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Original Article

Histological, Morphometric and Hormonal Assay Study on the Effects of Phenobarbital Sodium on the Different Endocrine Glands in Adult Male Albino Rats

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ABSTRACT

Introduction: Phenobarbital (PB) is a commonly used classical anticonvulsant drug. It is effective in treating partial tonic-clonic fits. Adverse effects of antiepileptic drugs on the endocrine glands were reported producing disturbed endocrine function.

Aim of the work: Was to study the possible effect of phenobarbital administration on rats' different endocrine glands using histological, hormonal assay and morphometric studies.

Material and Methods: Fifty adult male albino rats were used, divided into 3 groups; A control group (I); consisted of 10 rats, phenobarbital group (II); contained 20 rats, each rat received 7.5 mg phenobarbital sodium/100gm body weight orally for 30 days. PB withdrawal group (III); consisted of 20 rats, each received the same PB dose for 30 days orally and have been left without treatment for another 15 days.

Results: Examination of the pituitary, thyroid and adrenal gland sections of PB group showed dilatation and congestion of blood sinusoids, cytoplasmic vacuoles and irregularity of nuclei of pituitary secretory cells, marked decrease in both follicular size and colloid contents with destruction of the microvillus border of the thyroid follicles, increase in thicknesses of both adrenal cortex and medulla with disruption of the architecture of adrenal cortical layers. Examination of the PB withdrawal group showed evidence of improvement in the three glands as compared to the PB group.

Conclusion: Phenobarbital can cause harmful histopathological changes on the thyroid gland, adrenal cortex and pituitary gland and its withdrawal resulted in an improvement in these deleterious effects to a lesser extent.

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Key Words: Adrenal gland, phenobarbital, pituitary gland, rats, thyroid gland,.

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INTRODUCTION

Phenobarbital (PB) is a commonly used classical anticonvulsant drug. It is effective in treating partial tonic-clonic fits and has some sedative action. It may produce tolerance with long time usage[1].

It acts as a nonselective depressant of the central nervous system so it can produce all levels of central nervous system (CNS) mood alteration, ranging from excitation to mild sedation and hypnosis, and even administration of high doses of barbiturates induces anesthesia[2].

The anticonvulsant, hypnotic and sedative effects of barbiturates are due to their enhancement of the inhibitory synaptic action of gamma-amino-butyric acid (GABA), also PB blocks calcium and sodium channels and antagonizes glutamate receptors[3,4].

The physiological action of barbiturates is produced through depressing the sensory cortex, decreasing the motor activity that changes the cerebral function. also, barbiturates inhibit the conduction in the reticular formation, thus interfering the transmission of impulses from the thalamus to the cerebral cortex[5].

Phenobarbital can be absorbed from the gastro-intestinal tract in spite of being lipid-insoluble and may require an hour or more to reach the effective concentrations needed. About 45% of Phenobarbital is bound to plasma proteins and only a part of this amount can be metabolized in

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the liver, while about 25% of the dose is excreted in urine unchanged[2].

Phenobarbital is rapidly distributed to all tissues and body fluids and attains more concentrations in the brain, liver and kidneys, so it was reported that phenobarbital lowers serum bilirubin concentration probably by induction of glucuronyl transferase, the enzyme which conjugates bilirubin[6].

Several authors also reported teratogenicity produced by phenobarbital administration during pregnancy and the range of defects in the phenobarbital-exposed infants includes different forms of neural tube defects and cardiac anomalies[7].

Other articles reported adverse effects of antiepileptic drugs on the endocrine glands producing disturbed endocrine function and some metabolic hormone concentration of the serum can be affected. In addition, long-term antiepileptic treatment with valproate, carbamazepine, and phenobarbital in children suffering from epilepsy caused subclinical hypothyroidism[8, 9].

AIM OF THE WORK

The aim of the present research is to study the effect of long period administration of phenobarbital sodium on the endocrine glands (the pituitary, thyroid and the adrenal glands) of adult male albino rats using histological, morphometric and hormonal assay methodsxifylline on diabetic vasculopathy in the rat model.

MATERIAL AND METHODS

In this research 50 adult male albino rats weighing 150-200 grams were used. These rats were obtained from the Animal House of Faculty of Medicine-Tanta University. The animals were housed in suitable cages, each cage contained five animals and the rats were kept under standard environmental conditions having the same food and water supply.

Animals groups

Group I (control group):

This group contained 10 rats left without treatment and were furtherly subdivided into two subgroups. Subgroup Ia formed of 5 rats, which were sacrificed after 30 days and subgroup Ib formed of 5 rats, which were sacrificed after 45 days.

Group II: (phenobarbital group):

This group contained 20 rats, received 7.5 mg phenobarbital sodium/100gm body weight, once daily by orogastric tube for 30 days, then the rats have been sacrificed[10].

Group III: (PB Withdrawal group):

This group contained 20 rats, and the rats received 7.5 mg phenobarbital sodium / 100gm body weight daily for 30 days via orogastric tube, then, they have been left without treatment for another 15 days, after that the rats were sacrificed[10].

Phenobarbital was purchased from Alexandria Drug Company carrying a trade name called 'sominaletta syrup' with a concentration of 15mg\5ml.

Methods:

At the appropriate time of each group, the rats were anesthetized with ether, sacrificed by decapitation and then subjected for dissection. Specimens from the pituitary, thyroid and the adrenal glands were collected.

Specimens of the pituitary, thyroid and suprarenal glands were divided into two halves. One half was fixed in 10% formol saline for light microscopic examination and the second half was fixed in glutaraldehyde buffer solution for transmission electron microscopy.

Histological Techniques:

Preparation for light microscopic (LM) study: Specimens from the glands were immediately immersed in 10% neutral formol saline for 24 hours, then all the specimens were prepared for staining with Haematoxylin and Eosin (H & E) stain to be examined with the light microscope[11].

Preparation of ultrathin sections for electron microscopic (EM) examination:

Specimens from the pituitary, thyroid and adrenal glands from each animal were divided

into small pieces (1um in thickness), then they were fixed as soon as possible in 2.5-3 % phosphate buffered glutaraldehyde solution for two hours at 4 degrees centigrade, then washed and post fixed in 1% phosphate buffered osmium tetroxide for 1 – 2 hours at room temperature and then rinsed three times in phosphate buffer solution. The specimens passed through the dehydration steps and finally immersed in epoxy resin mixture to form capsules, which will be trimmed for preparing sections about (40 – 50 nm) in thickness, stained with uranyl acetate and lead citrate, examined and photographed on the JEOL 100 JEM (Tokyo Japan) electron microscopic unit of the Faculty of Medicine, Tanta University[12].

Morphometric study:

In all groups, the thyroid follicular size and the surface area of follicular colloid contents in thyroid sections were measured in 10 fields at magnification 400/slide (Fig. 3a).

In all groups, the adrenal cortex and medulla thicknesses of the adrenal gland sections were measured in 10 fields at magnification 100/slide (Fig. 5a)

The images were examined using Leica Qwin 500 LTD (Leica Germany) image analyzer computer system in the central research laboratory, Faculty of medicine, Tanta University.

Blood sampling for hormonal assay study:

Blood samples were obtained from all rats directly from left ventricle while the rats under anesthesia and still viable then the blood sample were centrifuged in Tanta University Central Laboratory unit and finally analyzed in clinical pathology department of Tanta university hospital to determine the serum levels of free T3 and cortisol hormones.

Statistical Analysis

Data obtained from hormonal assay and image analyzer were analyzed using Statistical Package for the Social Science (SPSS; SPSS Inc., Chicago, Illinois, USA) version 20. Data were compared by using mean, standard deviation and analytical statistics student's f- test to compare two groups and ANOVA test to compare three groups. $P \leq 0.05$ was considered statistically significant[13].

RESULTS

The current study has examined the effect of phenobarbital sodium over the thyroid, adrenal and pituitary glands of fifty adult male albino rats.

1. Histological results:

I: Pituitary gland:

A. light microscopic study:

H.&E. stained sections of the control group revealed the normal architecture of both adenohypophysis and neurohypophysis. The adenohypophysis showed secretory cells with the distinctive cell types (acidophils and basophils) and the color of staining denotes the chemical consistency of the hormone-laden granules inside these secretory cells. Normal shaped blood sinusoids were seen in between cells (Figs. 1a, 1b).

H.&E. stained sections from group II showed dilated and congested blood sinusoids between the pituitary secretory cells with cytoplasmic vacuolation of these cells (Figs.1c, 1d), while sections of the pituitary glands from group III showed mild congestion of blood sinusoids and cytoplasmic vacuoles could be detected in some secretory cells (Fig. 1e).

B. Electron microscopic study:

Electron microscopic examination of pituitary gland sections of the control group showed cells of the adenohypophysis containing central rounded nuclei with condensed peripheral chromatin, well developed rough endoplasmic reticulum (rER) and showed normal shaped mitochondria. Many small dark secretory granules were noticed inside the cells (Figs. 2a, 2b).

Ultrathin sections from group II showed cells of adenohypophysis containing irregular nuclei, dilated rER, irregular shaped mitochondria, many cytoplasmic vacuoles and few secretory granules could be seen within the cells (Figs 2c,2d).

Ultrathin sections of the pituitary gland from group III showed cells of the adenohypophysis containing irregular nuclei, slightly dilated rough endoplasmic reticulum and the cytoplasm contained few vacuoles and many secretory granules could be seen (Fig. 2e)

II: Thyroid gland:

A. Light microscopic study:

H.&E. stained sections of the thyroid glands from the control group showed the normal thyroid architecture in the form of thyroid follicles filled with homogenous colloid with peripheral active colloid vacuoles. The collections of the para-follicular cells form clumps between the thyroid follicles and the blood vessels in-between follicles could be seen. In higher magnification, the thyroid sections showed the thyroid follicles lined with cuboidal cells with basal basophilic nuclei and acidophilic cytoplasm (Figs. 3a, 3b).

H.&E. stained sections of the thyroid gland of group II, showed multiple small thyroid follicles with decreased colloid and increased colloid vacuoles inside their lumens, other follicles were devoid of colloid and others were distorted. Some follicular cells showed dark deeply stained pyknotic nuclei and the cytoplasm showed multiple vacuoles. Congested and dilated blood vessels in the interfollicular tissues could be seen (Figs. 3c, 3d).

Sections of the thyroid gland from group III showed some thyroid follicles with vacuolated colloid while other follicles were full of normal homogenous colloid. The cytoplasm of some follicular epithelial cells showed some vacuoles while other cells appeared normal (Fig. 3e).

B: Electron microscopic study:

Ultra-thin sections from the thyroid glands of the control group revealed the thyroid follicular cells with normal apical microvilli brush border, central nucleus with condensed peripheral chromatin. It also showed well developed rER and groups of rounded mitochondria (Figs. 4a, 4b).

Sections of the thyroid gland of group II showed the thyroid follicular cell containing destructed microvilli border, irregular nuclei, dilated rER, swollen mitochondria and the cytoplasm showed multiple vacuoles (Figs. 4c, 4d).

Examination of ultra-thin sections of the thyroid gland from group III showed the thyroid follicular cells with slightly irregular nuclei, mildly dilated rER, slightly affected mitochondria and the apical border facing the follicular colloid showed few microvilli (Fig. 4e).

III: suprarenal gland:

A: light microscopic study:

H & E stained sections of the suprarenal gland of control group revealed that the gland formed of an outer cortex and an inner medulla and surrounded by a connective tissue capsule. The cortex was divided into three zones; zona glomerulosa, zona fasciculata and zona reticularis. The zona fasciculata appeared as polyhedral cells with distinct cell membranes, large rounded nuclei and the cytoplasm contained numerous small lipid vacuoles (Figs. 5a, 5b).

Examination of H & E stained sections of the suprarenal gland from group II showed many vacuoles in the cytoplasm of zona fasciculata cells. Some zona fasciculata cells showed pyknotic nuclei while other nuclei showed karyorhexis (Fig. 5c).

H & E stained sections of the suprarenal gland from group III, showed the zona fasciculata cells with central rounded nuclei and some vacuoles could be seen in their cytoplasm (Fig. 5d).

B: Electron microscopic study:

Ultra-structural examination of the adrenal gland of the control group revealed cells of the zona fasciculata containing rounded nuclei with condensed peripheral chromatin. The cytoplasm contained rounded mitochondria and lipid droplets could be seen within the cytoplasm (Figs. 6a, 6b).

Ultrathin sections of zona fasiculata cells of group II showed irregular nuclei, irregularly shaped mitochondria with destructed cristae and cytoplasmic vacuoles could be seen (Fig. 6c).

Sections of zona fasiculata from group III showed zona fasiculata cells with apparent normal nuclei, their cytoplasm showed some vacuoles and rounded mitochondria were seen (Fig. 6d)

2. Morphometric study:

I: Thyroid gland

1. Measurements of the thyroid follicular size:

The mean of follicular size of group II were significantly decreased compared to the follicular size of the control group. There was a statistically

significant difference between the follicular sizes of both groups (*P value < 0.001*). The mean of follicular size of group III was slightly decreased with a statistically significant difference between follicular sizes of this group and the control group (*P value < 0.001*) with a significant difference between follicular sizes of group III and group II (*P value < 0.001*) (Table 1) (graph. 1).

2. Measurements of Follicular colloid surface area:

The mean of colloid surface area of group II was markedly decreased compared with the colloid surface area of the control group, with a statistically significant difference between the two groups (*P value < 0.001*). The mean of colloid surface area of group III showed a minimal decrease with a statistically significant difference between group III and the control group (*P <0.001*) with a statistically significant difference between groups II and III (*P value <0.001*) (Table 1) (graph 2).

II. Adrenal gland:

1. Measurement of the adrenal cortex thickness (Fig. 5a):

The mean of the adrenal cortex thickness in group II showed marked increase with a statistically significant difference between thicknesses in the control group and that of group II with *P value < 0.001*. The mean of the adrenal cortex thickness in group III was nearly normal compared with that of the control group with a statistically significant difference between the thickness in this group and group II with *P value < 0.001* (Table 2) (graph3).

2. Measurement of adrenal medulla thickness:

The mean of the adrenal medulla thickness of group II showed marked increase, with a statistically significant difference between the medullary thickness in this group and the control group with *P value < 0.001*. The mean of the adrenal medulla thickness of group III showed marked increase and there was a statistically significant difference between medullary thickness of this group and the control group with *P value < 0.001* (Table 2) (graph. 4).

3. Hormonal assay:

I. Free T3 level:

The mean of free triiodothyronine (T3) level in group II was markedly decreased while the mean of free T3 level in group III showed a minimal decrease. There was a statistically significant difference between free T3 levels of both control and group II with *P value < 0.001* and there was a statistically significant difference between groups III and II with *P value < 0.001*. There was no statistical difference in free T3 levels between the control group and group III with *P value 0.017*. (Table. 3) (graph.5).

II. Cortisol level:

The mean of cortisol level of group II was decreased and there was a statistically significant difference between this group and the control group. The mean of cortisol level of withdrawal group was decreased and there was a statistically significant difference between withdrawal and control group with *P value < 0.001* (Table 4) (graph 6).

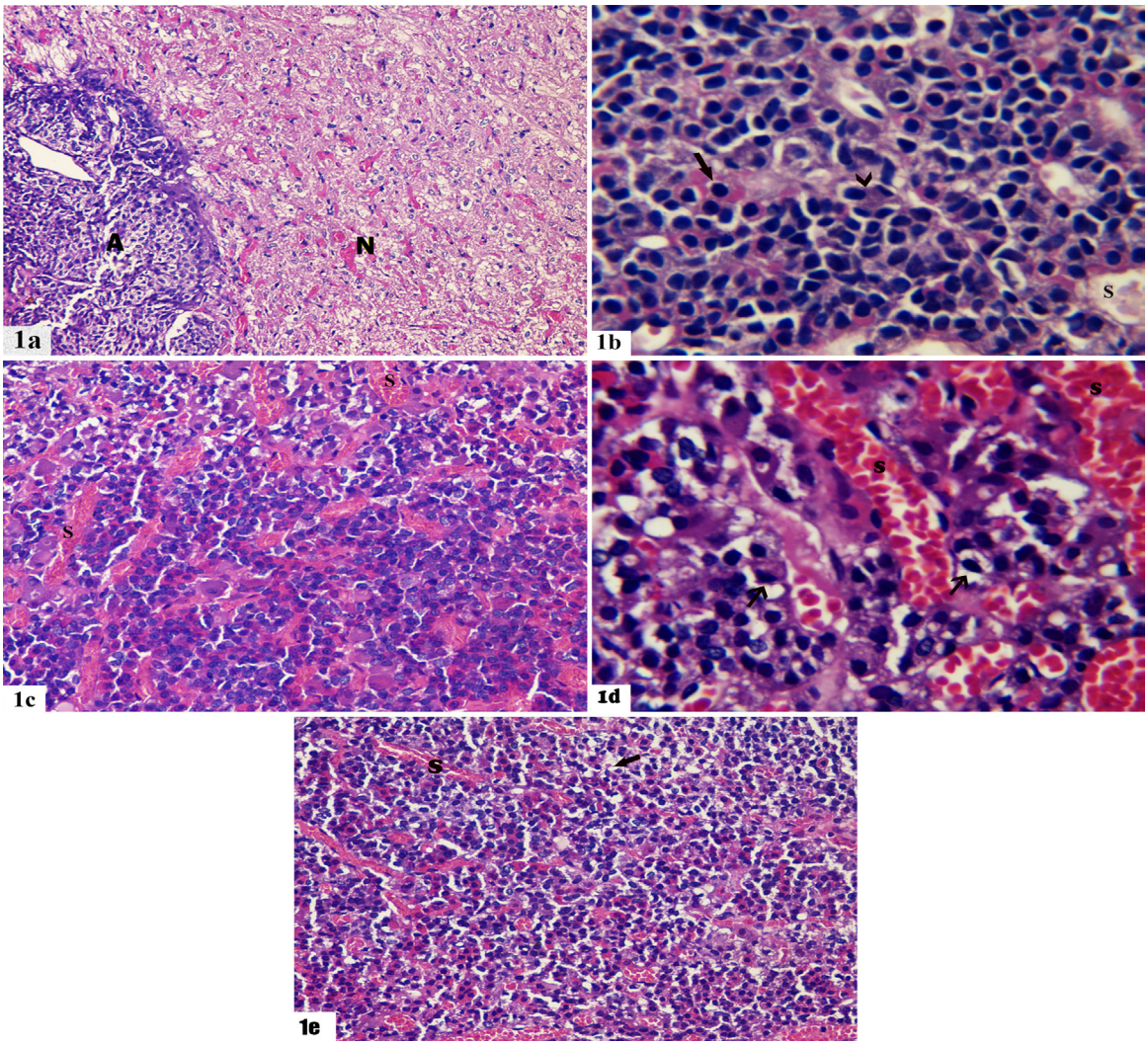


Fig. 1: Photomicrographs of sections in the pituitary gland showing:
(1a): The normal architecture of adenohypophysis (A) and neurohypophysis (N) in the control group (group I) . (H & E; X 200).
(1b): A section in the adenohypophysis from control group showing many acidophils (black arrow) and basophils (arrow head). The blood sinusoids (S) appear normal in between cells. (H & E; X 1000).
(1c): A section in the adenohypophysis from (group II) showing acidophils and basophils with dilated and congested blood sinusoids (S). (H & E; X 400).
(1d): A section in the adenohypophysis from (group II) showing dilated and congested blood sinusoids (S) and many vacuoles can be seen in the cytoplasm of the secretory cells (black arrows). (H & E; X 1000).
(1e): A section in the adenohypophysis from (group III) showing some secretory cells (black arrow) still affected with vacuolations of their cytoplasm and mildly congested blood sinusoids (S) can be seen. (H & E; X 400).

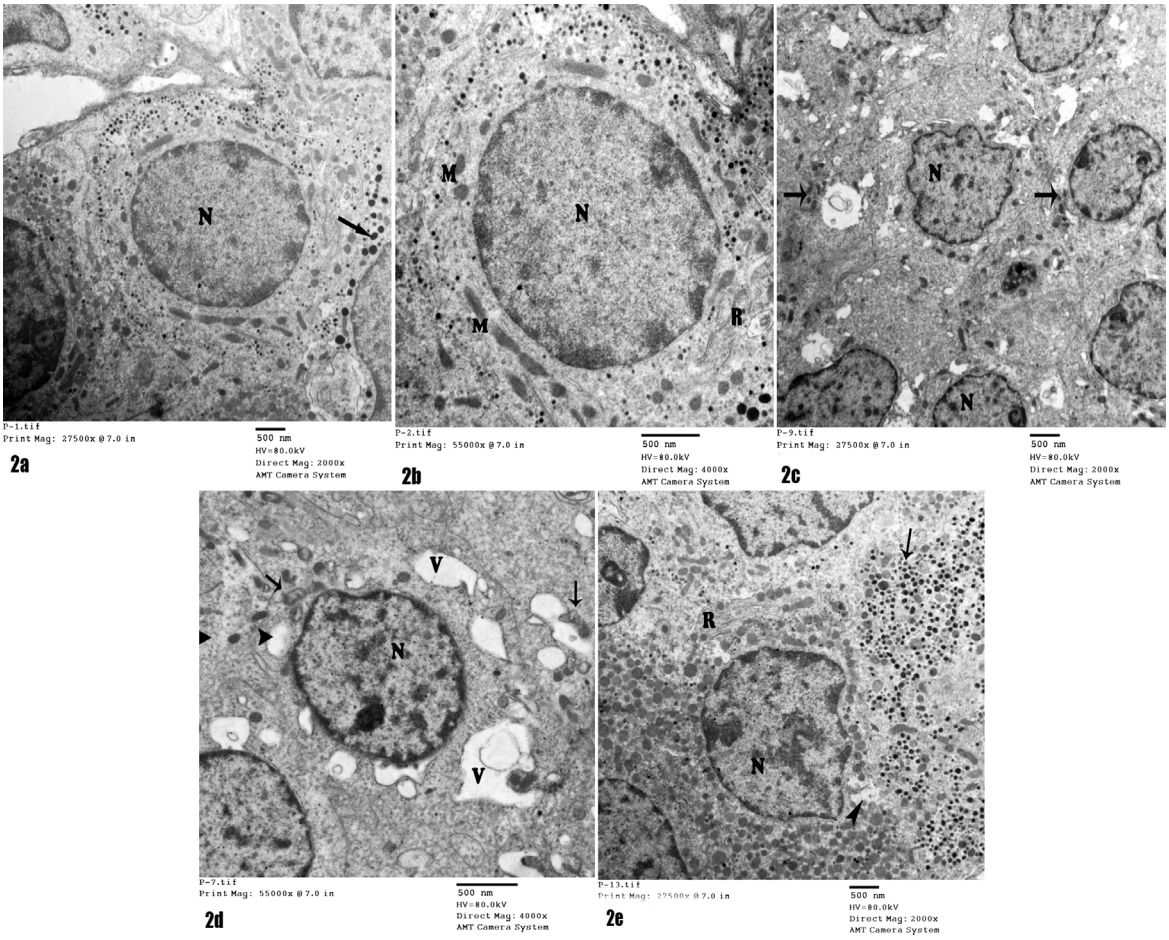


Fig. 2: Electron micrographs of ultrathin sections in the pituitary gland showing:
(2a): An ultrathin section in control group showing the adenohypophysis cells with central rounded nucleus (N) containing peripheral condensed chromatin and many secretory granules (arrow) can be seen. (X 2000).
(2b): A higher magnification of the previous micrograph showing rounded nucleus (N) with condensed peripheral chromatin, normal shaped mitochondria (M) and well developed rough endoplasmic reticulum (R). (X 4000).
(2c): An ultrathin section of the pituitary gland from (group II) showing adenohypophysis cells with irregular nucleus (N) and few secretory cells (arrow) in the cytoplasm. (X 2000).
(2d): An ultrathin section of (group II) showing a cell of adenohypophysis with irregular nucleus (N), irregular mitochondria (arrows), dilated rough endoplasmic reticulum (arrow heads) and many vacuoles can be seen in the cytoplasm (V). (X 4000).
(2e): An ultrathin section of (group III) showing cells of adenohypophysis with irregular nucleus (N) slightly dilated rough endoplasmic reticulum (R), few cytoplasmic vacuoles (arrow head) and many secretory granules (arrow) can be seen in its cytoplasm. (X 2000).

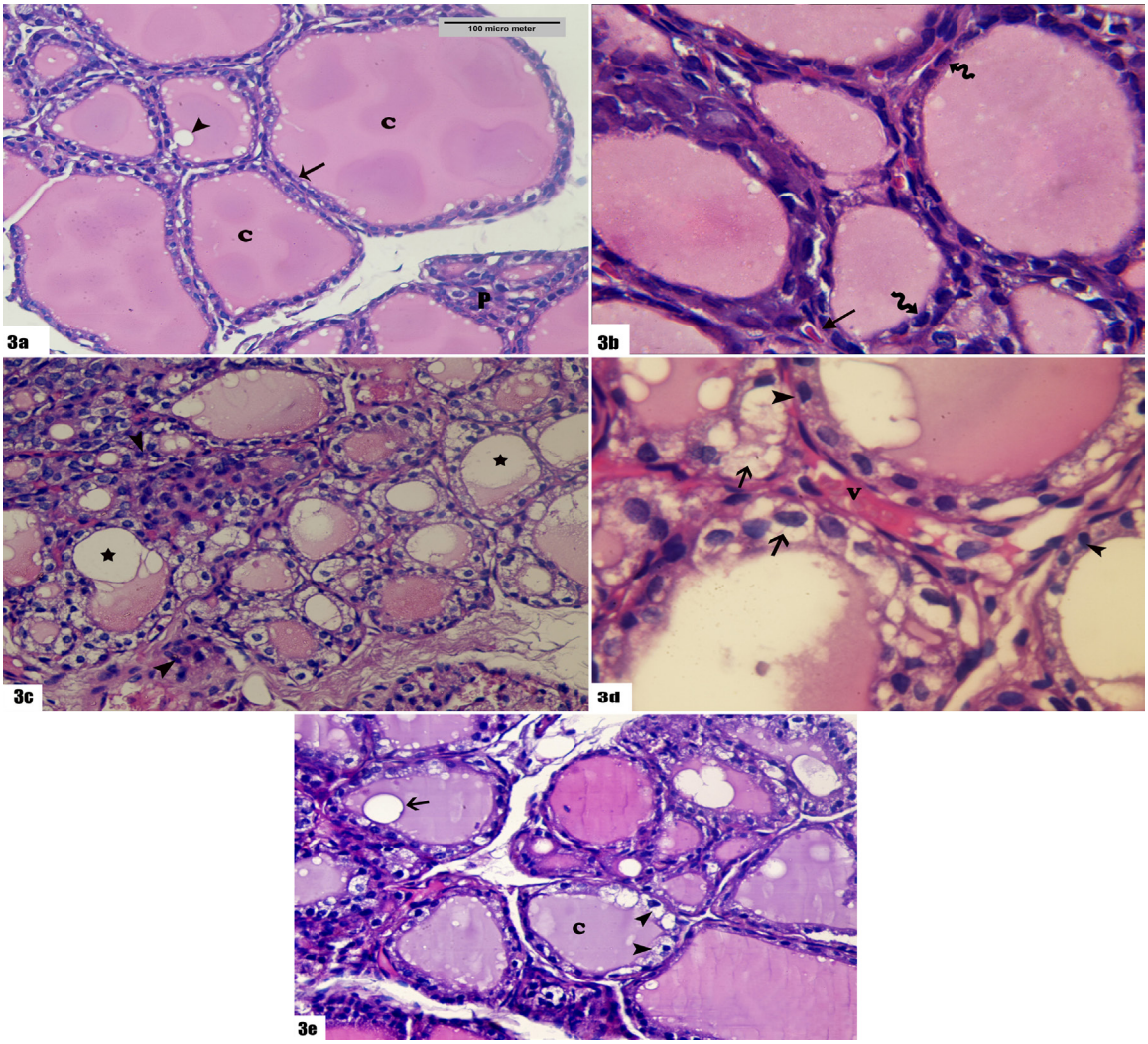


Fig. 3: Photomicrographs of sections in the thyroid gland showing:
(3a): A section in the control group (group I) showing thyroid follicles of various sizes lined with follicular cells (arrow), their lumen filled with homogenous colloid (C) showing peripheral colloid vacuoles (arrow head). Clumps of Para follicular cells (P) can be seen. (H & E; X 400).
(3b): A section in the control group showing the thyroid follicles lined with cuboidal cells (curved arrows) with basal basophilic nuclei and eosinophilic cytoplasm. Normal shape blood vessels can be seen between follicles (arrow). (H & E; X 1000).
(3c): A section in group (II) showing the thyroid follicles with various sizes and most of them are small in size, some follicles are devoid of colloid (star) and other distorted follicles (arrow heads) can be seen. (H & E; X 400).
(3d): A section in group (II) showing some follicular cells with dark deeply stained (pyknotic) nuclei (arrow heads) and other cells their cytoplasm showed multiple vacuoles (arrows). Some congested and dilated blood vessels (V) in the interfollicular tissues can be seen. (H & E; X 1000).
(3e): A section in group (III) showing thyroid follicles full of normal homogenous colloid (C) with vacuolated peripheral colloid (arrows). The cytoplasm of few follicular cells still shows vacuoles while other cells appear normal. (H & E; X 400).

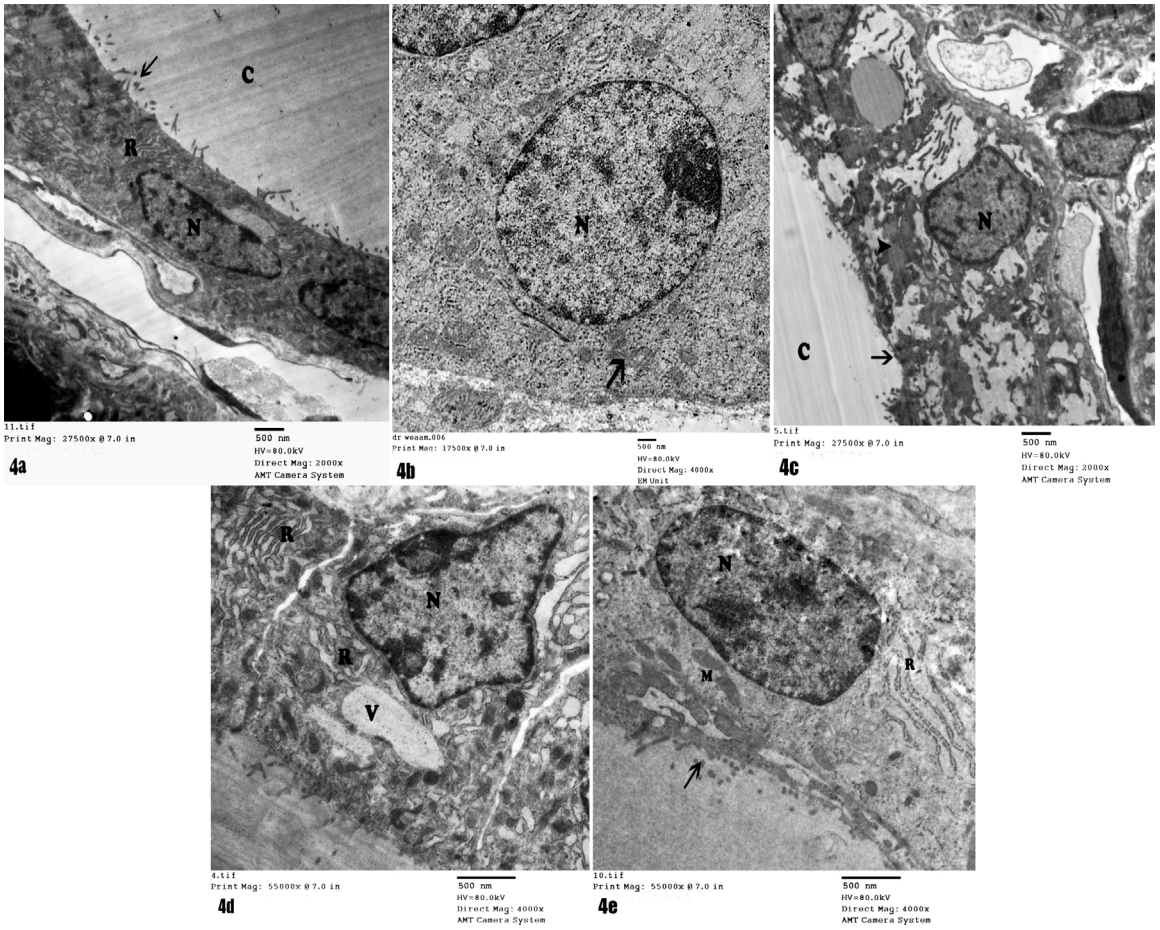


Fig. 4: Electron micrographs of ultrathin sections in the thyroid gland showing:
(4a): An ultrathin section in the control group (group I) showing a thyroid follicular cell with central nucleus (N) and well developed rER (R). The apical border facing the colloid (C) shows many microvilli (arrow). (X 2000).
(4b): An ultrathin section in the control group showing a thyroid follicular cell with rounded, central nucleus (N) with condensed peripheral chromatin and also shows many rounded mitochondria (arrow). (X 4000).
(4c): An ultrathin section in group II showing a thyroid follicular cell with irregular shaped nucleus (N), ballooned mitochondria (arrow head) and the apical surface facing the lumen that contains colloid (C) shows destructed microvilli (arrows). The cytoplasm shows multiple vacuoles (V) (X 2000).
(4d): An ultrathin section in group II showing thyroid follicular cells with irregular nucleus (N), dilated rER (yellow arrow). The cytoplasm shows multiple vacuoles (V). (X 4000).
(4e): An ultrathin section in group III showing a thyroid follicular cell with slightly irregular nucleus (N), mildly dilated rER (R), slightly affected mitochondria and the apical border showed few microvilli (arrows). (X 4000).

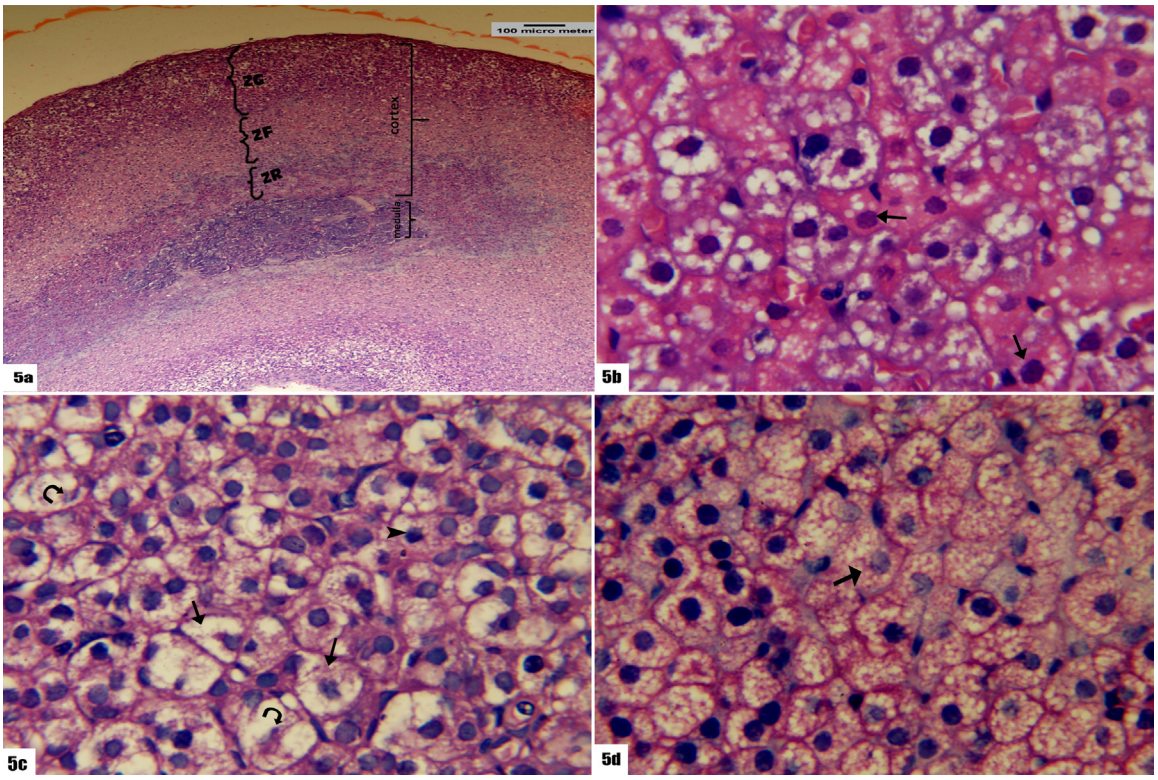


Fig. 5: Photomicrographs of sections in rat suprarenal glands showing:
(5a): A section in the control group (group I) showing the cortex divided into three zones; zona glomerulosa (ZG), zona fasciculate (ZF) and zona reticularis (ZR). Well demarcated suprarenal medulla can be also seen. (H &E; X 100).
(5b): A section in the control group showing zona fasciculata appeared as polyhedral cells with distinct cell membranes, large rounded nuclei and the cytoplasm has numerous small lipid vacuoles (arrows). (H &E; X 1000).
(5c): A section in group (II) showing the cells of zona fasiculata with many cytoplasmic vacuoles (arrows). Some cells appear with pyknotic nuclei (arrow head) and other nuclei shows karyorhexis (curved arrow). (H &E; X 1000).
(5d): A section in group (III) showing the cells of zona fasiculata with central rounded nuclei and some vacuoles (arrow) can be seen in its cytoplasm. (H &E; X 1000).

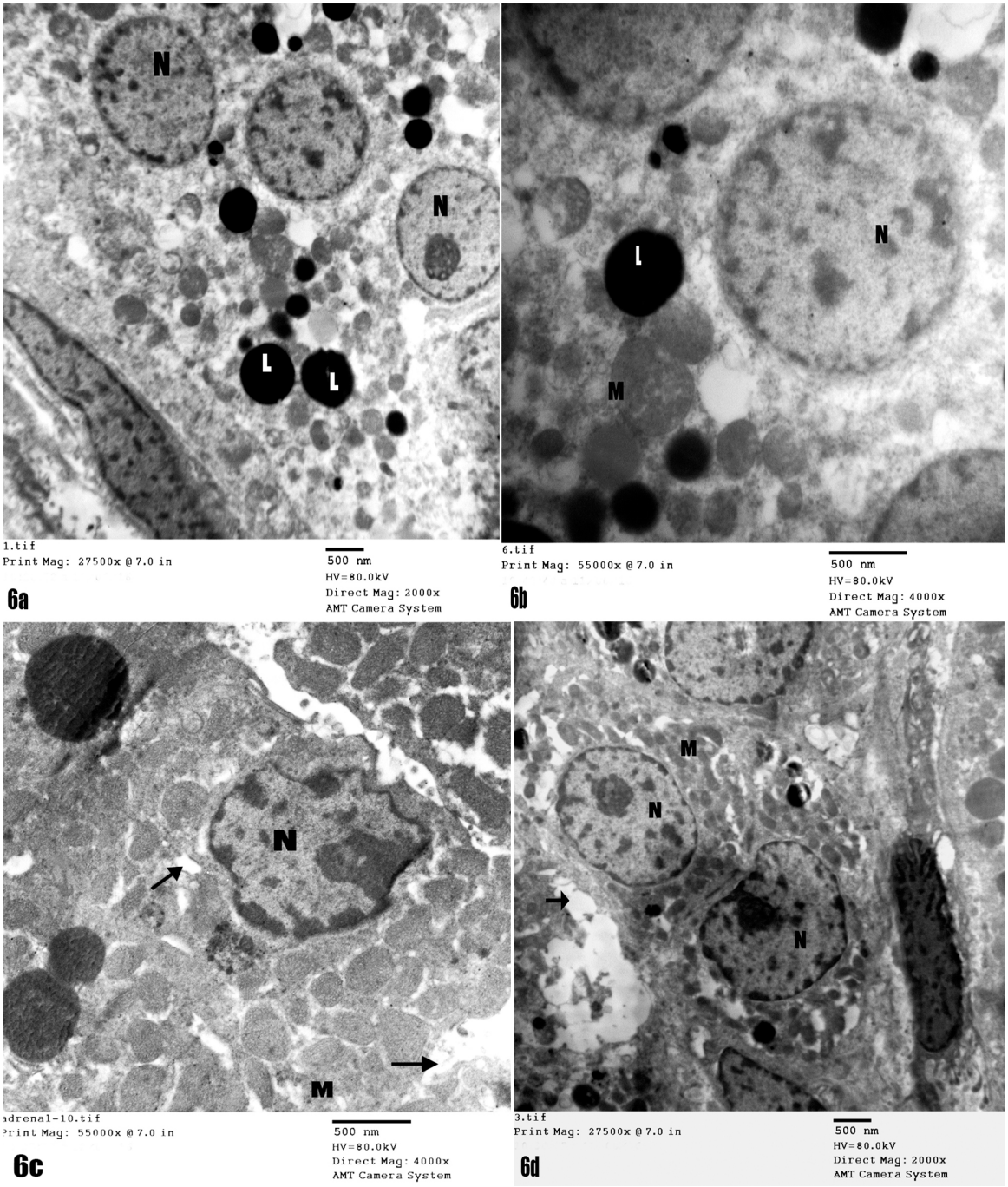
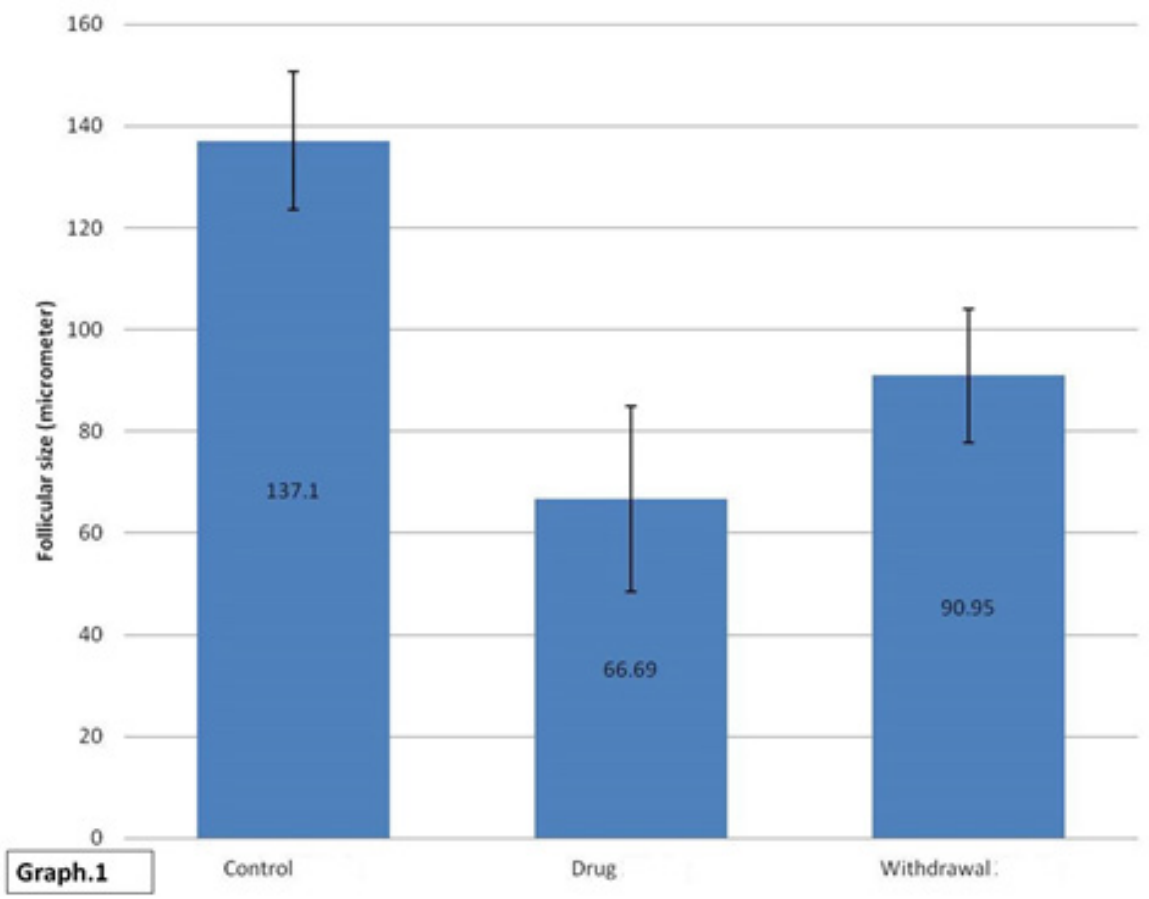


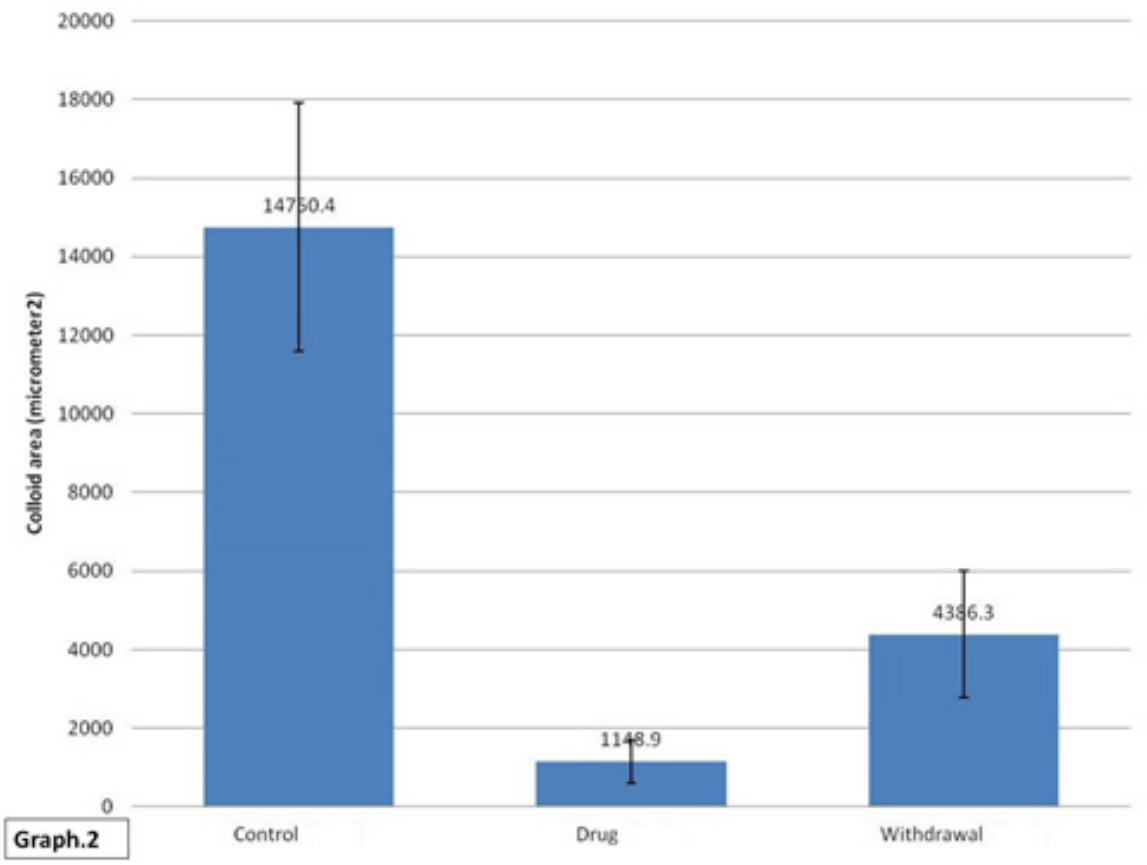
Fig. 6: Electron micrographs of ultrathin sections in the the zona fasiculata of the suprarenal gland showing:
(6a): An ultrathin section in the control group (group I) showing cells containing rounded nuclei (N) with nucleolus and many lipid droplets (L) in the cytoplasm. (X 2000).
(6b): An ultrathin section in the control group showing a cell in zona fasiculata containing rounded and regular nucleus (N), rounded mitochondria (M) and lipid droplets (L). (X 4000).
(6c): An ultrathin section in group (II) showing a zona fasiculata cell with irregular nucleus, irregularly shaped mitochondria with destructed cristae (M) and cytoplasmic vacuoles (arrow). (X 4000).
(6d): An ultrathin section in group (III) showing zona fasiculata cells with apparent normal nuclei (N) and rounded mitochondria (M).The cytoplasm shows some vacuoles (arrow). (X 4000).

Table. 1: Thyroid morphometric analysis.

	Control	Drug	Withdrawal	F test	*P value*
	Mean ± SD (range100%)	Mean ± SD (range100%)	Mean ± SD (range100%)		
Follicular size (micrometer)	137.1 ± 13.6 (108.4-157.45)	66.69 ± 15.285 (29.78-95.453)	90.95 ± 13.16 (44.78-104.89)	68.79	*< 0.001*
	P value Control and Drug	*P Value* Control & Withdrawal	*P value* Drug and withdrawal		
	< 0.001	*< 0.001*	*< 0.001*		
Colloid amount (micrometer)	14750.4 ± 3169.1 (7727.6-18241.2)	1148.9 ± 260.5 (220.01-2257.2)	4386.3 ± 1005.5 (554.2-8569.8)	202.6	*< 0.001*
	P value Control and Drug	*P Value* Control & Withdrawal	*P value* Drug and withdrawal		
	< 0.001	*< 0.001*	*< 0.001*		



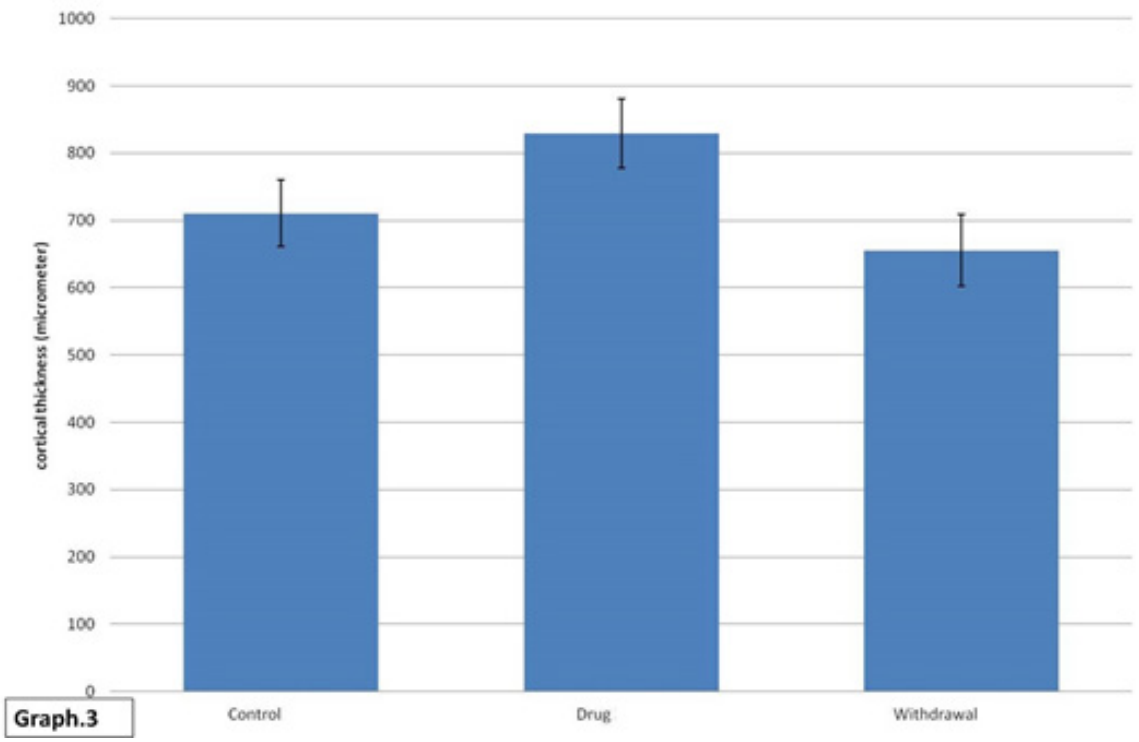
Graph. 1: The follicular size in micrometer in the three groups.



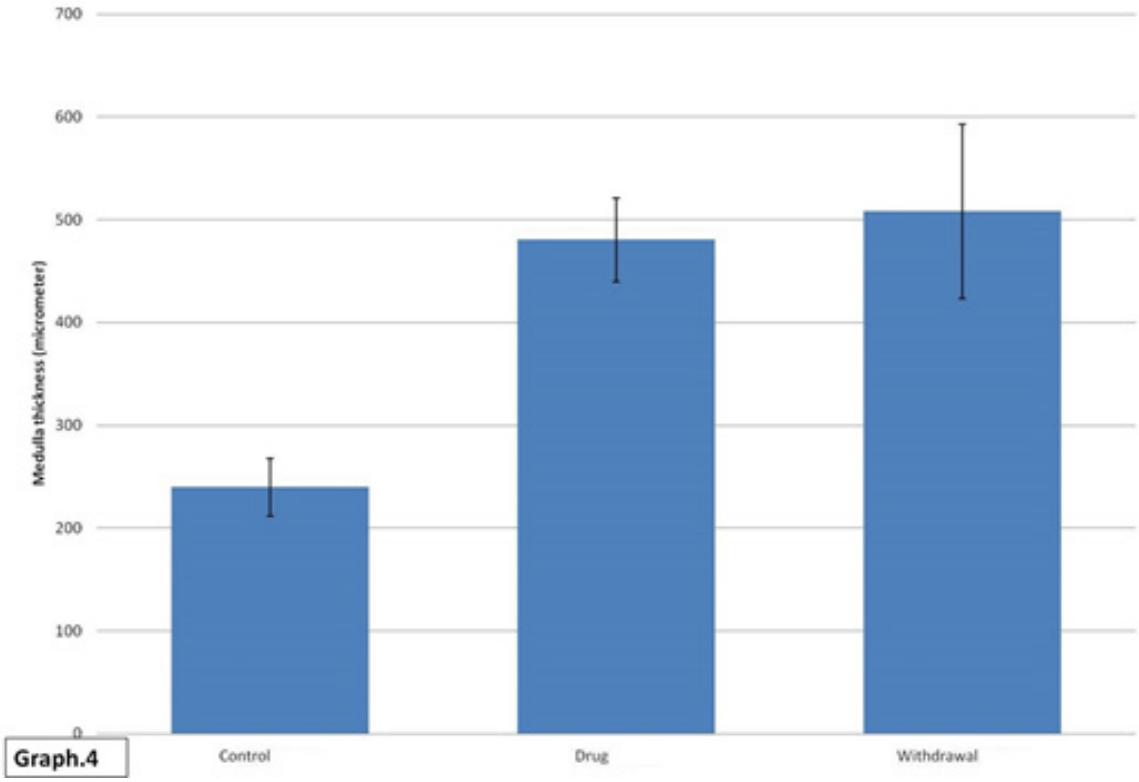
Graph. 2: the colloid area in micrometer among the three groups.

Table. 2: adrenal cortex and medulla thickening of all groups.

	Control Mean ± SD (range100%)	Drug Mean ± SD (range100%)	Withdrawal Mean ± SD (range100%)	F test	*P value*
Cortex thickness (micrometer)	710.5 ± 49.691 (649 - 810)	829.35 ± 51.41 (740 - 905)	655.6 ± 53.41 (567 - 720)	57.52	*< 0.001*
	P value Control and Drug	P Value Control & Withdrawal	P value Drug and withdrawal		
	< 0.001	*0.011*	*< 0.001*		
Medulla thickness (micrometer)	239.8 ± 27.72 (210 - 300)	480.55 ± 40.698 (388 - 533)	508.1 ± 84.74 (366 - 747)	70.68	*< 0.001*
	P value Control and Drug	*P Value* Control & Withdrawal	*P value* Drug and withdrawal		
	< 0.001	*< 0.001*	*0.198*		



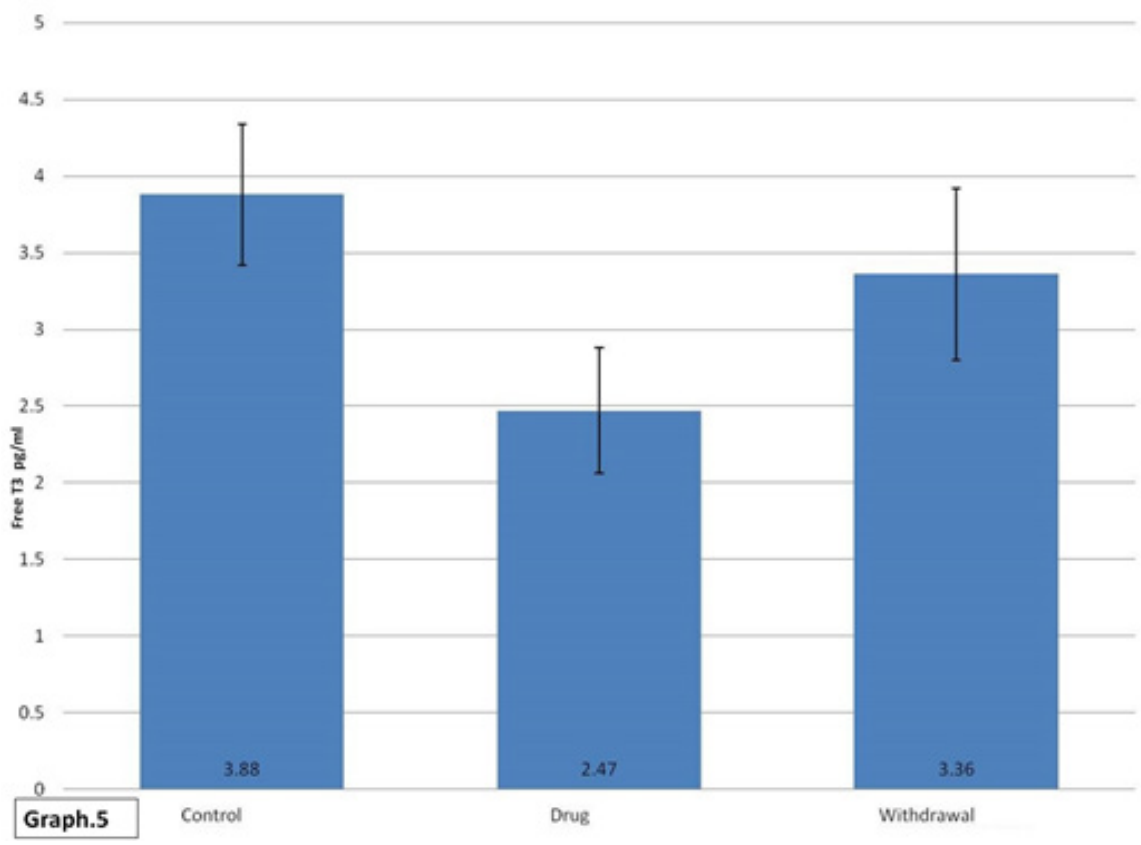
Graph. 3: The cortical thickness in micrometer among the three groups



Graph. 4: The medulla thickness in micrometers among the three groups

Table 3: free T3 level among groups.

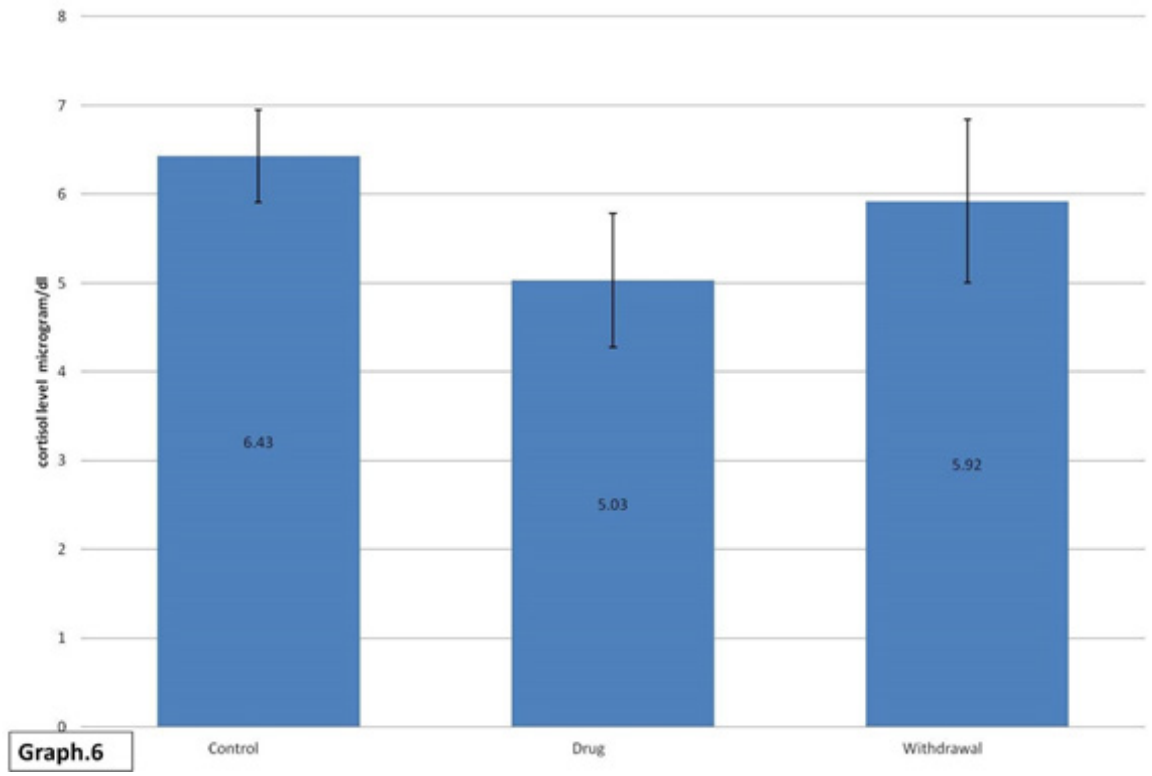
	Control Mean ± SD (range)	Drug Mean ± SD (range)	Withdrawal Mean ± SD (range)	F test	*P value*
Free T3 "pg/ml"	3. 88 ± 0. 46 (3.2-4.9) Control and Drug *P value < 0.001*	2. 47 ± 0. 41 (1.8-3.2) Control and withdrawal *P Value 0.017*	3. 36 ± 0.056 (2.4-4.8) Drug and withdrawal *P value < 0.001*	32.9	*< 0.001*



Graph. 5: Free T3 level in pg/ml among the three groups.

Table. 4: cortisol level among the groups.

	Control Mean ± SD (range 100%)	Drug Mean ± SD (range 100%)	Withdrawal Mean ± SD (range 100%)	F test	*P value*
Cortisol level (microgram/ dl)	6.43 ± 0. 52 (5.5 – 7.3) *P value* Control and Drug *< 0.001*	5.03 ± 0. 75 (3.9 – 6.6) *P Value* Control & Withdrawal *0.115*	5.92 ± 0. 92 (3.8 – 7.2) *P value* Drug and withdrawal *0.002*	12.05	*< 0.001*



Graph. 6: Cortisol level in microgram/dl among the three groups.

DISCUSSION

Phenobarbital (PB) is a clinically used drug mainly for treatment of epilepsy, despite it produces some adverse side effects especially behavioral, it is still widely used as the cheaper and effective classical antiepileptic drug[14].

In the present work we studied the effect of phenobarbital sodium over the pituitary, thyroid and adrenal glands of adult male albino rats. Light microscopic examination of the pituitary gland of PB group revealed dilatation and congestion of blood sinusoids between the secretory cells and also revealed vacuolations in the cytoplasm of secretory cells, these results coincide with Abdelhaliem and Mohamed[15], who reported similar results and attributed them due to free radicals and reactive oxygen species liberation.

In this study, ultrastructural changes of the pituitary gland with PB showed irregular nuclei, dilated rER, irregular shaped mitochondria and apparent decrease in the secretory granules, these findings are nearly similar to those reported by Japundzic[16] who discussed the electron microscopic changes in pituitary gland section of phenobarbital treated rats and reported morphological modifications of secretory cells with decrease in their number with some nuclear abnormalities and mitochondrial changes and cytoplasmic vacuoles.

On the other hand, Kojima *et al*.,[17] studied the effect of different doses administration of phenobarbital sodium over the pituitary gland and reported dose related toxicity regarding the pituitary gland, producing a decrease in the secretory cells function and with higher doses of phenobarbital sodium there were more destruction and decrease in pituitary gland secretory cells. Also, Kato *et al*., and Li *et al*.,[18,19] found a strong correlation between long period administration of phenobarbital sodium and the degenerative changes affecting the pituitary gland.

Histopathological changes detected in the pituitary gland sections in this research may be attributed to liberation of reactive oxygen species (ROS) and depletion of antioxidants in response to PB administration, producing apoptotic changes of the cells especially through affection of the

mitochondria[15]. Hence, partial improvement in the histological findings of the pituitary gland after PB withdrawal occurred due to decreased liberation of (ROS).

In the present work we studied the effect of PB on the thyroid gland, our results revealed multiple histological changes including congested blood vessels between thyroid follicles, decreased colloid and distorted thyroid follicles. These results coincide with Patil *et al*.[10] who reported similar results with different doses of PB administration over thyroid gland, found follicular degenerative changes like nuclear degeneration and highly vacuolated colloid contents of thyroid follicles.

Ultrastructural examination showed degenerated follicular cells and loss of microvilli. These results were in harmony with those of Hiasa *et al*., and Diwan *et al*.,[20,21] who reported degenerative histological changes in the thyroid gland and presence of follicular cell tumors following long term administration of phenobarbital sodium. Other researchers reported increased incidence of thyroid gland follicular cell tumors accompanying the chronic use of different antiepileptic drugs specially PB[22,23,24].

Yamaguchi *et al*.,[25] reported histopathological changes in the thyroid gland with decreased T3 level and attributed these results to indirect role of PB, by affection of the liver and hepatic cells in the form of hepatocellular hypertrophy and disturbed hepatic microsomal enzymes, these hepatic changes in turn produces injury of the thyroid gland and testis.

In the present research the adrenal cortex sections under PB treatment showed different histological abnormalities like increased cytoplasmic vacuoles of zona fasiculata cells, irregularities of their nuclei and abnormal shaped mitochondria. Similar histological findings were reported by Patil *et al*.,[10] who revealed marked distortion of architecture of all layers of adrenal cortex and adrenal medulla in PB treated animals.

Our study also examined the effect of PB withdrawal over the adrenal gland which revealed partial improvement in tissues and cells of adrenal cortex, decreased adrenal cortex and medullary thickness compared with that of PB group, which may be attributed to decreased cytoplasmic vacuoles. Ultrastructurally we noticed apparently normal shape nuclei and decreased vacuoles in the cytoplasm of most zona fasiculata cells.

Morphometric studies in our research revealed a decrease in both the thyroid follicular size and the amount of follicular colloid content in thyroid glands of PB group. These results agreed with Peter and Leslei[26] who found a decrease in the follicular sizes of thyroid gland in phenobarbital treated rats. Also, the adrenal gland of PB group revealed an increase in the thicknesses of the adrenal cortex and adrenal medulla, the same findings were reported by Singh *et al*., and Patil *et al*.,[10,27] which may be attributed to vascular congestion of the adrenal cortex or due to increased cytoplasmic vacuoles of most adrenal cortex cells.

In the present work we found marked decrease in T3 level in PB group with a minimal decrease in the withdrawal group, these results were in agree with Kato *et al*.,[18] who reported a decrease in T3 and T4 serum levels with PB administration in rodents, this response may be explained either by the direct affection of PB on the thyroid follicular cells or indirect through hepatic affection after PB long term administration and increased biliary excretion of T4 through bile or may be due to accumulation of T4 in different body tissues specially in the liver[28,29].

Regarding the effect of PB withdrawal, we noticed a minimal decrease in the free T3 hormone level and similar results were reported by Morten *et al*.,[30] who measured the serum level of T3 and serum T4 in PB children and noticed marked improvement in the thyroid hormones levels after the drug withdrawal.

The serum cortisol level had been measured in this study and was markedly decreased in PB treated rats; also, our results showed a reversible effect of PB over serum cortisol level and this was evident by elevation of the hormone level after PB withdrawal. Singh *et al*.,[27] found a direct relation between chronic administration of PB and depression of serum cortisol level, while Svalheim *et al*.,[31] suggested that this decrease is due to increased metabolism of cortisol hormone through induction of hepatic microsomal enzymes[32].

CONCLUSIONS

Finally, it could be concluded that administration of phenobarbital sodium resulted in several histopathological, morphometric and hormonal harmful effects on the pituitary, thyroid and adrenal glands of albino rats, which regressed to a lesser extent after its withdrawal.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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ملخص البحث

المقدمة: يعد عقار الفينوباربيتال من عقارات الصرع الكلاسيكيه وله دور فعال ضد التشنجات الكلية والجزئية وقد تم الإبلاغ عن الآثار الجانبية الضارة للأدوية المضادة للصرع على الغدد الصماء مسببة اضطراب فى وظائف تلك الغدد.

الهدف من البحث: يهدف هذا البحث إلى دراسة التأثير المحتمل للعلاج بعقار الفينوباربيتال على الغدد الصماء المختلفة للفئران دراسه هيستولوجية وقياسية شكلية وهرمونية.

مواد وطرق البحث: استخدم في هذه الدراسه خمسون فأراً من ذكور الفئران البيضاء البالغة و تم تقسيمهم إلى ثلاث مجموعات: مجموعة ضابطة (١) تكونت من ١٠ فئران و مجموعة الفينوباربيتال (٢) واحتوت على ٢٠ فأراً حيث أعطى كل فأر عن طريق الفم ٧,٥ مج فينوباربيتال /١٠٠ جم من وزن الجسم لمدة ثلاثون يوماً و مجموعة انسحاب الفينوباربتال (٣) وتكونت من ٢٠ فأراً وأعطى كل فأر نفس الجرعة من الفينوباربيتال مدة ثلاثون يوماً ثم تركت الفئران بدون علاج لمدة ١٥ يوماً اخرى.

النتائج: لقد أظهر فحص عينات الغدة النخامية والغدة الدرقية والغدة الكظرية من مجموعة الفينوباربيتال توسع واحتقان الأوعية الدموية ووجود فجوات بالسيتوبلازم وعدم انتظام نوى خلايا إفراز الغدة النخامية كما أظهرت انخفاض ملحوظ في كل من حجم جريبات الغدة الدرقية وانخفاض محتواها من المادة الغروية وعدم انتظام وتمزق في الزغيبات الصغيرة المقابلة للمادة الغروية كما أظهرت النتائج ايضا زيادة فى سمك كل من قشرة الغدة الكظرية ونخاعها مع وجود إخلال فى بنية الطبقات القشرية الكظرية وأظهرت نتائج فحص مجموعة انسحاب الفينوباربيتال دليلاً على التحسن في أنسجة الغدد الثلاثة مقارنة بمجموعة الفينوباربيتال.

الخلاصة: من هذه الدراسة يمكن أن نخلص إلى أن عقار الفينوباربيتال يمكن أن يسبب العديد من التغييرات النسيجية في أنسجة الغدة الدرقية و الغدة الكظرية والغده النخامية ومع ذلك فإن انسحابه قد حسن وقلل إلى حد ما من هذه التأثيرات الخطيرة.