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Can Somatotrophs and Thyrotrophs Recover from the Effects of Diazepam after its Withdrawal? An Ultrastructure Study

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Abstract. This study aimed to detect the effects of long term administration of diazepam on the ultrastructure of somatotrophs and thyrotrophs of the anterior pituitary, and the possibility of recovering from its effects after withdrawal. Forty male adult rats used in this study were divided into a control and experimental groups. The latter was divided into two subgroups; first received diazepam intraperitoneally at a daily dose of 0.18 mg/200 gm body weight for 21 days. The second was left for one month after stopping the diazepam, which was given for same period. The pars distalis of the pituitary glands were dissected and processed for light and electron microscopic study. Most of the somatotrophs showed signs of degeneration following administration of diazepam for 21 days. Thyrotrophs showed variable degrees of effect, but to a lesser extent compared to somatotrophs. After withdrawal of diazepam, most of the somatotrophs showed irreversible changes, apart from few cells that appeared intact. Some thyrotrophs recovered from the effects of diazepam while others could not. In conclusion, withdrawal of diazepam did not allow the affected somatotrophs and thyrotrophs to recover from its harmful effect. Therefore, diazepam should be given cautiously, especially when treating children.

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Introduction

The pituitary, the master gland of most endocrine tissues, consists of three distinct anatomical and functional parts: the pars distalis (anterior pituitary), pars intermedia, and pars nervosa. The anterior pituitary cells, through their secretory activity, control important bodily functions, such as growth, development, reproduction, and responses to stress. The cells release their hormones in response to blood-borne hypothalamic factors[1].

Two cells are the focus of interest in the present study: thyrotrophs secrete trophic hormone, which controls the activities of an important peripheral endocrine gland and the thyroid; somatotrophs secrete growth hormone, which controls body growth *via* its influence on protein synthesis in different cells[2]. Both are involved in body growth and cellular differentiation.

Prototypic benzodiazepines, such as diazepam (DZ), are not only anxiolytic, but they also produce sedation. These effects are mediated by GABA (A) receptors[3,4] that contain a α1, α2, α3, or α5 subunit, at which the positive modulatory effects of benzodiazepines are mediated *via* a specific benzodiazepine recognition site[5].

Diazepam is used in the treatment of a variety of psychosomatic conditions. However, there is increasing evidence that the BD class of drugs may exert neuroendocrine effects in addition to their well-known profile of central action[6]. Diazepam might exert its effect directly on the anterior pituitary gland, a concept that was described by Grandison, who demonstrated specific binding sites for DZ on rat pituitary cells in culture[7]. Diazepam also acts indirectly through GABA (A) receptors, as described above.

The effect of DZ on the morphology of the different anterior pituitary cells has not been investigated thoroughly to date. Therefore, this study was conducted to detect the effects of long-term administration of DZ on the microscopic structure of somatotrophs and thyrotrophs of the anterior pituitary. The study also aimed to answer the question of whether somatotrophs and thyrotrophs can recover from the effects of DZ after its withdrawal.

Materials and Methods

Forty healthy adult male rats (Rattus norvegicus) were used in this study. Their body weights ranged from 200 to 250 gm, and they were fed a standard diet. The animals were divided into the following groups: GI, control (n=10) and GII, experimental (n=30). The GII group was subdivided into two subgroups (GIIa and GIIb). The animals in the GI group were also subdivided into negative control, which were left without any treatment, and positive control, which were injected intraperitoneally with propylene glycol (used as a vehicle) at a dose of 0.2 ml/200 gm body weight for 21 days (to exclude the effect of the solvent). Experimental groups GIIa and GIIb were injected intraperitoneally with pure DZ dissolved in propylene glycol at a daily dose of 0.18 mg/200 gm body weight for 21 days. The GIIa (n=15) rats were sacrificed the day after the last injection. The GIIb (n=15) animals were left for one month after stopping the DZ injection, after which they were sacrificed to study the effects of DZ withdrawal. Pure DZ (kindly provided by the Pharmaceutical Department, Faculty of Pharmacy, Assuit University, UAR, and obtained from Hoffman-La Roche, Montreal) was chosen, to avoid the harmful effects of the additives in commercial injections (ethyl alcohol, benzoic acid, and sodium benzoate).

The animals in the GIIa group (n = 15) were injected intraperitoneally with pure DZ dissolved in propylene glycol at a daily dose of 0.18 mg/200 gm body weight for 21 days, then sacrificed 24 h after the last injection. The animals in group GIIb (n = 15) were injected intraperitoneally with pure DZ at the same daily dose for 21 days, left for one month after stopping the drug, then sacrificed to study the effects of DZ withdrawal.

The pituitary glands were removed immediately after sacrificing the animals by decapitation to avoid the harmful effects of the anesthetic drugs. For light microscopic (LM) examination, the glands were fixed in Bouin's fixative, processed, cut into sections for paraffin (5-7 microns), and then stained with hematoxylin and eosin. Performic Acid/Alcian Blue/Periodic Acid Schiff/Orange G. (PFA/AB/PAS/OG) stain was further used to differentiate the various types of pars distalis cells[8]. This stain distinguishes between two types of basophil cells—S cells, because the Alcian blue demonstrates sulphur-containing amino acids and R cells,

as they are resistant to oxidation and subsequent staining with Alcian blue [8].

Very small parts of the pars distalis were fixed in 5% cold glutaraldehyde for 24 hours, then processed and embedded in Epon 812, using gelatin capsules[9]. Semi-thin sections (0.5–1 microns) were obtained to detect the selected areas. The blocks were trimmed and cut into ultrathin sections, collected on copper grids, and stained with uranyle acetate and lead citrate; then examined with a transmission electron microscope (TEM-100 CX II Jeol) and photographed at 80 KV.

Results

Light Microscopic Study

Hematoxylin- and eosin-stained sections showed that the parenchyma of the pars distalis was formed of groups of cells arranged in cords or clusters, and separated by wide, irregular blood capillaries. The cells were comprised mainly of two distinct types: chromophobes and chromophils. The chromophobes are the smallest and most numerous in the pars distalis; they have scanty cytoplasm. Chromophils are classified according to their shape, size, position of the nucleus, and the staining affinity of their granules into two major types: acidophils and basophils (Fig. 1).

The PFA/AB/PAS/OG-stained sections showed most pars distalis cell types, depending on their cytoplasmic staining. Acidophils were stained orange with orange G, so they were called orangophils. Two types of basophils were distinguished: the R cells, which stained deep purple, and the S cells, which stained blue (Fig. 2).

After 21 days of DZ administration, the different cell types of the pars distalis were difficult to identify in the PFA/AB/PAS/OG-stained sections. On the other hand, after withdrawal of DZ, the PFA/AB/PAS/OG-stained pars distalis sections showed that most of the cells had well-defined outlines. Orangophils appeared more or less similar to those of the controls (Fig. 3). Cells in other sections of the pars distalis were still affected, where orangophils appeared irregular, small, and deeply stained (Fig. 4).

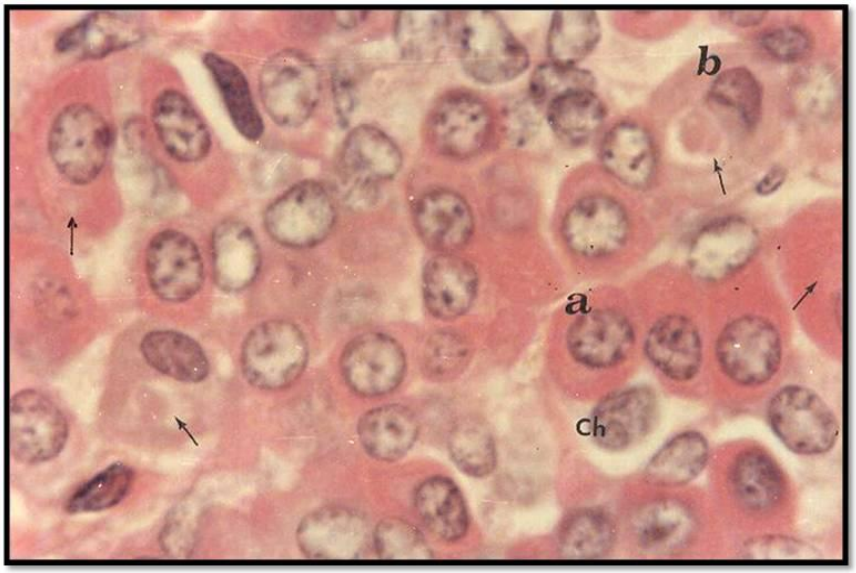

Fig. 1. A section of pituitary gland (pars distalis) of control rat showing chromophobes (ch), acidophils (a) appear smaller with polyhedral or oval shape, arranged in groups or clusters and basophils (b) with prominent Golgi areas (arrows). (H&E stain X 1250).

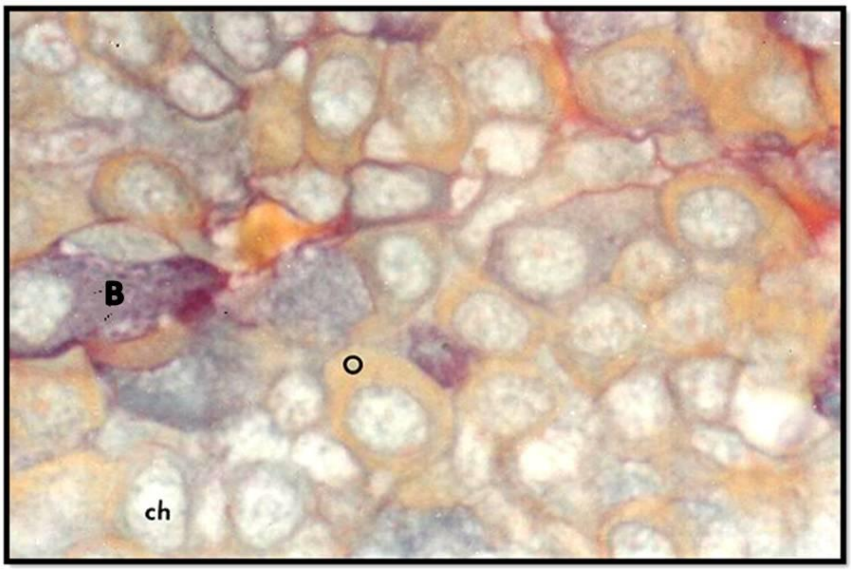

Fig. 2. A section of pituitary gland (pars distalis) of control rat showing different types of its cells; chromophobes (ch), orangophils (o) and thyrotrophs (T) distributed at the periphery of the lobe, and usually in direct contact with blood capillaries by long processes (PFA/AB/PAS/OG stain X 1250).

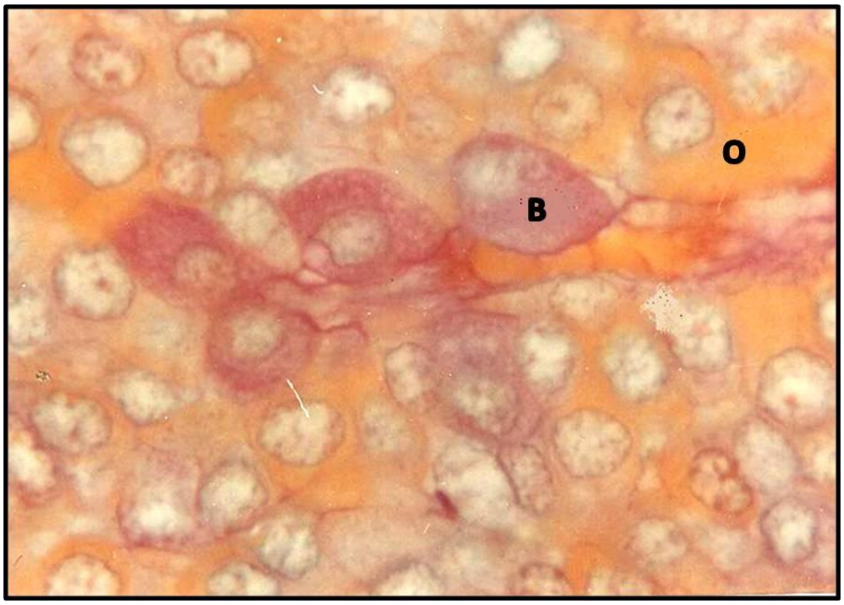

Fig. 3. A section of pituitary gland (pars distalis) of albino rat after withdrawal of DZ showing orangophils, most probably somatotroph (S) and large purple cells, most probably gonadotrophs (G) the (PFA/AB/PAS/OG stain X 1250).

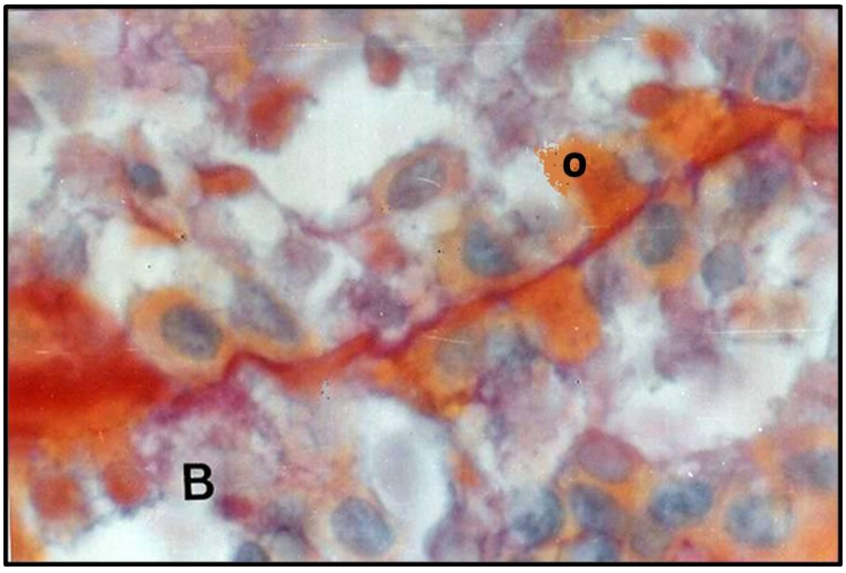

Fig. 4. A section of pituitary gland (pars distalis) of albino rat after withdrawal of DZ showing irregular distributed deeply stained acidophil, most probably somatotroph (S) and large Basophil (B) with remnant of cytoplasm (PFA/AB/PAS/OG stain X 1250).

Electron Microscopic Study

Semi-thin sections prepared from the pars distalis of the control groups (negative or positive) showed that somatotrophs were predominant. In the ultrathin section, they appeared rounded or oval, plus they had rounded eccentric nuclei with dispersed chromatin. They were easily distinguished from other cell types by the presence of numerous round electron-dense secretory granules (the largest granules in the anterior pituitary cells). In some cells, these secretory granules were randomly scattered throughout the cytoplasm, except in the perinuclear region; in other cells, they were concentrated in one part of the cell periphery. The rough endoplasmic reticulum (rER) was found as a series of flattened cisternae arranged in parallel rows in the perinuclear area. Hence, somatotrophs contained large mitochondria (Fig. 5 and 6).

Thyrotrophs were polygonal in shape. They were identified by their secretory granules, which were present all over the cytoplasm. Their secretory granules were the smallest of all types of pars distalis cells. They were spherical and often arranged in a row near the cell membrane. In those cells, rER was poorly developed and found as scattered flattened cisternae. The Golgi apparatus was small (Fig. 5 and 7).

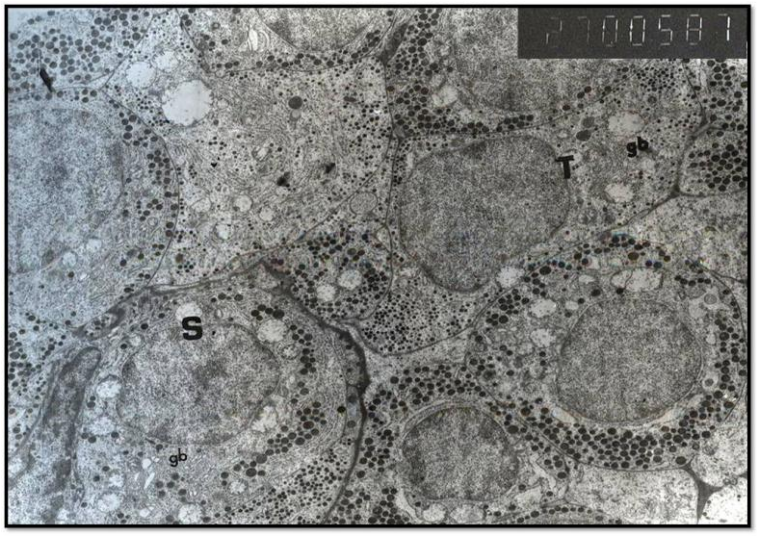

Fig. 5. An electron micrograph of pituitary gland (pars distalis) of control rat showing somatotrophs (S) with large granules and justa nuclear Golgi bodies (gb). Thytotrophs (T) have the smallest secretory granules with the Golgi body (gb) located juxtanuclear (TEM X 2,700).

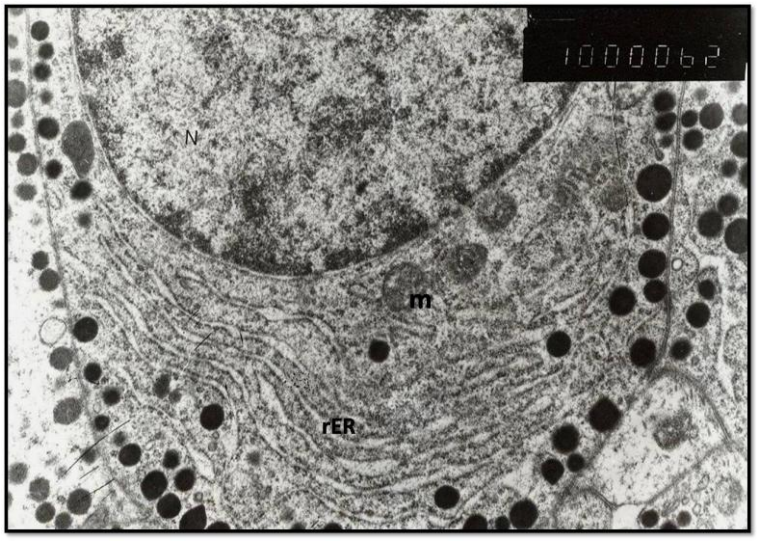

Fig. 6. An electron micrograph of pituitary gland (pars distalis) of control rat shows part of somatotrophs with electron dense, membrane bound large and rounded granules. Well developed Rough endoplasmic reticulum (rER) in relation with the granules and mitochondria (m) are seen (TEM X 10,000).

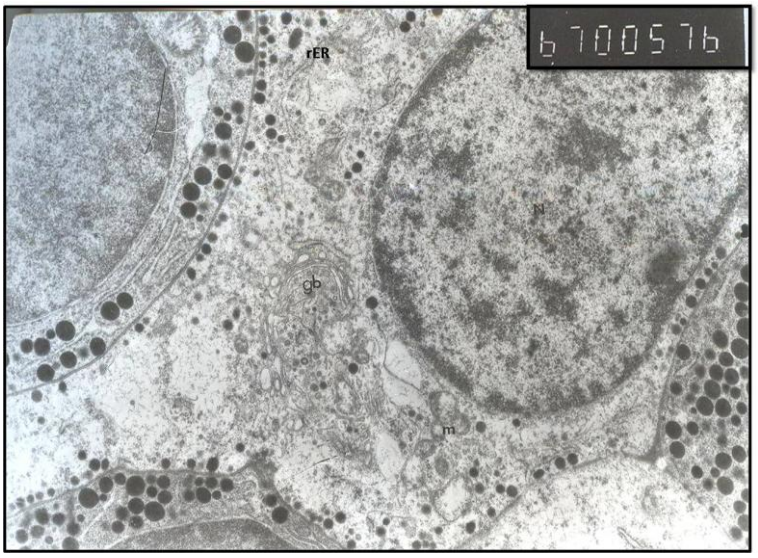

Fig. 7. An electron micrograph of the pituitary gland (pars distalis) of control rat shows part of thyrotrophs having large and rounded nucleus (N). The cytoplasm contains small sized granules, rER, Golgi body (gb) and mitochondria (m) (TEM X 11,000).

After 21 days of DZ administration, distinct morphological changes were observed in most of the pars distalis cells. The changes observed in the somatotrophs varied in degree. Some showed small, dense nuclei, an electron-dense matrix, and large and heterogeneous secretory granules. Multiple vesicles and secondary lysosomes were seen in their cytoplasm (Fig. 8). Other cells were severely affected and could only be identified by their few remaining characteristic granules. The mitochondria possessed disrupted cristae (Fig. 9).

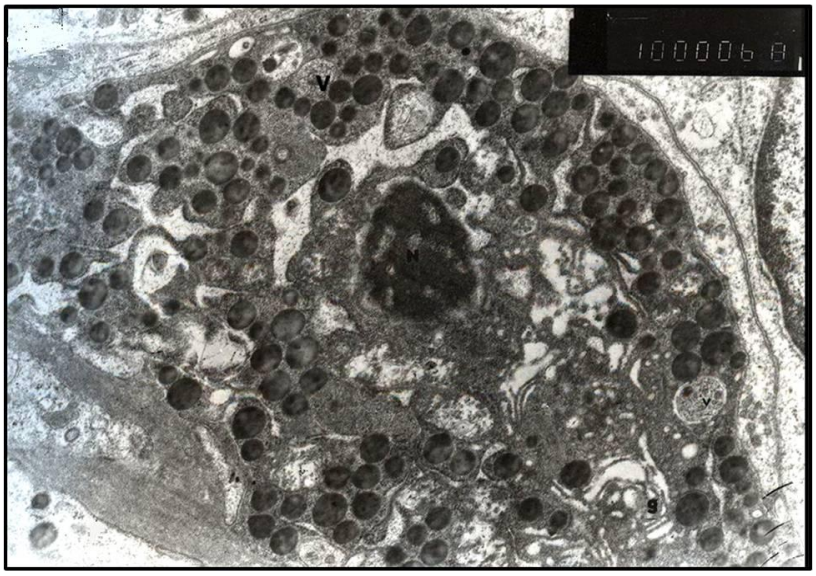

Fig. 8. An electron micrograph of pituitary gland (pars distalis) of albino rat treated for 21 days with DZ shows somatotroph with small dense nucleus (N) and electron dense cytoplasm containing large altered heterogeneous secretory granules, prominent Golgi body (g), some vesicles and secondary lysosomes (V) (TEM X 10,000).

Thyrotrophs were also affected and could hardly be identified by a few small characteristic granules remained in their cytoplasm. The affected thyrotrophs (most probably) had a moderately electron-dense cytoplasm. They contained few organelles, few small secretory granules, and numerous vesicles (mostly pinocytotic vesicles), in addition to some mitochondria with disrupted cristae (Fig. 10).

The effects of DZ withdrawal were obvious by TEM. Most of the cells appeared more or less similar to those of the controls. However, some cells still showed signs of degeneration (*e.g.*, cytoplasm contained

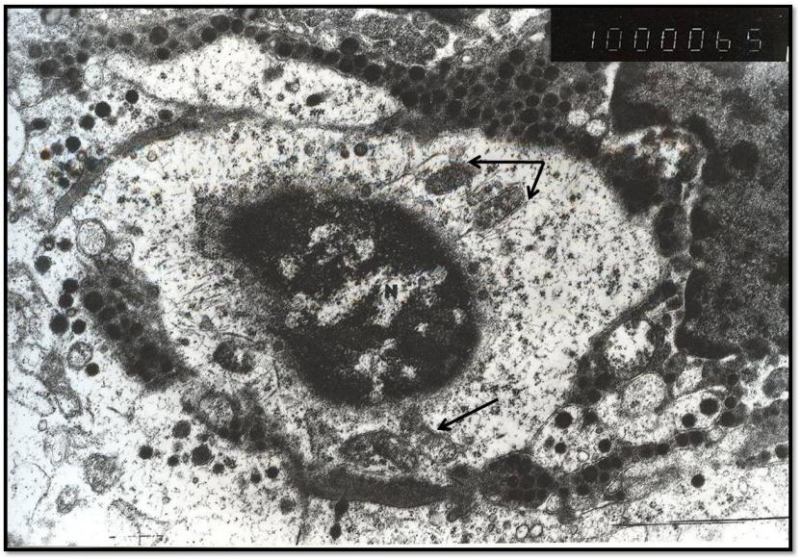

Fig. 9. An electron micrograph of pituitary gland (pars distalis) of albino rat treated for 21 days with DZ shows somatotroph having ill defined boundaries, dense nucleus (N) and electron lucent cytoplasm containing few organelles and granules at the cell periphery. The mitochondria (arrow) show disrupted cristae (TEM X 10,000).

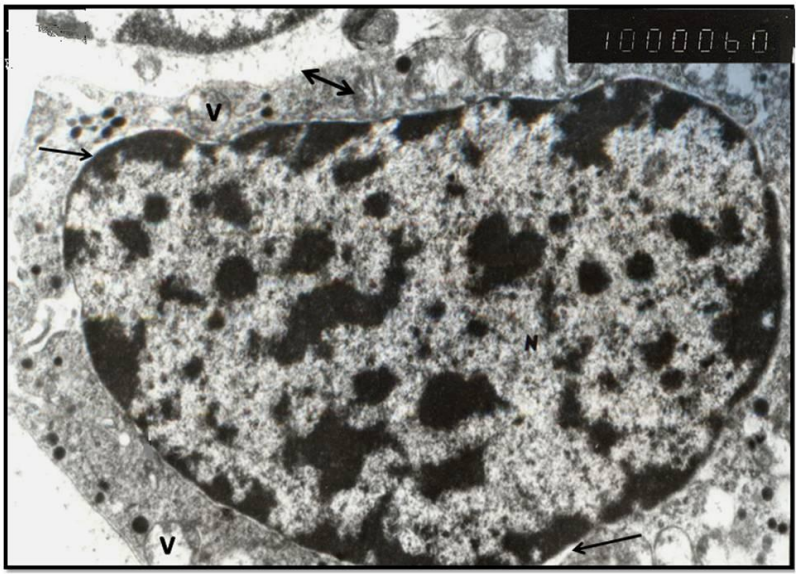

Fig. 10. An electron micrograph of pituitary gland (pars distalis) of albino rat treated for 21 days with DZ shows thyrotroph (most probably) having large nucleus (N) with clumps of chromatin, irregular widely dilated perinuclear space (arrow). Few small secretory granules and numerous vesicles (V), and mitochondria with disrupted cristae (bihead arrow) are seen (TEM X 10,000).

multiple vacuoles) (Fig. 11). Some somatotrophs appeared intact, with the cytoplasm engorged with homogenous secretory granules. They had large nuclei with prominent nucleoli (Fig. 12). A residual effect was seen in the form of dilated cistern of rER and Golgi apparatus, as well as some degenerated mitochondria. They had large nuclei, with prominent nucleoli, and their cytoplasm was engorged with homogenous secretory granules (Fig. 12). Other somatotrophs were even more severely affected. They showed small and electron-dense nuclei and ill-defined cell boundaries. The cytoplasm was moderately electron-dense and contained markedly dilated cisternae of the rER and Golgi body (Fig. 13).

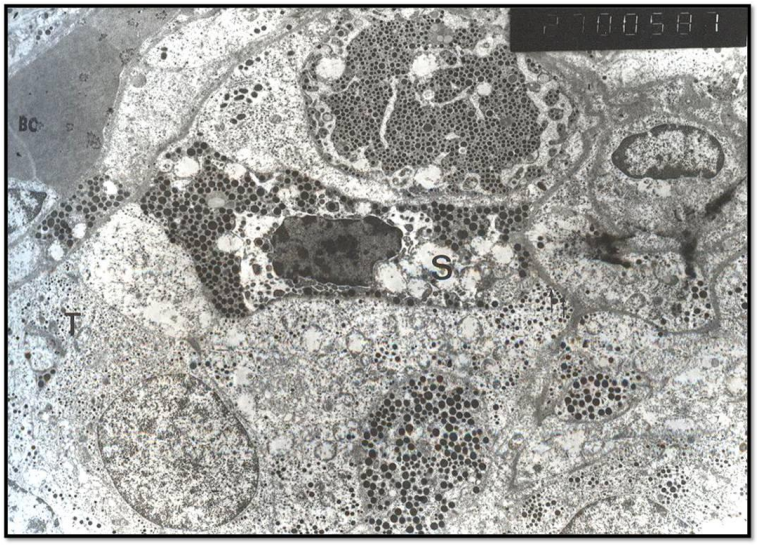

Fig. 11. An electron micrograph of pituitary gland (pars distalis) of albino rat after withdrawal of DZ shows group of chromophilic cells adjacent to dilated blood capillary (BC). Thyrotrophs (most probably) (T) appear intact, while some somatotrophs (S) still show multiple vacuoles (TEM X 2,700).

The majority of thyrotrophs (most probably based on their characteristically small fine granules) that recovered from DZ effects showed signs of recovery. Their nuclei were large and rounded, with prominent nucleoli. They contained small secretory granules and some intact mitochondria, in addition to some dilated rER (Fig. 14). Few thyrotrophs (most probably) could not recover from the effects of DZ and appeared markedly affected. They showed highly dilated rER filled with homogenous moderately electron-dense material (Fig. 15). It was noted

that the cytoplasm of some chromophobes contained some electron-dense granules that were not noticed in the control group (Fig. 16 and 17).

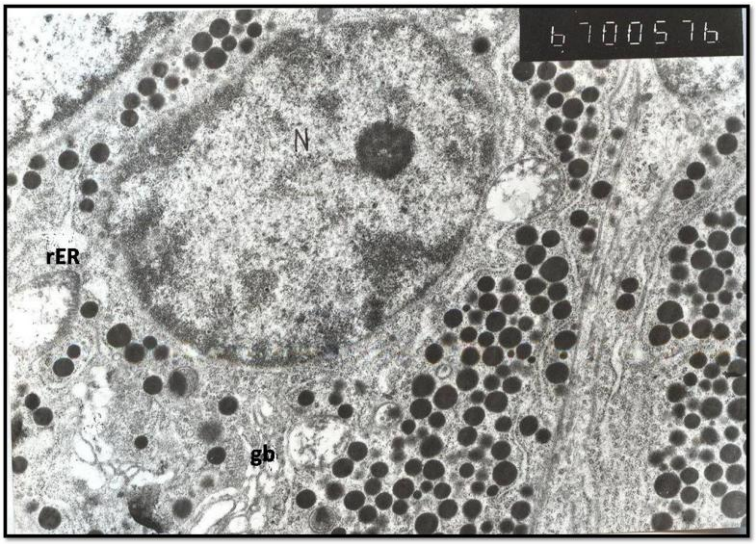

Fig. 12. An electron micrograph of pituitary gland (pars distalis) of albino rat after withdrawal of DZ shows somatotroph with large nucleus (N), and slightly dilated rER, Golgi body (gb) mitochondria (m) with disrupted cristae. Note, the cytoplasm is engorged with homogenous secretory granules (TEM X 6,700).

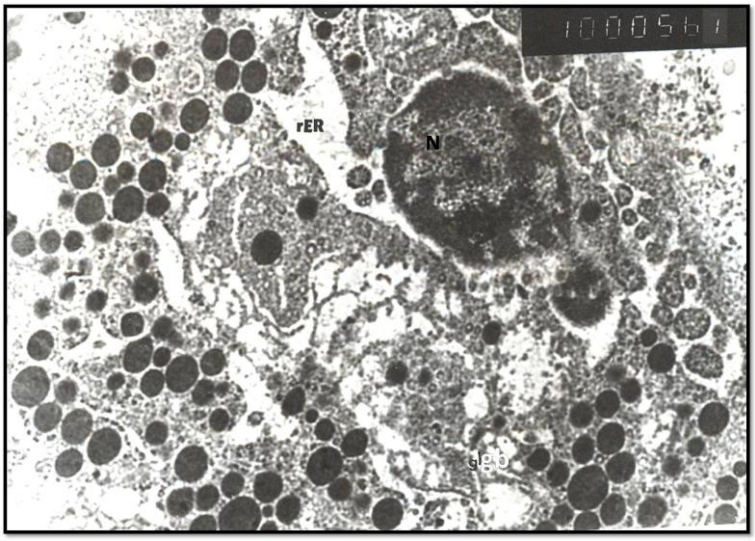

Fig. 13. An electron micrograph of pituitary gland (pars distalis) of albino rat after withdrawal of DZ shows somatotroph with ill-defined boundaries and dense nucleus (N). The cytoplasm is moderate electron dense and contains markedly

dilated rER and Golgi body (gb) cisternae, in addition to some secretory granules (TEM X 10,000).

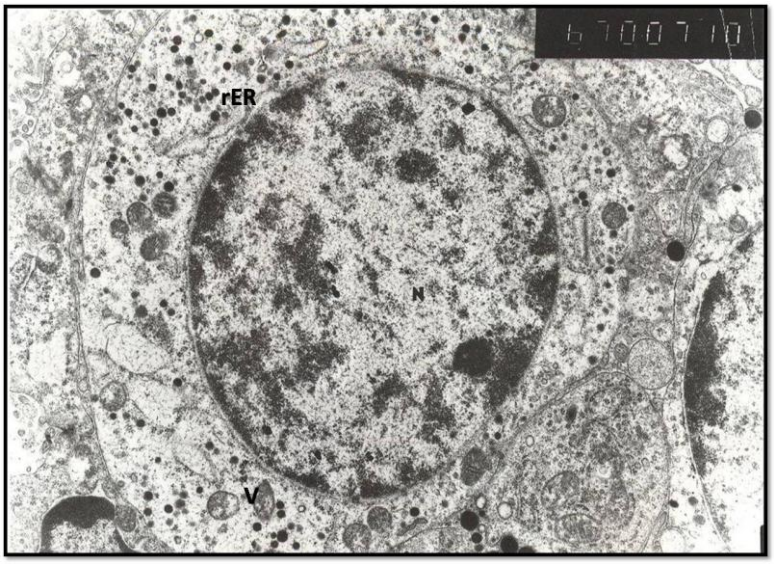

Fig. 14. An electron micrograph of pituitary gland (pars distalis) of albino rat after withdrawal of DZ shows thyrotroph (most probably) having large nucleus (N) with prominent nucleolus and clumps of chromatin. Small sized granules, mitrochondria (arrow) and multiple vesicles (V) are seen. Note the presence of some dilated rER (TEM X 6,700).

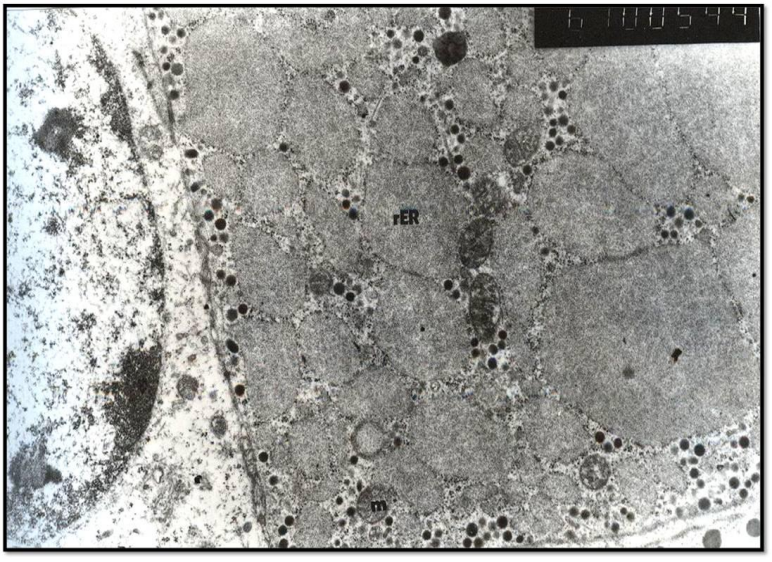

Fig. 15. An electron micrograph of pituitary gland (pars distalis) of albino rat after withdrawal of DZ showing thyrotroph, most probably based on their

characteristic small fine granules having markedly dilated rER filled with homogenous moderate electron dense material in addition to some secretory granules and mitochondria (m) (TEM X 6,700).

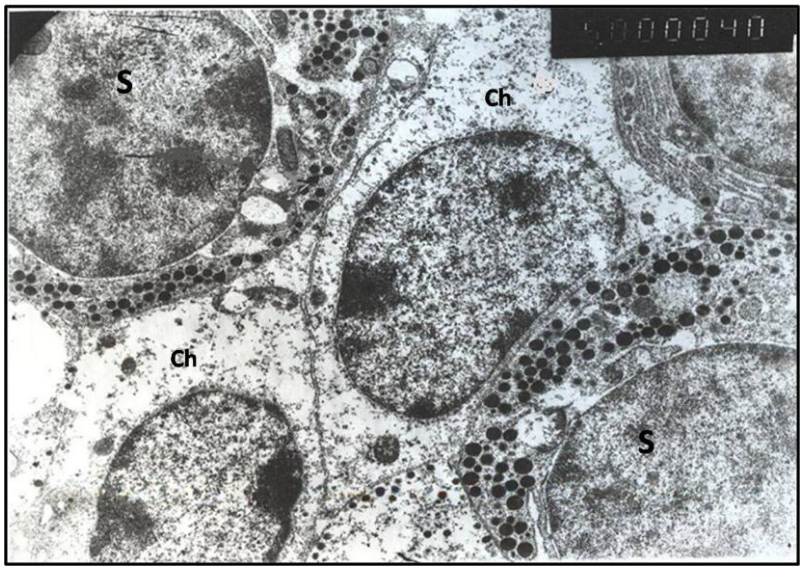

Fig. 16. An electron micrograph of pituitary gland (pars distalis) of control rat showing two chromophobes (ch) enclosed between two somatotrophs (S). The cytoplasm of the chromophobes is scanty and has no secretory granules (TEM X 6,000).

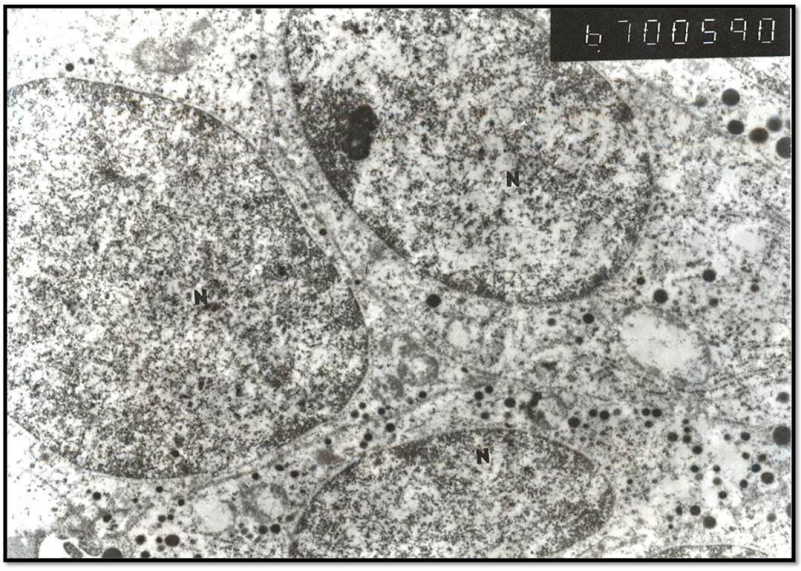

Fig. 17. An electron micrograph of pituitary gland (pars distalis) of albino rat after withdrawal of DZ showing group of chromophobes having large nuclei (N), small and few secretory granules with no characteristic distribution (TEM X 6,700).

Discussion

Diazepam (DZ), the well-known anxiolytic drug, has been reported to have a stimulatory effect on the activity of mammotrophs, gonadotrophs, and corticotrophs[10]. Therefore, the authors decided to study the effect of DZ when administrated for long periods, on both thyrotrophs, and somatotrophs and to explore if this effect is reversible or not.

Somatotrophs make up about 40–50% of the pars distalis cells. The lateral regions contain the greatest number. They are characterized by the presence of numerous round electron-dense secretory granules approximately 350 nm in diameter[11,12].

The results of this study revealed that DZ administration altered the morphology of somatotrophs. The degree of alternation of these cells varied among the rats in the study, and even in the same gland. Most of the somatotrophs showed degenerative changes, while other cells showed lesser degrees of affect. These findings may be attributed to differences in cellular sensitivity to DZ. It could be also explained based on what was reported by Kurosumi and Tosaka, who proposed that somatotrophs are not homogenous, and can be classified into different groups[13]. Hence, their response to various inhibitory or stimulating agents may be different.

Unfortunately, there is no available literature dealing with alternation in the structure of somatotrophs structure after DZ administration. Subsequently, these results can be interpreted, in view of hormonal assays concerning serum growth hormone level following DZ administration. Laakmann *et al*. reported that DZ causes significant dose-dependent growth hormone stimulation in man. The degree of stimulation also depends on the route of administration. In addition, the same authors hypothesized that there are individual variations regarding whether they are growth hormone responders or non-responders[14-16]. Short-period administration (tolerance not yet developed) may explain such stimulating effects with subsequent release of stored growth hormone.

Thyrotrophs make up about 10% of the pars distalis cells. They are concentrated in the medial anterior region and possess the smallest granules compared to other hypophysis parenchymal cells[11,12].

The results of this study showed that DZ administration altered the morphology of thyrotrophs. They contained few organelles, few small-sized secretory granules, and numerous vesicles, which suggested decreased secretory activities. These finding were in contradiction with those of Ugalde and Calderón, who concluded that chronic treatment with diazepam did not produce any visible ultrastructural effects in the secretory cells of the adenohypophysis. They attributed this to the short lifetime of DZ or the lesser effect of DZ metabolites in this species[17]. In the present study, relying on these findings or explanations could not have been possible as it is well known that the action of DZ after being metabolized, is further prolonged by a long half-life of two to five days by its principle active metabolite into nordiazepam[18]. Again, the route and duration of DZ administration by Ugalde and Calderón was not clear.

Unfortunately, research concerning the histological changes of thyrotrophs after DZ administration has been meager. Therefore, again, it could only explain; these results in light of the studies conducted on the effect of DZ on thyroid stimulating hormone (TSH).

Many researchers have reported that TSH released in response to thyrotropin-releasing hormone is inhibited by DZ administration[19-21]. They proved that this inhibition occurs through the central type benzodiazepine receptors *via* competitive inhibition at the pituitary level. In addition, Saleem *et al*., proved that there is a significant decrease in TSH after DZ administration. Their results indicated that cell organelles of thyrotrophs undergo degenerative changes after prolonged duration of DZ administration[22]. Based on the above, one can conclude that the signs of histological degeneration observed in this study were most probably due to lack of stimulation, which resulted from blocking of thyrotropin releasing hormone binding sites of thyrotrophs by DZ.

In this study, it was found that discontinuation of treatment with DZ resulted in varying degrees of response on the part of the individual cells. Thyrotrophs behaved differently from somatotrophs. Most of the somatotrophs that were severely affected after 21 days and showed signs of degeneration still showed a picture of irreversible changes, and were on their way to complete degeneration.

Most of the thyrotrophs recovered from the effects of DZ, apart from a few cells that showed dilated cisternae of rER filled with homogenous moderate electron-dense substance. Accumulation of this substance in the degenerated areas of rat anterior pituitary had been described by Baillif under a severe stimulus of cold exposure. He suggested that this substance is not associated with specific secretion of the gland, but rather with cellular disintegration resulting from long exposure to severe stimulus[23]. This seems to be what happens in cases of DZ administration.

It was observed that some chromophobes showed small secretory granules that were not identical to any of those of the known chromophils, suggesting that these cells might start to compensate for the cells lost due to DZ administration. This is supported by Mescher, as he described chromophobes as a heterogeneous group, including stem and undifferentiated progenitor cells, as well as any degranulated cells present[24]. On the other hand, Junqueira and Carneiro stated that chromophobes had been observed by electron microscope to include two populations of cells. One has few secretory granules, and the other has none[25].

Conclusion

Chronic administration of DZ altered the normal structure of pars distalis cells, particularly the somatotrophs and thyrotrophs that regulate somatic function and metabolism, especially in developing individuals. Discontinuation of DZ administration did not allow the affected cells to return to their normal state. Thus, DZ should be given cautiously, especially in young ages and before puberty. Hormonal monitoring during DZ administration to children should be conducted. Future studies on the possible protective effect of antioxidants against DZ-induced harmful effects on somatotrophs and thyrotrophs will be conducted by the authors.

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References

[1] **Kreft M, Zorec R.** Anterior pituitary cells excited by GABA. *J Physiol* 2008; **586**(13): 3023-3024.

[2] **Guyton AC.** The pituitary hormones and their control by the hypothalamus, In: *Textbook of Medical Physiology*. 7th ed. chp 75, London: Saunders, 1986. 889-896.

[3] **Wiens SC, Trudeau VL.** Thyroid hormone and γ -aminobutyric acid (GABA) interactions in neuroendocrine systems, A review. *Comp Biochem Physiol A Mol Integr Physiol* 2006; **144**(3): 332-344**.**

[4] **Katzung BG, Masters SB, Trevor AJ.** Basic and clinical pharmacology, and seizure drugs. chp 24. 11th ed, New York: McGraw–Hill/Lange., 2009. 23-33.

[5] **Atack JR.** GABA (A) Receptor Subtype-Selective Modulators. I. α2/α3-Selective Agonists as Non-Sedating Anxiolytics. *Curr Top Med Chem* 2011; **11**(9): 1176-202.

[6] **Tyrer P, Rutherford D, Huggett T.** Benzodiazepine withdrawal symptoms and propranolol. *Lancet* 1981; **1**(8219): 520-523.

[7] **Grandison L.** Suppression of prolactin secretion by benzodiazepines in vivo. *Neuroendocrinology* 1982; **34**(5): 369-73.

[8] **Drury RAB, Wallington EA.** Carleton's Histological technique. 5th ed. Oxford, New York, Toronto: Oxford U P, 1980. 42.

[9] **Gupta PD.** Ultrastructural study on semithin section. *Sci Tools* 1983; **30**(1): 6-7.

[10] **Mohamed SA, Ali SA, Saleh MM, Shehata MM.** Effect of Diazepam on the structure of the rat pars Distalis. *Assuit Vet Med J.* 1995; **33**(65): 34-50.

[11] **Ichihara I.** Electronmicroscopic studies of anterior pituitary glands of vitamin E-deficient male mice. *J Anat* 1969; **104**(3): 455-465.

[12] **Krause WJ.** Krause's Essential Human Histology for Medical Students. 3rd ed University of Missouri Columbia, Missouri. 2005. 265-268.

[13] **Kurosumi K, Tosaka H.** Prenatal development of growth hormone producing cells in the rat anterior pituitary as studied by immunology electron microscopy. *Arch Histol Cytol* 1988; **51**(2): 193-204.

[14] **Laakmann G, Hinz A, Voderholzer V, Caffner C, Muller OA, Neuhanser H, Neulinger E, Wittmann M.** The influence of psychotropic drugs and releasing hormones on anterior pituitary hormones secretionin the healthy subjects and depressed patients. *Pharmacopsychiat* 1990; **23**(1): 18-25.

[15] **Laakmann G, Treusch J, Eichmeier V.** Inhibitory effect of phentolamine on diazepam induced growth hormone secretion and lack of effect of diazepam on prolactin secretion in man. *Psychoneuroendocrinol* 1982; **7**(2-3): 135-139.

[16] **Laakmann G, Treusch J, Schmasuss M, Schmitt B, Trensch V.** Comparison of growth hormone stimulation induced by Desimipramine, Diazepam and metaclazepam in man. *Psychoneuroendocrinol* 1982; **7**(2-3): 141-146.

[17] **Ugalde HS, Calderón FU.** Ultrastructure of adenohypophyseal cells of white rats subjected to the effect of a benzodiazepine in chronic form. *Rev Biol Trop* 1987; **35**(1): 15-19.

[18] **Martindale WJ, Reynolds EF.** The extrapharmacopoeia. 29th ed. London: Pharmaceutical P, 1989. 122-126.

[19] **Russel JP, Astier H, Tapia AL.** Benzodiazepines inhibit thyrotropin (TSH)- releasing hormone- induced TSH and growth hormone releasing from perifused rat pituitaries. *Endocrinol* 1986; **119**(6): 2519-2526.

[20] **Tapia AL, Alonso R, Astier H.** Evidence of the role of central type benzodiazepine receptors in the inhibition of thyrotropin releasing hormone induced from rat perefused pitutariey. *Neurosci. Letters* 1986; **71**(3): 329-334.

[21] **Pivac N, Perićić D.** Inhibitory effect of diazepam on the activity of the hypothalamic-pituitary-adrenal axis in female rats. *J Neural Transm Gen Sect* 1993; **92**(2-3): 173-186.

[22] **Saleem TH, Abdel Ghany SM, Mohamed SA.** Effect of diazepam and midazolam on thyroid function and structure. *Assiut Vet Med J* 1991; **26**(51): 67-80.

[23] **Baillif FN.** Microscopic changes in the hypophysis of the albino rat following exposure to cold and their relationship to the physiology of section. *Am J Anat* 1973; **62**(4): 475-495.

[24] **Mescher AL.** Junqueira s Basic Histology. Text and Atlas. 12th ed. Toronto: McGraw Hill Medical. 2010. 351.

[25] **Junqueira LC, Carneiro J.** Basic Histology, text and atlas. Toronto: McGraw-Hill. 2005. 393.

هل تستطيع الخلايا الحافزة للنمو والحافزة للغدة الدرقية التعافى من تأثيرعقار الديازيبام بعد توقف تعاطيه؟ دراسة للتركيب الدقيق

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المستخلص. هدفت هذه الدراسة لفحص تأثير عقار الديازيبام على التركيب الدقيق لخلايا حافزات النمو وحافزات الغدة الدرقية. وقد استخدم لهذا الغرض أربعين فأراً بالغاً قسمت إلى مجموعتين: الأولى ضابطة والثانية تجريبية، وقد قسمت بدورها إلى مجموعتين فرعيتين حقنت أولاهما داخل الغشاء البريتونى بعقار الديازيبام الخام يوميا لمدة ٢١ يوم، بينما تركت الثانية لمدة شهر بعد حقنها بنفس جرعة العقار لمدة ٢١ يوم. وقد تم تجهيز عينات من الجزء القاصي من الغدة النخامية من جميع الفئران وفحصها بكل من الميكروسكوب الضوئي والإلكتروني. وقد أظهرت النتائج أن الديازيبام اسفر عن تحلل أغلب الخلايا الحافزة للنمو بينما ظهر القليل منها غير بالغ التأثر. كما ظهرت حافزات الغدة الدرقية ذات درجات متفاوتة من التأثر بالعقار. أما انسحاب العقار فلم يسفر عن توقف عملية التحليل

فى الخلايا الحافزة للنمو شديدة التأثر. وقد تمكنت بعض حافزات الغدة الدرقية من التعافى من تأثير العقار، بينما لم يتمكن البعض الآخر من ذلك. لذا يوصى بعدم استخدام هذا العقار إلا عند الحاجة الضرورية، وكذالك بالحذر الشديد عند استخدامه وخاصة من قبل الأطفال لتفادى تأثيره الضار على الغدة النخامية مع متابعة مستوى هرمونات الغدة النخامية.