam City, Cairo, Egypt). It is commercially available in form of tablets. Each contains 250mg of Hypericum perforatum. Both drugs have property to dissolve in distilled water. The drugs were freshly prepared and administered orally by oral gavage at the same time daily in the morning.

### *Animals*

Fifty six adult male albino rats were used in the study. Their age ranged from 100–110 days old and they were 180-200 gm weight. The rats were habituated to their environment for one week before the beginning of the experiment. Rats were housed in clean, properly ventilated cages at the same environmental conditions, and were fed on a standard diet and had ad libitum access to tap water. All procedures conducted in accordance with the Guide of Ethical Committee for Scientific Research at the Faculty of Medicine, Menoufia University.

### *Experimental design*

Rats were divided randomly into four equal groups (14 rats each). The duration of the experiment was 8 weeks.

**Group I:** (Control group): Rats received distilled water orally in a volume equivalent to that used to dissolve the drugs by oral gavage once daily.

**Group II:** (HP supplemented group): Rats received Hypericum perforatum at a dose of (80 mg/kg/day) dissolved in distilled water by oral gavage once daily for 8 weeks. This dose was equivalent to human therapeutic dose[10]. Each rat received 1 ml of a solution formed by dissolving 250 mg of Hypericum perforatum in 15 ml distilled water.

**Group III:** (OLZ treated group): Rats received Olanzapine at a dose of (10 mg/kg/day) dissolved in distilled water by oral gavage once daily for 8 weeks .This chosen dose was identical to those inducing an antipsychotic effect in rats models for schizophrenia[11]. Each rat received 2 ml of a solution formed by dissolving 10 mg of OLZ in 10 ml distilled water.

**Group IV:** (OLZ and HP group) Rats received a combination of Olanzapine (10 mg/kg/day) and Hypericum perforatum (80 mg/kg/day) by oral gavage once daily for 8 weeks.

### *Determination of the animal's body weight*

Rats were weighed at the end of the experiment. Then, all parameters of animal's body weights were calculated for statistical analysis

### *Blood samples for biochemical study*

At the end of experiment all animals were anaesthetized at the next morning following last doses by intra peritoneal injection of phenobarbital sodium at a dose of 40 mg / kg bw.[12]. Blood samples were collected from retro-orbital venous plexus into a clean glass tubes without anticoagulant. Samples were centrifuged at a speed of 4000 rpm for 10 min at 4 °C by using a cooling centrifuge (Beckman model L3-50, USA) and stored at − 20 °C until use . The sera were used to assess the hormones of anterior pituitary gland: growth hormone (GH); prolactin hormones; Thyroid stimulating hormone (TSH); luteinizing hormone (LH); follicle stimulating hormone (FSH) and adrenocorticotrophic hormone (ACTH), Using a commercially available ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. This procedure was carried out in the central lab at Faculty of Medicine, Menoufia University.

### *Tissue sampling*

Skulls of animals in all groups were carefully dissected. The whole pituitary gland was excised carefully from each rat, and then they washed with normal saline. In each group half numbers of pituitary gland were used for light microscopic study and the other half for transmission electron microscopic study.

### *Histological study*

#### Light microscopic study

The specimens were fixed in Bouin's solution for three days, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, impregnated in soft paraffin followed by hard paraffin and sectioned in 5-µm-thickness. Section were stained with hematoxylin and eosin (H&E) staining for routine histological examination[13]. Toluidine blue (TB) staining; sections were obtained from the semi-thin sections of 1 µm thick during the electron microscopic preparation[14].

#### Transmission Electron Microscopic (TEM ) study

Tiny pieces of 1x1 mm² were obtained from each pituitary gland, fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetraoxide, dehydrated and embedded in epoxy resin. Semi thin sections of 1 µm thick were stained by toluidine blue. Ultrathin sections (70–90 nm) were contrasted with lead citrate and uranyl acetate[14]. All sections of all groups were examined by using transmission electron microscope (Seo-Russia) in Tanta E.M Unite at Faculty of medicine, Tanta University, Egypt.

#### Immunohistochemical technique for Caspase-3

Caspase-3 immune- staining (an apoptotic marker)

was performed on 4-mm thick Paraffin sections by using streptavidin–biotin complex technique[15]. Serial sections were deparaffinized on charged slides, then, incubated in 0.1% hydrogen peroxide for 30 min to block the endogenous peroxidase. The sections were incubated overnight at room temperature with primary antibodies of anti-caspase-3; mouse monoclonal antibody (Catalog number: C9598; Lab Vision, Fremont, CA USA) for 60 min. Finally, the slides were washed with diluted phosphate- buffered saline (PBS) and incubated with the secondary anti-mouse antibody "universal kits" for about 30 min at room temperature. In each run the controls slides were included. The positive control slide was mouse appendix . Negative controls were prepared by exclusion of primary antibody. After that, the sections were counterstained by Mayer's haematoxylin stain, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and mounted in Canada balsam.

### *Morphometrical study*

All measurements were done for 10 randomly selected non-overlapped sections from each group at the same magnification. The study was carried out by using Leica Qwin 500 LTD software image analysis computer system (Cambridge, England) at the Department of Histology, Faculty of Medicine, Menoufia University. The following parameters were calculated: the percentage of chromophobes per field (TB x1000) and the average number of positive caspase-3 immune-stained pars distalis cells per field (caspase-3 x400).

### *Statistical analysis*

Data were statistically analyzed by SPSS, version 20 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean and SD and analyzed by using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test for comparison between control group and other groups. *P value* > 0.05 was considered non-significant and *P values*< 0.05 were considered statistically significant but *P values* < 0.001 were considered high significant results[16].

## RESULTS

### *General observations*

- Two rats in group III and one rat in group IV died before the end of the experiment
- Group I and II had identical histological and immunohistochemical results.

Analysis of body weight: Olanzapine treated group (group III) exhibited a high significant increase in rat`s body weight (*P value*<0.001) when compared to the control group. While group IV (Olanzapine and HP group) showed a significant increase in body weight ($P < 0.05$) when compared to the control (Table 2 and Histogram 1).

### *Biochemical Results*

Olanzapine treated group (group III) revealed a high significant decrease (*P value*<0.001) in the mean plasma level of LH and FSH hormones and a significant increase ($P < 0.05$) in the plasma level of TSH and prolactin hormones. Meanwhile, there were no significant statistical differences (*P value*>0.05) in the levels of GH and ACTH hormones comparing to control group.

OLZ and HP group (group IV) showed none significant differences (*P value*>0.05) in the plasma level of GH, ACTH, TSH, and prolactin hormones compared to control group. But levels of LH and FSH hormones revealed a significant reduction ($P < 0.05$) when compared to control group. (Table 1).

### *Histological result*

#### Hematoxylin and eosin staining

Control group and HP supplemented group (group I and II) showed normal morphology of the pituitary gland that formed of three parts; pars nervosa, pars intermedia and pars distalis(Figure 1A). Pars distalis was formed of anastomosing cords of cells separated by numerous blood sinusoids. Chromophils exhibited darkly stained cytoplasm. There were two main populations of chromophils; acidophils and basophils. Acidophils appeared polygonal cells with acidophilic cytoplasm arranged in cords around the blood sinusoid. Basophils were large cells with light basophilic or purple cytoplasm. They were arranged in scattered clusters. Chromophobes appeared with pale staining cytoplasm scattered throughout the two cells types of chromophils (Figure 1B).

Chronic administration of Olanzapine in group III displayed considerable morphological changes in the arrangement and structure of anterior pituitary gland (Figure 1C). Where, cells of pars distalis appeared amalgamated with inability to distinguish between the cells types. Meanwhile many cells had small dark nuclei and other showed vacuolated cytoplasm. Eosinophilic homogenous material was noticed between the cells cords of anterior pituitary(Figure 1D). Blood sinusoid appeared irregular and dilated, even some appeared congested and surrounded by few cellular infiltration (Figure1E ).

Combination of Hypericum Perforatum with Olanzapine in group IV achieved high preservation of normal histological feature of the anterior pituitary gland. Pars distalis cells restored their histological feature and normal rearrangement but some cells still contained small dark nuclei. Slight dilatation of blood sinusoids was still observed (Figure 1F).

#### Toluidine blue staining

Semi-thin sections of anterior pituitary gland of control and HP supplemented groups (group I and II) demonstrated regular arrangement of pars distalis cells into clusters around typical blood sinusoids. Number of chromophiles was observed such as somatotrophs that appeared with light cytoplasm and prominent nucleoli, thyrotrophs with elongated nuclei, corticotrophs; large angular basophilic cell and gonadotrophs; large rounded with deep basophilic

granules. Concerning chromophobes, they appeared light cells with no cytoplasmic granules (Figure 2A).

Olanzapine treated group (group III) revealed shrunken degenerated pars distalis cells. Cells had deeply stained cytoplasm and dark nucleus. Numerous chromophobes were noticed in sections of this group with margination of their nuclear chromatin. Meanwhile, widening of the inter-cellular spaces was observed. Blood sinusoids were markedly dilated and congested (Figure 2B).

OLZ and HP group (group IV) showed normal cells of pars distalis, more or less identical to those of control group but some cells show darkness of their nuclei. Slight dilatation of blood sinusoids was still occurred (Figure 2C).

**Transmission electron microscopic results**

Control and HP supplemented groups (group I and II)

With electron microscope, cells of pars distalis of control group and HP supplemented groups appeared well organized. As for chromophils, they clarified distinctive secretory granules that characterized each cell type. The shape, size and position of the granules help to identify each type of chromophils. Somatotrophs appeared polygonal in shape and represent the main bulk of the chromophils. Somatotrophs had euchromatic nuclei with prominent nucleoli. The cytoplasm was densely packed with numerous specific secretory granules. The granules were multiple large, spherical and electron–dense. The cytoplasm contained few strands of rough endoplasmic reticulum (rER). Other cytoplasmic organelles were not prominent as the granules occupied all cytoplasm (Figure 3A).

Gonadotrophs appeared large rounded cells with euchromatic nuclei. They had electron dense secretory granules that appeared variable in size and smaller than that of somatotrophs. The cytoplasm contained numerous rod shaped mitochondria and rER (Figure 3B).

Corticotrophs were stellate medium sized cells with expressed long cytoplasmic process. They had large central euchromatic nuclei and pale electron lucent cytoplasm containing rode shaped mitochondria. Their secretory granules were small, uniform, dense and rounded granules concentrated mainly at the periphery, arranged just beneath the cell membrane (Figure 3C).

Thyrotrophs were large elongated cells. They contained oval euchromatic eccentric nuclei with prominent nucleoli. The inner surface of the nuclear membrane had a rim of condensed heterochromatin. The cytoplasm exhibited long dense numerous mitochondria & rER. Their granules were small uniform spherical, electron-dense and were located mainly at the periphery of the cytoplasm (Figure 3D).

Mammotrophs were large triangular cells. The cytoplasm contained numerous mitochondria and well developed profiles of rough endoplasmic reticulum. Their cytoplasmic granules appeared pleomorphic in shape, electron-dense, variably sized almost large granules and mainly collected near the nucleus. The nuclei appeared eccentric and euchromatic (Figure 3E).

As for chromophobe cells appeared large cell and contained oval large euchromatic nucleus, rER and numerous mitochondria. The cytoplasm was free from specific granules (Figure 3F).

**OLZ treated group (group III)**

Ultra structurally, sections of the pituitary gland of OLZ treated group (group III) exhibited distortion and disorganization of the cells of anterior pituitary with observable degenerative changes in most cells of pars distalis. Meanwhile, this group showed increase in the number of chromophobes. The blood sinusoids were highly congested and dilated (Figure 4A). Concerning the gonadotrophs, the most obvious changes were in their mitochondria where, most of them were vacuolated and swollen with loss of the internal cristae, some gonadotrophs were apparently hypertrophied and contained indented irregular nuclei with fragmentation and margination of the nuclear chromatin and others had shrunken electron-dense degenerated nuclei. The cytoplasm was filled with numerous small, ovoid vacuoles. Their granules were scanty and scattered between these vesicles. The rough endoplasmic reticulum exhibited marked dilatation (Figure 4B). Additionally, chronic administration of olanzapine led to remarkable changes in thyrotrophs. Where, their granules were apparently increased in number and size compared to control group. Moreover, their cytoplasm contained irregular nucleus and dilated rER (Figure 4C). Regarding the mammotrophs they appeared with indented euchromatic nuclei, and the cytoplasm showed marked rarefaction and vacuolization due to marked dilatation of their rER. Their mitochondria revealed obvious degeneration (Figure 4D).As for corticotrophs, they exhibited some changes. Their cytoplasmic process became short. The nuclei appeared irregular with clumps of heterochromatin (Figure 4E).The somatotrophs of this group showed slight changes. Where, their cytoplasm contained numerous specific secretory granules, mildly degenerated mitochondria and dilated rER (Figure 4F).

**OLZ and HP group (group I V)**

Ultrathin sections of OLZ and HP group exhibited restoration of the normal structure of most pars distalis cells. The somatotrophs had euchromatic nuclei and their cytoplasm contained few strands of rER, and numerous secretory specific granules like those of control group (Figure 5A). The thyrotrophs had the identical ultra-structure. They contained euchromatic nuclei with prominent nucleoli, rER, mitochondria and small secretory granules (Figure 5B). Features of corticotrophs were more or less similar to those of the control group (Figure 5C).. Mammotrophs had eccentric euchromatic nuclei with mild irregularity. Their cytoplasm contained numerous mitochondria, well developed rER and variable shaped granules (Figure .D). On the other hand the gonadotrophs still revealed some changes. They had irregular

euchromatic nuclei with fragmented chromatin. Their cytoplasm contained mildly degenerated mitochondria and slightly dilated rER. But their granules were numerous and variable in size (Figure 5E).

**Caspase-3 immunohistological results**

Control group and HP supplemented group (group I and II) showed negative caspase-3 immune expression of pars distalis cells (Figure 6A). In contrast OLZ treated group (group III) revealed intense positive cytoplasmic caspase-3 immune expression (Figure 6B). But OLZ and HP group (group IV) revealed weak positive caspase-3 immune expression (Figure 6C).

### *Morphometric results*

The percentage of the chromophobes per field: OLZ treated group (group III) revealed a significant increase (*P values* < 0.05) in the percentage of chromophobes compared with the control group. There were no significant statistical differences between OLZ and HP group (group IV) and control groups ($P < 0.05$) (Table 2, Histogram 2).

**Average number of positive caspase-3 immune-stained pars distalis cells per field**

There was a high significant increase(*P values* < 0.05) in the number of positively caspase-3 immune-stained cells in OLZ treated group (group III) as compared with control group.The number of positive caspase-3 cells showed no statistical differences between OLZ and HP group (group IV) and control groups ($P < 0.05$) and control groups (Table 2, Histogram 3).

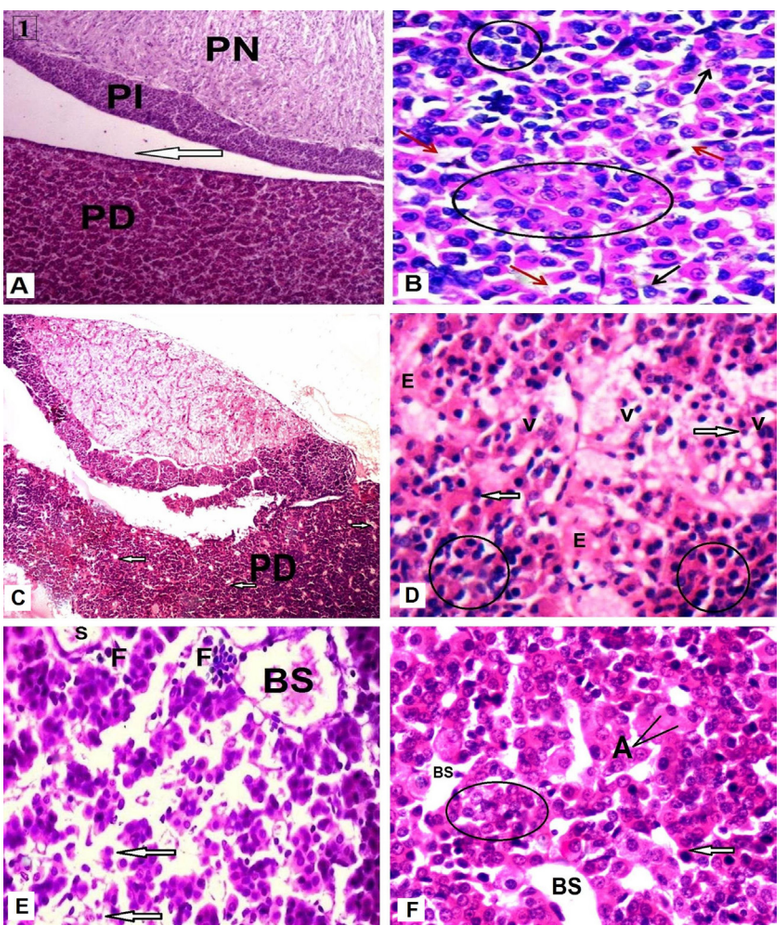



**Fig. 1:** (A–B) A photomicrograph of a section of pituitary gland of control group (group I) showing A): typical organization of pituitary gland with its three parts: pars nervosa (PN), pars intermedia (PI) and pars distalis (PD). (H&E. x 100) B): normal morphological structures of pars distalis cells. Acidophils; polygonal cells with acidophilic cytoplasm arranged in cords (oval shape) around the blood sinusoid (red arrows). Basophils; large cells with light basophilic cytoplasm (purple color) arranged in scattered clusters (circle).Chromophobes; scattered cells with pale staining cytoplasm (black arrows). (H&E. x 1000). (C-E): A photomicrograph of a section of pars distalis of anterior pituitary gland in OLZ treated group (groupIII) showing C): disorganized anterior pituitary gland with obvious dilatation of blood sinusoid (arrows). (H&E. x 100) D): Pars distalis cells appear indistinguishable and amalgamated to each other (circle) and most of them contain small dark nuclei (arrows). Some cells have vacuolated cytoplasm (V) Notice: eosinophilic homogenous material in between the cells of anterior pituitary (E). (H&E. x 1000) E): congested and dilated blood sinusoid (BS) surrounded by cellular infiltration (F). Notice: apparent hypo cellularity of Pars distalis cells (arrows). (H&E. x 1000) (F): A photomicrograph of a section of pars distalis of anterior pituitary gland in group IV (OLZ and HP group) showing typical structure of Pars distalis cells. Acidophils (A) have dark acidohilic cytoplasm and are arranged in cords around regular blood sinusoid (BS). Basophils (B) have light purple cytoplasm and arranged in cluster (oval shape). Notice: small dark nucleus of pars distalis cell (arrow). (H&E. x 1000)

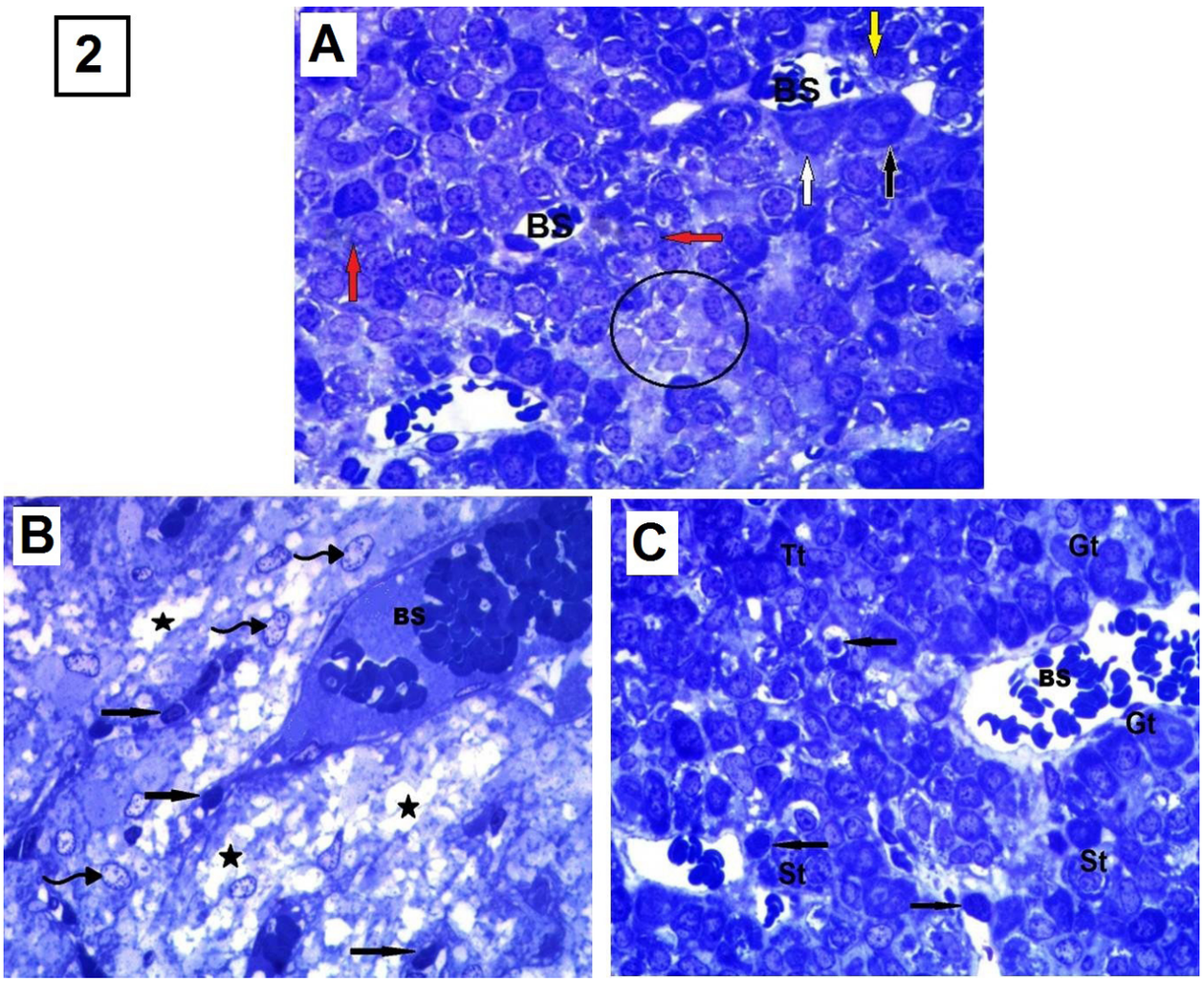



**Fig. 2:** A): a semithin section of the pars distalis of the anterior pituitary gland in control group showing cells of pars distalis regularly arranged into clusters around blood sinusoids (BS). Many chromophobes with no cytoplasmic granules (circle) and a number of chromophils such as somatotrophs (red arrows) with light cytoplasm and prominent nucleoli, thyrotrophs (yellow arrow) with elongated nucleus, Corticotrophs; large angular basophilic cell (white arrow), gonadotrophs; large rounded with deep basophilic granules (black arrow), are noticed. B) a semithin section of pars distalis of anterior pituitary gland of OLZ treated group (group III) showing relative increase in the number of chromophobes with margination of nuclear chromatin (curved arrows). Pars distalis cells appear shrunken degenerated with deeply stained cytoplasm and dark nuclei (arrows). Notice: wide spaces between the cells of pars distalis (star) and markedly dilated congested blood sinusoids (BS). C): a semithin section of pars distalis of anterior pituitary gland of group IV (OLZ and HP group) showing identical chromophiles, such as somatotrophs (St), thyrotrophs (Tt), gonadotrophs (Gt). Notice: dilatation of blood sinusoids (BS) and darkness of some nuclei of pars distalis cells (arrows). (T.B.1000).

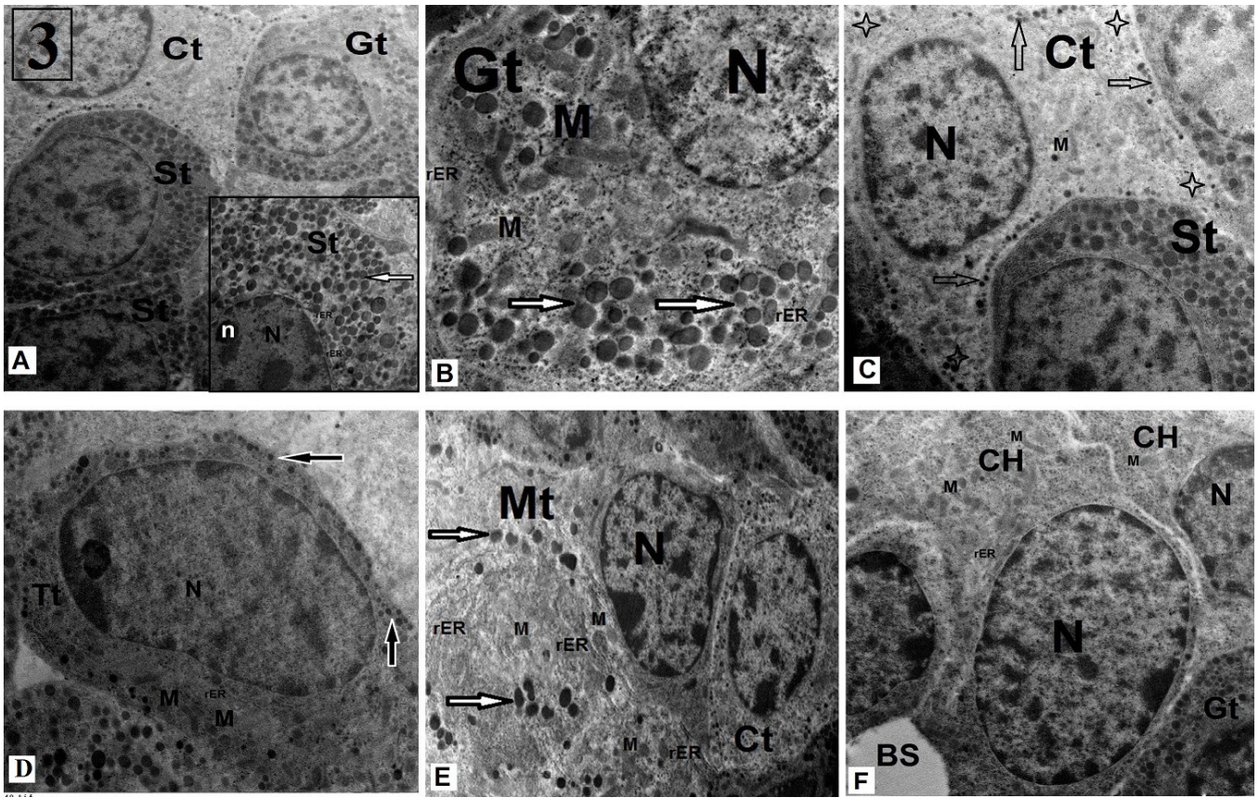



**Fig. 3:** An electron micrograph of ultrathin section of the pars distalis of pituitary gland in control group (group I) showing A):typical polygonal Somatotrophs (St), gonadotrophic cell (Gt) and corticotrophic cell (Ct). (TEM×1500). Inset:showing somatotrophic cell (St) having euchromatic nucleus (N), with prominent nucleolus (n). The cytoplasm is densely packed with multiple spherical larger electron dense granules (arrow) and rough endoplasmic reticulum (rER) (TEM×4000). B): gonadotrophic cell (Gt) with euchromatic nucleus (N) and rER (rER). Numerous rode shaped mitochondria (M) and variable sized electron dense secretory granules (arrows). (TEM×4000).C): corticotrophic cell (Ct); stellate cells with long cytoplasmic process (stars), having rounded euchromatic nucleus (N), pale electron lucent cytoplasm containing rode shaped mitochondria (M) and small uniform, dense and rounded electron dense granules studied the outer part of the cell (arrow) Notice: part of polygonal somatotrophs ((St). (TEM×2500). D): large elongated thyrotrophic cell (Tt). The nucleus (N) appears eccentric oval euchromatic with prominent nucleolus with a rim of peripheral heterochromatin. The cytoplasm contains small uniform spherical granules mainly at the periphery (arrows), multiple elongated dark mitochondria (M) and rER (rER) (TEM×2500). E): mammotroph (Mt); triangular cell contains eccentric euchromatic nucleus (N), well developed profiles of rER (rER) and scattered pleomorphic electron- dense secretory granules collected near the nucleus (arrows). Notice: part of corticotrophic cell (Ct) (TEM×3000). F): chromophobe cell (CH); large cell free from specific granules, containing oval large euchromatic nuclei (N), rER (rER) and numerous mitochondria (M). Notice: the blood sinusoid (BS) and part of gonadotrophic cell (Gt). (TEM×2500)

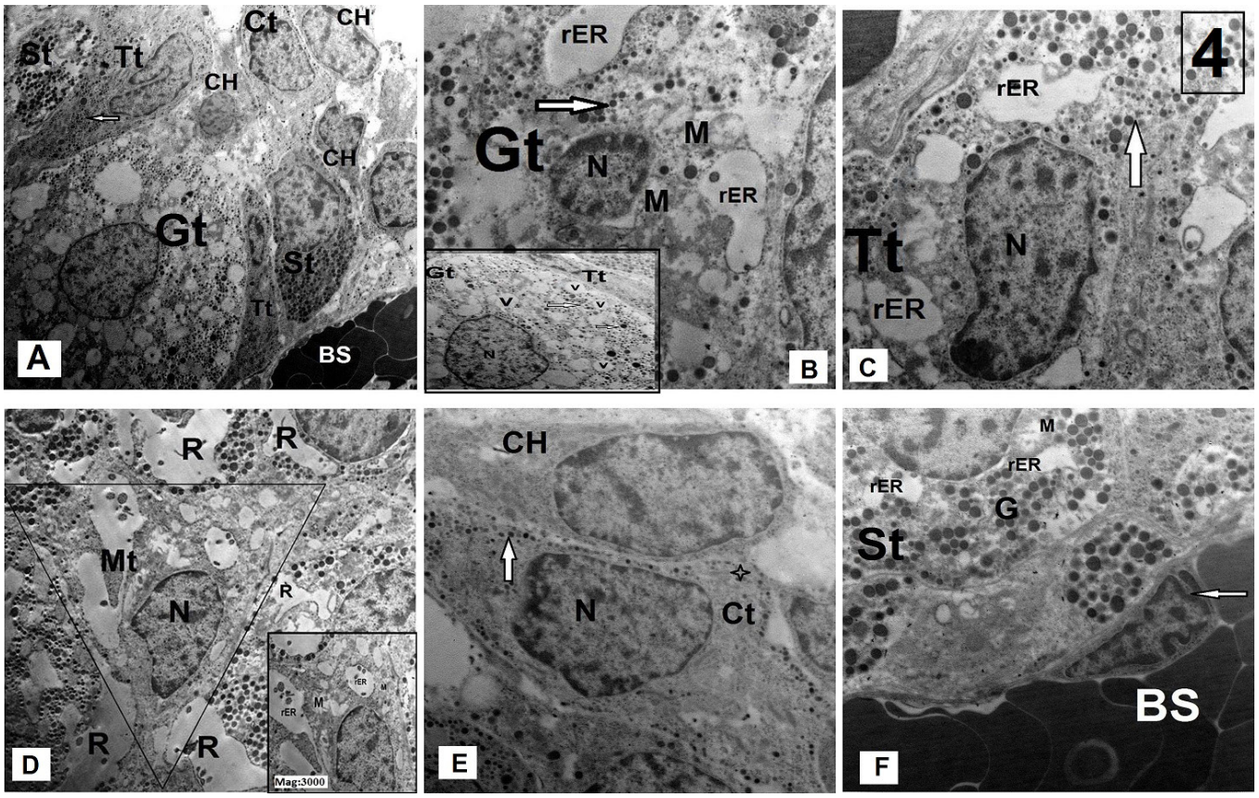
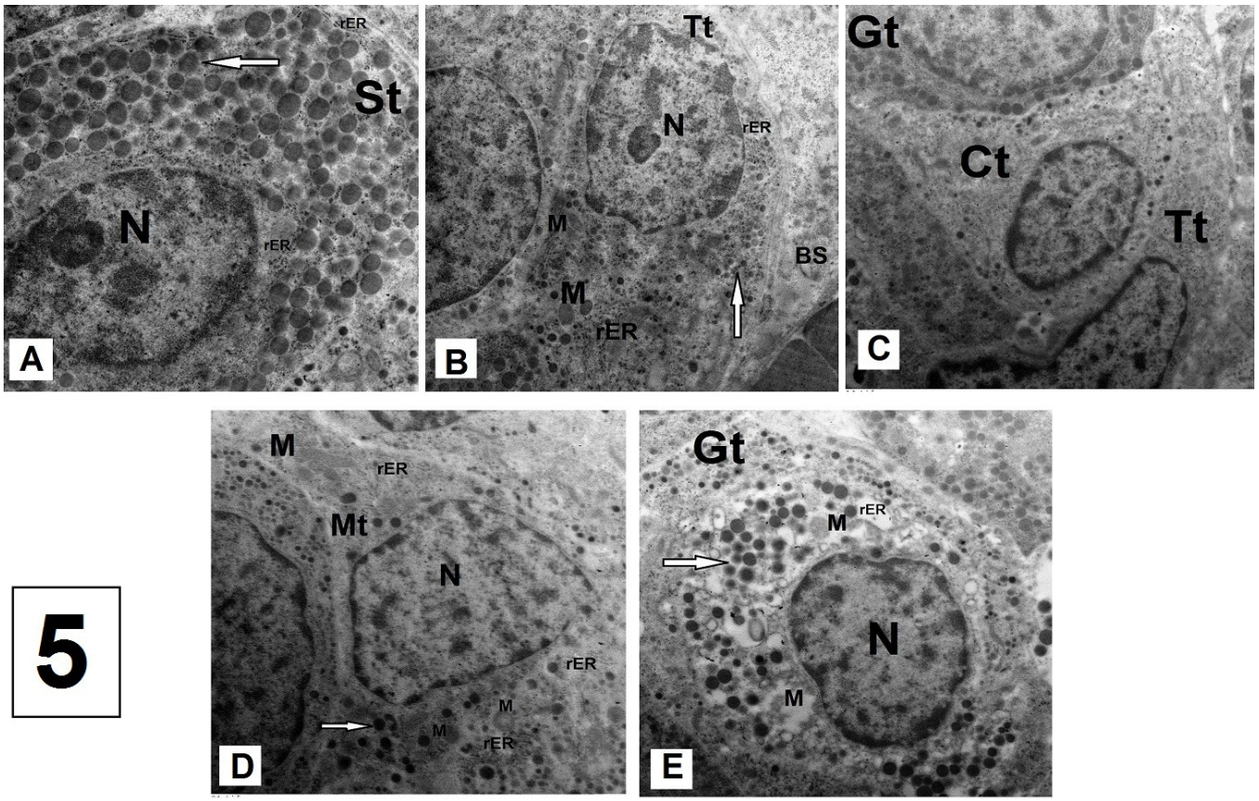



**Fig. 4:** An electron micrograph of an ultrathin section of the pars distalis of pituitary gland in OLZ treated group (group III) showing A): numerous chromophobe cell (CH) with no specific granules, somatotrophs (St), corticotrophs (Ct), enlarged hypertrophied gonadotrophs (Gt) and thyrotrophs ((Tt) with noticeable increased granules (arrow). Notice: congested blood sinusoid (B.S). (TEM×1000). B): gonadotrophs (Gt) containing shrunken degenerated nucleus (N) and rarified cytoplasm containing dilated cisternae of rough endoplasmic reticulum (rER), vacuolated swollen mitochondria (M) and few secretory granules(arrow) (TEM×3000). Inset: enlarged gonadotrophic cell (Gt) contains indented euchromatic nucleus with fragmentation and margination of nuclear chromatin (N) and numerous small, ovoid vacuoles fill the cytoplasm (V). Granules are scanty and scattered between the vesicles (arrow) notice: compressed thyrotrophic cell ((Tt). (TEM×3000). C): elongated thyrotrophic cell (Tt) with irregular nucleus (N) and dilated rER (rER). Their granules were apparently larger in number and size than that of control group (arrows). (TEM×2500). D): marked rarefaction of the cytoplasm of most chromophiles (R). The mammotroph cell (Mt) appears triangular and having indented euchromatic nucleus (N). (TEM×1500) Inset: higher magnification of the triangle area in pervious image showing mammotrophic cell with dilated rER (rER) and degenerated mitochondria (M). (TEM×3000). E): irregular corticotrophic cell (Ct) with short cytoplasmic process (star) containing irregular nucleus with clumps of heterochromatin (N) and many fine granules at the periphery of cytoplasm beneath the cell membrane (arrow). Notice: chromophobe cell with no cytoplasmic granules (CH). (TEM×2000). F): Somatotrophic cell (St) with numerous regular granules (G), degenerated mitochondria (M), mildly dilated rER. Notice: congested dilated blood sinusoid (BS) lined by endothelial cell with irregular nucleus (arrows). (TEM×2500).




5

**Fig. 5:** An electron micrograph of ultrathin section of pars distalis of anterior pituitary gland in OLZ and HP group (group IV) showing A): Somatotrophic cell (St) having euchromatic nucleus (N). The cytoplasm contains numerous spherical large granules (arrow) and few rER (rER). (TEM×4000).B): elongated thyrotrophic cell (Tt) having an euchromatic nucleus(N) with prominent nucleoli , rER (rER), mitochondria (M), and small electron dense secretory granules(arrow). (TEM×3000)

C): identical corticotrophic cell (CT). Notice: Part of gonadotrophic (Gt) and thyrotrophic cells (Tt) (TEM×2500). D): Mammotrophic cell (Mt) with irregular eccentric euchromatic nucleus (N). The cytoplasm contained numerous mitochondria (M), well developed rER and variable shaped granules (arrow) (TEM×3000). E): gonadotrophic cell (Gt) contains numerous variable sized secretory granules (arrows), irregular euchromatic nucleus (N), mildly degenerated mitochondria (M) and slightly dilated rER (rER). (TEM×2500)

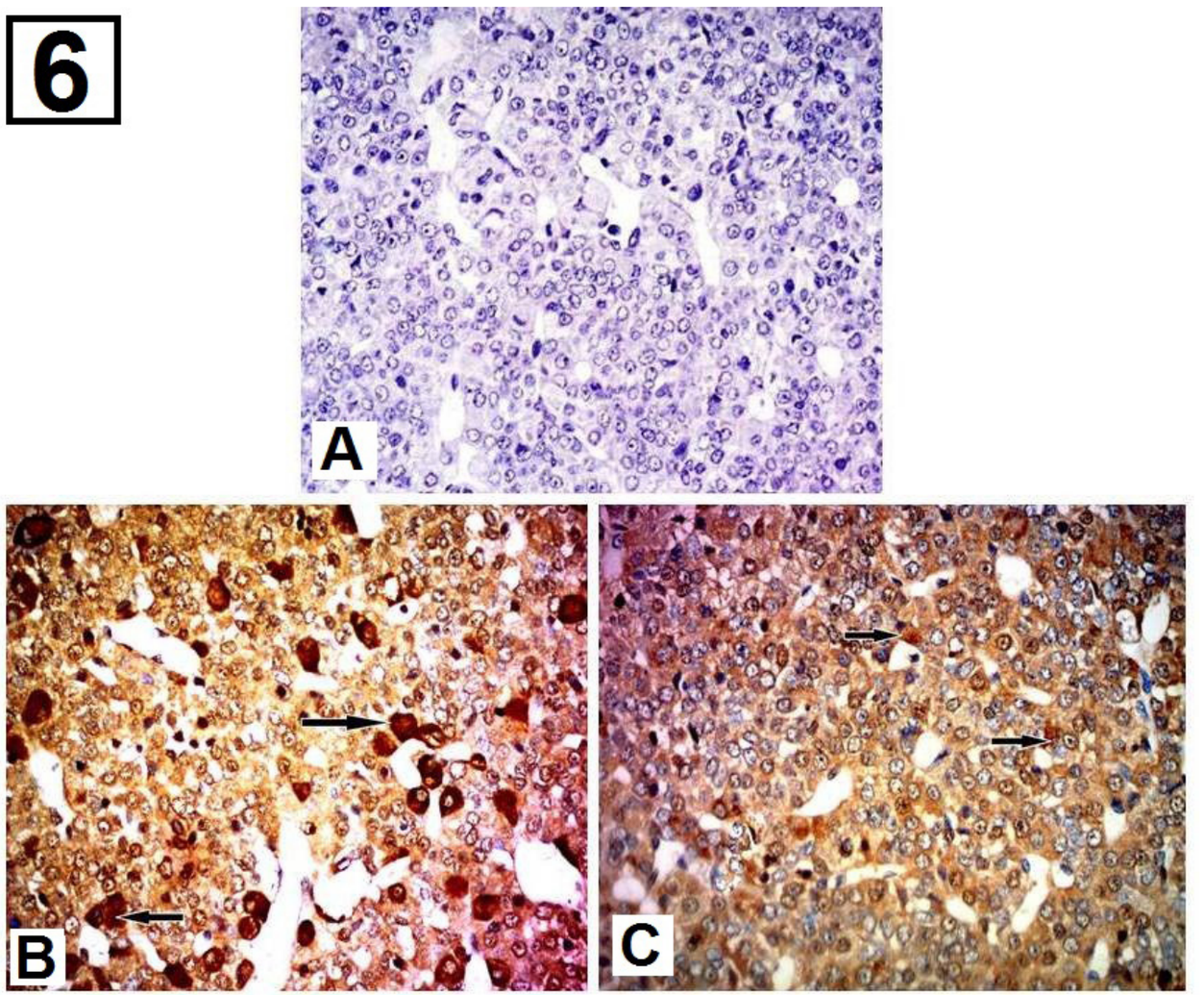



**Fig. 6:** A): a photomicrograph of a section of pars distalis of anterior pituitary gland in control group (group I) showing negative caspase-3 immune expression of pars distalis cells. B): anterior pituitary gland of OLZ treated group (group III) showing intense positive cytoplasmic caspase-3 immune expression in pars distalis cells (arrows). C): pars distalis of anterior pituitary gland of group IV (OLZ and HP group): showing weak positive caspase-3 immune expression (arrows) (caspase-3 x 400).

**Table 1:** The mean serum levels of different hormones of pars distalis in all studied groups.

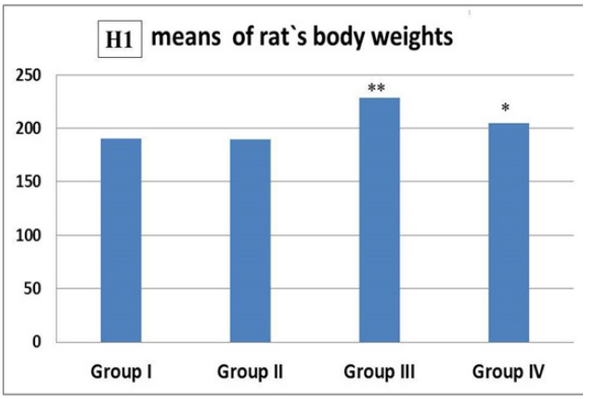
| | Group I | Group II | Group III | Group IV | *P value* |
|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | |
| GH | 0.76 ± 0.25 | 0.77± 0.21 | 0.72 ± 0.27 | 0.74 ± 0.23 | P1=0.924<br>P2=0.735<br>P3=0.854 |
| Prolactin | 9.2 ± 0.4 | 9.6± 0.45 | 12.9 ± 4.6 | 9.9 ± 6.4 | P1=0.133<br>P2=0.021<br>P3=0.734 |
| FSH | 1.5 ± 0.109 | 1.6 ± 0.122 | 1.2 ± 0.2 | 1.3 ± 0.15 | P1=0.099<br>P2= 0.004<br>P3=0.002 |
| LH | 4.52 ± 2.06 | 5.02 ± 2.5 | 0.625 ± 3.7 | 1.1 ± 5.8 | P1=0.631<br>P2=0.009<br>P3=0.042 |
| TSH | 5.06 ± 0.57 | 4.97 ± 0.682 | 11.6 ± 9.3 | 7.5 ± 25.6 | P1=0.753<br>P2=0.04<br>P3=0.766 |
| ACTH | 60 ± 2.1 | 61.1 ± 2 | 48.6 ± 28.4 | 55.4 ± 20.57 | P1=0.246<br>P2=0.222<br>P3=0.487 |

P1: HP treated group (group II) as regards control group (group I).
P2: OLZ treated group (group III) as regards control group (group I).
P3: OLZ and HP treated group (group IV) as regards control group (group I).
*P value* > 0.05 = non-significant.
*P value* < 0.05 = Significant.
*P value* < 0.001 = highly significant.

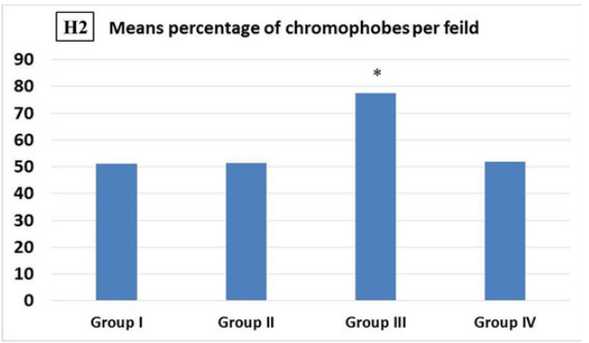
**Table 2:** The mean body weights and morphometric results in all studied groups.

| | Group I | Group II | Group III | Group IV | *P value* |
|---|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | |
| Body weights | 190.2 ± 7.2 | 189.8± 6.9 | 228.5 ± 9.3 | 204.8 ± 17.5 | P1 = 0.9<br>P2 = 0.001<br>P3=0.253 |
| Percentage of chromophobe cells per feild | 51.27 ± 0.656 | 51.03 ± 0.657 | 77.42 ± 0.475 | 51.8 ± 0.721 | P1 = 0.424<br>P2 = 0.001<br>P3 = 0.103 |
| positive caspase-3 immune-stained cells per feild | 1.1 ± 1.2 | 1.4±1.1 | 7.7±1.2 | 1.5±1.0 | P1=0.547<br>P2 = 0.00<br>P3=0.182 |

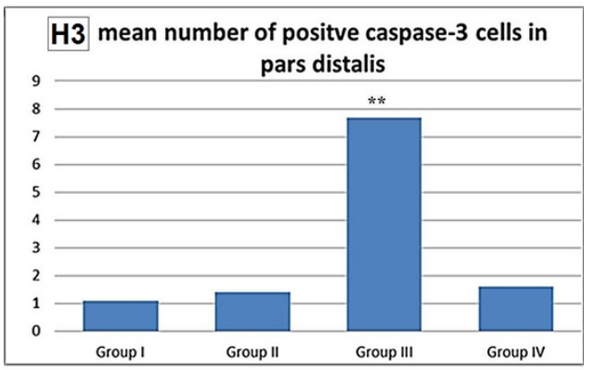
P1: HP treated group (group II) as regards control group (group I).
P2: OLZ treated group (group III) as regards control group (group I).
P3: OLZ and HP treated group (group IV) as regards control group (group I).
*P value* > 0.05 = non-significant.
*P value*< 0.05 = Significant.
*P value* < 0.001 = High significant.




**Histogram 1:** the means of body weight in all studied group.
* Significant versus control group.
** Highly significant versus control group.




**Histogram 2:** mean percentage of the chromophobes per field in all studied group.
* Significant versus control group.




**Histogram 3:** mean number of positive caspase-3 immune-stained pars distalis cells per field.
** Highly significant versus control group.

## DISCUSSION

Olanzapine is an atypical antipsychotic drug that is commonly used in the treatment of schizophrenia and other psychiatric disorders. Schizophrenic patients are usually medicated by olanzapine for prolonged time periods[17]. Thus the previous experiments in rats treated by OLZ were typically run for long time up to 12 weeks. These studies revealed that olanzapine induced weight gain, increased plasma lipid levels and some hormonal changes[18].

Recently, natural products can play an important protective role when they used as a clinical therapeutic regimens with calculated dose[19]. Hypericum perforatum is a perennial plant that is markedly distributed all over the world. Through centuries, it has been used for treatment of many disorders in traditional medicine, such as mild to moderate depression, anxiety and minor burns. Moreover, recent studies proved its neuroprotective and anti-inflammatory effects[20]. So, the present study was designed to assess the biochemical, histological, and immunohistochemical changes in the anterior pituitary gland induced by the chronic administration of olanzapine and the potential protective role of Hypericum perforatum.

Our data showed that long-term use of olanzapine was accompanied by a high significant increase in the rat's body weights. These changes were recorded previously by other researchers[18]. The molecular mechanisms of increased body weights have been suggested previously by Skrede *et al*.,[21]. They mentioned that, olanzapine-induced hyperphagia leading to the up regulation of hypothalamic AMP-activated kinase and activation of hypothalamic orexigenic neuropeptides.

In this study, chronic administration of OLZ led to a significant decrease in mean plasma level of FSH, and LH but significant increase in TSH and prolactin hormones. There was a non-significant relation in GH, ACTH hormones, compared to the control group. These biochemical results are coincided with our electron microscopic results. The clinical study of Iversen *et al*., 2018[22] could support our biochemical results. They reported a significant increase of TSH hormone in schizophrenic patients treated with olanzapine, comparing to healthy controls.

In accordance with our results Ardic *et al*., 2021[23], reported a significant decrease in the serum levels of FSH, LH, and testosterone hormones in rats treated with olanzapine. They found that OLZ evaluated the oxidative stress markers such as glutathione (GSH), malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD).

In agreement with our results some authors found that olanzapine is associated with a significant increase in the level of prolactin hormone[24,25]. Prolactin hormone is under the inhibitory effect of dopamine. The an¬tischizophrenic drugs led to hyperprolactinemia through their direct impact on tuberoin¬fundibular tract; one of the four dopamine-associated tracts. Olanzapine act by blocking dopamine receptors and inducing the conversion of androgens to estrogens hormones leading to the elevation of plasma estrogen levels resulting in impotence, loss of libido and hyposper-matogenesis[26]. In contrast, some studies showed that olanzapine, has no significant changes on the level of prolactin hormone[27].

In this experimental study, Olanzapine treated group showed degenerative changes in pars distalis in the form of

disturbance as the cells appeared amalgamated with each other with failure to differentiate them. Most pars distalis cells showed small dark nuclei. Some cells appeared with vacuolated cytoplasm. The number of chromophobes was significantly increased compared to those of control. In agreement with our results, Shah *et al*, 2018[28] found that beta cells of islets of Langerhans treated by olanzapine showed extensive necrosis, degeneration and vacuolization of the cytoplasm. The nuclei exhibited pyknosis and chromatin condensation. This also goes with the finding of the Batarfi and Almeflehie, 2018[29]. They demonstrated that olanzapine induced degenerative and necrotic changes in the hepatocytes. As well as Akram *et al*, 2020[30] reported that spermatogenic cells exposed to olanzapine showed signs of degeneration in the nuclei and cytoplasm.

Our histological results revealed some cellular infiltration around the dilated blood sinusoids. These results could be in line with Batarfi and Almeflehie, 2018[29]. They recorded that schizophrenic patients treated with olanzapine had an inflammatory syndrome this syndrome is known to be associated with an increased risk of insulin resistance that occurred with olanzapine treatment. Additionally, olanzapine has a strong and selective effect on expression of tumor necrotic factor α (TNFα)[31].

In our study, the acidophilic homogenous material detected in between the pars distalis cells is in harmony with previous investigators[17]. They reported acidophilic substance between liver cells treated by olanzapine and explained this by production of reactive oxygen species induced by olanzapine.

Degenerative changes recorded in our study were explained recently by other authors[32,28,33], they stated that olanzapine induced oxidative stress and enhance production of reactive oxygen species. Moreover, olanzapine has direct toxic effect on pituitary gland as it has a high affinity to dopamine receptors subtype 2 (D2 receptors) which is present in the pituitary gland[34].

Olanzapine treated group also showed dilation and congestion of blood sinusoids with cellular infiltration. These findings are in agreement with Akram & Faruqi, 2019[32] who found that olanzapine caused dilated capillaries in the kidney tissue surrounded by inflammatory cells. Also previous study,[10] reported dilatation and congestion of central vein and portal vein in liver tissue exposed to olanzapine. The vascular dilatation induced by Olanzapine is secondary to blockage of alpha1 (α1) and 5-hydroxytryptamine2 (5HT2) receptors. This led to vasodilatation by increasing cyclic adenosine monophosphate (CAMP)[17].

The ultra-structural changes found in the current study came in agreement with the histological and biochemical result. Examination of OLZ treated rats revealed that most cells in the pars distalis as gonadotrophs, mammotrophs and thyrotrophs" exhibited degenerative changes. Their cytoplasm had irregular degenerated nuclei with chromatin fragmentation and clumps of heterochromatin, dilated cisternae of rER, and vacuolated swollen mitochondria with loss of the internal cristae and changes in the numbers of their granules. These changes might be in accordance with previous study[35], that concluded that hepatotoxicity of olanzapine was associated with increased mitochondrial degeneration and frequent presence of dilated rER. As reference to Batarfi, 2020[35], cytotoxicity induced by olanzapine was mediated by mitochondrial degeneration, degradation of lysosomal membrane, and lipid peroxidation.

Corticotrophs and somatotrophes showed no massive changes in their morphology that could explain the none significant changes in their secretory hormones measured in our result.

In the current study, there was a significant increase in the number of chromophobes in the OLZ treated group. Previous studies reported a compensatory increase in the number of the chromophobes after partial hypophysectomy[36]. The presence of chromophobes with mitotic figures act as stem cells to compensate the degeneration occurred to chromophils[37].

In this study olanzapine treated group showed a high significant increase in the number of positive caspase-3 immune-stained cells. This finding goes with the results of a previous study[38], which postulated that the atypical antipsychotic drugs were associated with increased levels and activity of caspase-3 in frontal cortex of albino rats.

The current study indicates that Hypericum perforatum provides a good protection against structural alterations in the anterior pituitary glands induced by chronic administration of olanzapine. Where H & E sections of OLZ and HP group (IV) exhibited normal histological feature of Pars distalis cells except, some cells still contained small dark nuclei. In agree with our finding Barnes *et al*, 2001[39], reported that Hypericum perforatum has antioxidant and neuroprotective effects and plays an important role in reducing the inflammatory disorders. This protective effect of Hypericum perforatum explained previously as it contains high content of phenolic compounds that have high ability to scavenge the free radical[10]. Hypericum perforatum have the ability to bind iron and ions. It also has a moderate to high direct scavenging action for the hydroxyl radical, independent of any enzymatic activity[40].

Furthermore, other investigators[10] postulated that Hypericum perforatum inhibit the damage of liver tissue induced by olanzapine. They proved that HP restored Superoxide dismutase (SOD) enzymes and GSH levels in the liver to the normal values. Also, it decreased lipid peroxidation and oxidative stress caused by olanzapine.

The antioxidant effects of Hypericum perforatum were attributed by Silva *et al*, 2008[41] who stated that HP protect against mitochondrial dysfunction by maintaining trans-membrane potential, leading to reduction of lipid peroxidation in the mitochondria. This could explain our TEM finding where, ultrathin sections of OLZ and HP

group exhibited restoration of the normal structure of most pars distalis cells. Scientists have studied its properties as antidepressant in the past years, they found that several of its major molecular structures protect from any neural insults, either through their antioxidant properties or through the mechanisms of neuroprotection directly[20].

The current study demonstrated the mechanisms of the ameliorative effects of Hypericum perforatum that proved by its antiapoptotic effect. This effect was evidenced in our results. Where there were non-significant changes in the number of positive caspase-3 immune-stained cells in OLZ and HP group as compared to control group. Regarding to Uruç *et al*., 2020[42], Hypericum perforatum has antiapoptotic effect and significantly reduced the activity of caspase-3 in osteocytes treated by methotrexate. Hypericum perforatum has the ability to block the apoptotic cascade and induced down regulation of Bax gene[43].

## CONCLUSION

In conclusion, the current study revealed negative effects of chronic use of Olanzapine on the function and structure of anterior pituitary gland. Hypericum Perforatum reversed the associated functional and structural changes via its antiapoptotic mechanisms. Hence, the present study could provide a potential scope for the future clinical use of Hypericum Perforatum as a natural herb for patients received Olanzapine for long period.

## CONFLICT OF INTEREST

There are no conflicts of interest.

## REFERENCE


1. Parasuraman, S., Zhen, K. M., Banik, U., & Christapher, P. V. Ameliorative Effect of Curcumin on Olanzapine-induced Obesity in Sprague-Dawley Rats. Pharmacognosy research (2017), 9(3), 247–252. doi: 10.4103/pr.pr_8_17.

2. Kim, D., Ryba, N.L., Kalabalik, J: Critical Review of the Use of Second-Generation Antipsychotics in Obsessive–Compulsive and Related Disorders. Drugs R D 18, 167–189 (2018). doi.org/10.1007/s40268-018-0246-8.

3. Yang LH, Chen TM, Yu ST, & Chen YH. Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. Pharmacol Res (2007); 56(3):202. doi: 10.1016/j.phrs.2007.05.007.

4. Maloney AE & Sikich L Olanzapine approved for the acute treatment of schizophrenia or manic/mixed episodes associated with bipolar I disorder in adolescent patients: Neuropsychiatr Dis Treat (2010), 10; 6:749- 66. doi: 10.1016/j.phrs.

5. Pivonello, R., Waaijers, M., Kros, J.M: Dopamine D2 receptor expression in the corticotroph cells of the human normal pituitary gland. Endocrine 57, 314–325 (2017) . doi.org/10.1007/s12020-016-1107-2.

6. Correll, C. U., Rubio, J. M., & Kane, J. M: What is the risk-benefit ratio of long-term antipsychotic treatment in people with schizophrenia? World psychiatry. Official journal of the World Psychiatric Association (WPA), (2018), 17(2), 149–160. doi.org/10.1002/wps.20516

7. Sertié, A.L., Suzuki, A.M., Sertié, R.A.L., Andreotti, S., Lima, F.B., Passos-Bueno, M.R., Gattaz, and W.F. Effects of antipsychotics with different weight gain liabilities on human in *vitro* models of adipose tissue differentiation and metabolism. Prog. Neuro-Psychopharmacol. Biol. Psychiatry (2011), 35 (38), 1884–1890. doi: 10.1016/j.pnpbp.2011.07.017.

8. Oliveira AI, Pinho C, Sarmento B and Dias ACP: Neuroprotective Activity of Hypericum perforatum and Its Major Components. Front. Plant Sci. (2016), 7:1004. Doi: 10.3389/fpls.2016.01004.

9. Wang P. and Si T: Use of antipsychotics in the treatment of depressive disorders. Shanghai Arch Psychiatry J. (2013); 25(3):134–140. doi: 10.3969/j.issn.1002-0829.2013.03.002..

10. Abd El-Hameed AM, Eskandrani AA & Elroby FA: Assessment of the ameliorative effect of Hypericum perforatum on olanzapine-induced hepatic oxidative stress and metabolic abnormalities in experimental male albino rats. Journal of Taibah University for science. (2020), V. 14, 1 pp. 1496-1502. DOI: 10.1080/16583655.2020.1834757

11. Pouzet B, Mow T, Kreilgaard M, Velschow S. Chronic treatment with antipsychotics in rats as a model for antipsychotic-induced weight gain in human. Pharmacol Biochem Behav. (2003); 75(1):133-140. doi: 10.1016/s0091-3057(03)00042-x.

12. Kalleny, N.K. and Soliman, N.B. Light and electron microscopic study on the effect of topically applied hyaluronic acid on experimentally induced corneal alkali burn in albino rats". Egyptian journal of histology (2011), 34: 829-848. DOI:10.1097/01.EHX.0000407699.63226.2a

13. Kiernan JA: Histological and histochemical methods, theory and practice.5rded. Scion Publishing limited Press (2015). 1;12(6):479. doi: 10.4081/ejh.2016.2639

14. Dykstra, M.J. and Reuss, L.E. staining methods for semi thins and ultra-thins. In: Biological electron microscopy, theory, techniques and troubleshooting. . (2003), 2nd ed. Kluwer Academic Publishers/Plenum Publishers. pp.175- 196. DOI:10.1007/978-1-4419-9244-4_7

15. Huang KH, Fang WL, Li AF, Liang PH, Wu CW, Shyr YM, Yang MH. Caspase-3, a key apoptotic protein, as a prognostic marker in gastric cancer after curative surgery. International Journal of Surgery (2018), 52:258-263. DOI: 10.1016/j.ijsu.2018.02.055

16. Dawson B &Trapp RG: Basic and clinical biostatistics. 5th ed. New York: McGraw-Hill Education Medical; (2020). ch2; P1117.

17. Elbakary RH: Histological Study of the Effects of Olanzapine on the Liver of Adult Male Albino Rat with and without Vitamin C. (2017) ISSN: 1110-0559, Vol. 40, No. 1. DOI: 10.21608/EJH.2017.3692

18. Ersland KM, Myrmel LS, Fjære E, Berge RK, Madsen L, Steen VM, Skrede S. One-Year Treatment with Olanzapine Depot in Female Rats: Metabolic Effects. Int J Neuropsychopharmacol. (2019)May 1; 22(5):358-369. Doi: 10.1093/ijnp/pyz012.

19. Kassab, A.A. and El-Aaser, M.:" Effect of Avocado pulp extract on chlorpyrifos-induced thyroid gland injury in rats: A histological and morphometric study". Egyptian Journal of histology (2018). ISSN: 1110-0559. 41 (1): 82-93. DOI: 10.21608/EJH.2018.7524

20. Elhadidy, M. E., Salama, A. A., El-Kassaby, M., & Omara, E. A. Protective effect of Hypericum perforatum on dexamethasone-induced diabetic depression in rats. Journal of the Arab Society for Medical Research, (2019). 14(1), 25. DOI:10.4103/jasmr.jasmr_7_19

21. Skrede S, Martins L, Berge RK, Steen VM, López M, Fernø J Olanzapine depot formulation in rat: a step forward in modeling antipsychotic-induced metabolic adverse effects. Int J Neuropsychopharmacol (2014), 17:91–104. doi.org/10.1017/S1461145713000862

22. Iversen T, Steen NE, Birkeland KI, *et al*. T229. Antipsychotic Drug Use and Thyroid Function In Patients With Severe Mental Disorders. Schizophrenia Bull. (2018); 44(Suppl 1):S205-S206. doi:10.1093/schbul/sby016.505.

23. Ardic, C., Ilgın, S., Baysal, M., Karaduman, A.B., Kılıç, V., Aydoğan-Kılıç, G., Uçarcan, Ş., & Atlı-Eklioğlu, O. Olanzapine induced reproductive toxicity in male rats. Scientific Reports (2021), 11; 407. doi: 10.1038/s41598-021-84235-4.

24. Konarzewska B, Wołczyński S, Szulc A, Galińska B, Popław¬ska R, Waszkiewicz N: Effect of risperidone and olanzapine on reproductive hormones, psychopathology and sexual functioning in male patients with schizophrenia. Psych neuroendocrinology. (2009); 34:129–139. doi: 10.1016/j.psyneuen.2008.08.015.

25. Wieck A, Haddad PM. Antipsychotic-induced hyperprolac¬tinaemia: Mechanisms, clinical features and management. Drugs J. (2004); 64:2291–2314. DOI: 10.2165/00003495-200464200-00003

26. Soliman HM, Wagih HM, Attia GM, Algaidi SA. Light and electron microscopic study on the effect of antischizophrenic drugs on the structure of seminiferous tubules of adult male albino rats. Folia Histochem Cytobiol J (2014); 52:335-49. Doi, 10.5603/FHC.a2014.0038

27. Fell MJ, Marshall KM, Williams J, Neill JC. Effects of the Atypical Antipsychotic Olanzapine on Reproductive Function and Weight Gain in Female Rats. Journal of Psychopharmacology. (2004); 18 (2):149-155. Doi:10.1177/0269881104042613.

28. Shah R, Subhan F, Sultan SM, Ali G, Ullah I, and Ullah S: Comparative evaluation of pancreatic histopathology of rats treated with olanzapine, risperidone and streptozocin. Braz. J. Pharm. Sci. (2018); 54(3): e17669. doi.org/10.1590/s2175-97902018000317669

29. Batarfi N M & Almeflehie A. The Preventive Effect of Nigella Sativa Oil on the Liver of Male Albino Rats Treated with Anti-Depressants (Olanzapine) is Histological Study. International Journal of Pharmaceutical Research & Allied Sciences (2018), 7(4):30-46.

30. Akram W, Syed M & Faruqi NA: Histopathological and Histomorphometric Studies on the Effects of Olanzapine on Testis: An Experimental Study in Albino Rats. Journal of the Anatomical Society of India (2020). Vol 68, Issue 3. DOI: 10.4103/JASI.JASI_43_19

31. Van Brunt DL, Gibson PJ, Ramsey JL and Obenchain R. Outpatient use of major antipsychotic drugs in ambulatory care settings in the United States, 1997-2000. MedGen Med. (2003); 5:16. PMID: 14600653.

32. Akram W & Faruqi NA: Histopathological And Histomorphometric Studies On the Effects Of Olanzapine On Kidney: An Experimental Study In Albino Rats. J. Anat. Science, 27(1): June (2019), 1-4.

33. Heiser P, Sommer O, Schmidt AJ, Clement HW, Hoinkes A & Hopt UT: Effects of antipsychotics and Vitamin C on the formation of reactive oxygen species. J Psychopharmacology (2009); 24:1499-504. 10.1177/0269881109102538.

34. Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J,Wilson AA: 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: A PET investigation. Am Psychiatry J (1998); 155:921-8. DOI: 10.1176/ajp.155.7.921

35. Batarfi NM. Ultrastructure and Pathology Study of the Effect of an Antidepressant (Olanzapine) on the Health of Mitochondria in liver of male Albino Rat. J Histol Histopathology. (2020); Volume 7, Article 3, pp 37-45 .doi.org/10.7243/2055-091X-7-3. ISSN 2055-091X

36. Saeger W, Warnecke H. Ultrastructural examination of the regeneration of the rat adenohypophysis after partial hypophysectomy. Virchows Archiv (1980); 387:279–288. doi.org/10.1007/BF00454831

37. Abdel-Aziz HO and El Haliem NG: Ultrastructural study of the effect of carbon tetrachloride on the pars distalis of the anterior pituitary gland of mice and the possible protective role of ginger The Egyptian Journal of Histology (2012), vol 35: PP 598-606. DOI: 10.1097/01.EHX.0000418458.34353.62

38. Jarskog LF, Gilmore JH, Glantz LA, Gable KL, German TT, Tong RI and Lieberman JA: Caspase-3 Activation in Rat Frontal Cortex Following Treatment with Typical and Atypical Antipsychotics. Neuropsychopharmacology (2007), 32, 95–102. doi:10.1038/sj.npp.1301074.

39. Barnes J, Anderson A, Phillipson JD: St John's wort (Hypericum perforatum L.): A review of its chemistry, pharmacology and clinical properties J. Pharm (2001), 53, No. 5, P. 583. DOI: 10.1211/0022357011775910

40. Oliveira AI, Pinho C, Sarmento B and Dias ACP: Neuroprotective Activity of Hypericum perforatum and Its Major Components. Front. Plant Sci. (2016), 7:1004. Doi: 10.3389/fpls.2016.01004

41. Silva BA, Malva JO, Dias ACP: St. John'Wort (Hypericum perforatum) extracts and isolated phenolic compounds are effective antioxidants in several in *vitro* models of oxidative stress. Food Chem J. (2008), 110, P.611. DOI:10.1016/j.foodchem.2008.02.047

42. Uruç, V., F. Salimov and H.M. Kaplan.Protective Effect of Hypericum perforatum extract on methotrexate-induced osteotoxicity via reducing oxidative stress and MAPK activity. Int. J. Pharmacol.( 2020), 16: 430-436. DOI: 10.3923/ijp.2020.430.436

43. Savici, JA., Boldura, O M., Balta, C.. L., Brezovan, DA., Muselin, FN., Mircu, C. N & Tulcan, CA: Protective Effect of Hypericum perforatum L. Extract on Hexavalent Chromium Induced Toxicity in Rat Adrenal Gland. Revista de Chimie (Rev.Chim.) (2017), 68(9), 2014-2017.doi.org/10.37358/RC.17.9.5813

**الملخص العربى**

# أثار التناول المزمن للأدوية المضادة للذهان (الأولانزابين) على الغدة النخامية الأمامية في ذكور الجرذان البيضاء البالغة والدور الوقائي المحتمل لعشب العرن المثقوب

**إيمان شحاتة الرغي وريهام عبدالله البربرى**

**قسم الهستولوجيا وبيولوجيا الخلايا، كلية الطب، جامعة المنوفية، مصر**

**المقدمة:** الأولانزابين هو دواء غير نمطي مضاد للذهان، وقد تم استخدامه كعلاج معتمد لمرض انفصام الشخصية والاضطراب ثنائي القطب. العرن المثقوب هو نبات عشبي وُصف بأنه يساعد علي حماية اللأعصاب. كما أن له تأثيرقوي كمضاد للأكسدة والالتهابات.

**الهدف من الدراسة:** لتقييم آثار الاستخدام المزمن للأولانزابين على الغدة النخامية الأمامية والدور الوقائي المحتمل لعشب العرن المثقوب.

**المواد المستخدمة وطريقة البحث:** تم تقسيم ستة وخمسين من ذكور الجرذان البالغة إلى ٤ مجموعات، وتلقيت العلاج عن طريق الفم لمدة ٨ أسابيع. وهي كالأتي: (١) المجموعة الضابطة، (٢) المجموعه المدعومة بعشب العرن المثقوب (٨٠ مجم / كجم / يوم), (٣) المجموعة المعالجة بالأولانزابين (١٠ مجم / كجم / يوم)، (٤) المجموعة المعالجة بالأولانزابين وعشب العرن المثقوب معا (١٠ مجم OLZو٨٠ مجم HP/ كجم / يوم). في النهاية، تم وزن الفئران وتخديرها والتضحية بها. تم جمع عينات الدم لعمل الدراسة البيوكيميائية. تم تشريح الغدة النخامية ومعالجتها للدراسة النسيجية.

**النتائج:** أظهرت المجموعة المعالجة بالأولانزابين زيادة كبيرة في وزن جسم الجرذان. كما انخفضت مستويات بلازما الدم من الهرمون اللوتيني ((LH, الهرمون المحفز للحوصلة (FSH), ولكن تم زيادة الهرمون المحفزللغدة الدرقية (TSH) وكذلك هرمون البرولاكتين. لقد تسبب الأولانزابين في احداث ضرر في خلايا الفص الصدغى للغدة النخامية. حيث كانت الخلايا تحتوي على نوى مظلمة صغيرة مع وجود فجوات في السيتوبلازم. لوحظ ترسب خلايا التهاب واحتقان في الأوعية الدموية. احتوت معظم الخلايا المحبة للتصبغ على ميتوكوندريا متحللة وشبكة إندوبلازمية خشنة متوسعة. علاوة على ذلك كانت هناك زيادة كبيرة في عدد الخلايا المصبوغة مناعيا بواسطة كاسباس-٣. أدي التناول المتزامن لعشب العرن المثقوب في تحسين هذه التغييرات المحدثة بواسطة الأولانزابين.

**الاستنتاج:** تسبب الأولانزابين في تغيرات مورفولوجية وكيميائية في الغدة النخامية الأمامية. كما يمكن لعشب العرن المثقوب أن يؤدي إلى تحسين هذه التغييرات.