# Identification of Immunohistological Changes in the Thymus Gland of Male andFemale Rats with Endocrine Glands Disorder

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**KEYWORDS**
Indocrine Disorders, Thymus Glandn , Prolactin (PRL), Hyperprolactinemia

**ABSTRACT**
Endocrine disorders is the result of a hormone imbalance, a condition characterized by a gland producing too much or too little of a hormone. This imbalance can be caused by:Issues with the endocrines feedback system−its main job is to keep hormones in the body perfectly balanced but it can malfunction and cause an imbalance a genetic disorder or infection or disease or injury to an an endocrine gland.The study amied to know the changes that occur in the thymus gland as a result of endocrine disorders in order to explain and analyze them accurately,as well as an immunological comparison between males and females.Twenty−four male and female rats Rattus norvegicus were divided into three groups of eight rats each : The first group (G1) received normal saline for 21 day; The second group male(G2), rats were given 6 mg / kg metoclopramide by intraperitoneal injection for hyperprolactinemia induction for 21 days; The third group femals (G3)was given metoclopramide 6 mg / kg intraperitoneally to induce hyperprolactinemic rats.After the end of experimental [21 days ], Measure prolactin hormone, and the thymus gland was removed, processed and examine for histomorphological changes. The result of the study revealed that is a significant ($p<0.05$) in prolactin and( WBC) in the two hyperprolactinemia group when compared with the control group.And when compared a group of hyperprolactinemia the males and agroup hyperprolactin females.Note a significant difference(increase)in the male group compared to the female group.And the resuit of the study showed asignificant decrease($p<0.05$) in weight and size the thymus gland in hyperprolactinemia male and female group compared with the control group.The histologicale study in male and female laboratory rats of the two hyperprolactinemia group also showed clear histological changes compared to the negative control group,reprsented the cortex area increased compared with the medulla area ,where the cortex−to−medulla ratio was about 3:1 .The limited numbers of epithelial cells were observed in the medulla area where the increase in lymphocyte numbers was significantly observed.Also, Hassall‘s corpuscles were not observed in the medulla.AS note the limited deposition or almost absence of collagen fibre in the medulla area.The study concluded endocrine disruption has a significant and noticeable effect on the size and weight of the thymus gland on the one hand and a histological effect on the other hand.

## 1. Introduction

Endocrine disorder sare disorders of the endocrine glands such as hypogonadism ,insulin−dependent diadetes, hypothyroidism and hypoparathyroidism which can be seen in some hereditary hemolytic diseases to treatment [ Sayehmiri et al.,2016;Azami et al.,2016].The endocrine disorder is classified in to classes: First, the complication arises when a gland generates more or less of some hormone which is called allows for hormone imbalance. Second,the disorder is caused by tumor formation in the endocrine system,which may or may not impair hormone levels[Espirito et al.,2016].Among these disorders is hyperprolactinemia is an increase in the concentratio of prolactin in the blood as a result of disorder in the pituitary gland [Halperin Rabinovich et al.,2013;Hoskova et al.,2022].The most common cause of hyperprolactinemia is the presence of tumors in the pituitary gland or using of certain drugs[Zeng et al.,2023 ].Among the druges that lead to an increase in the level of prolactine in the blood is metoclopramide[Tomova & Pharmacist,2016;Vilar et al.,2019].Which is a common drug used to increase the movement of the digestive system and empty the stomach.Metoclopramide was first described by Jastin−Besaucon and Lavillen in 1964[ McCallum et al.,1976].Its long−term using leads to hyperprolactinemia[Molitch,2005]. The thymus is a superior mediastinal retrosternal organ. It is bilobed and has two subcomponents:the cortex and the medulla and is made up of epithelial, dendritic,mesenchymal,and endothelial cells[Yan et al.,2017].The thymus (T) is an essential component of the adaptive immune system comserved in all vertebrates[Hess&Boehm,2012;Boehm et al.,2012]. It is a specialized primary lymphoid organ that supports T−cell (and Natural−Killer cell) development and maturation, and its absence (athymia) results in severe or complete immunodeficiency[Flangan&Nude,1966&Auricchio et al.,2005].

## 2. Methodology

Twelve adult females and twelve males, weighing approximately 200-250 g, were purchased. Animals were kept in a standard plastic cage (four rats per cage). They were housed under standard condition maintained at room temperature and under natural daylight rhythm. Animals were kept to acclimatize for 10 days before the onset of the experiment. They were given standard rodent diet ad libitum as well as water supply.

### Experimental design

The animals were randomly divided in to three groups (8 rat in each group) as follows Group (1) serves control ; received normal saline(N.S) intraperitoneal(IP) Group (2)(Hyperprolactinemia male group ): The animals received a single dose of metoclopramide 0.6 mg/ kg bwt to induce hypreprolactinemia, ratdaily given by injection for (21) days. Group (3)(Hyperprolactinemia female group ): The animals received a single dose of metoclopramide 0.6 mg/ kg bwt to induce hypreprolactinemia, ratdaily given by injection for (21) days. .

### Blood and tissue sampling

Blood samples were collected using . After the collection of blood samples, rats were by an of die by ether. The sera were separated from plain tubes at 3000 rpm for 15 min after blood clotting. The thymus gland of each rat were excised all groups, washed by the saline solution to remove blood, dried by blotting between filter papers. The thymus gland tissue of were preserved in 10% formalin solution for histopathological examination.

### Thymus weight

Measurement of the thymus gland was taken for all study groups after its removal during autops. Note an increase in the weight of the thymus gland for the two groups exposed to hyperprolactinemia.

### Serum biochemical paramenters

Determination of serum prolactin(PRL) , Withe blood cells (WBC) using commercial kite(Elisa).

### Histopathology

Formalin-fixed thymus of were dehydrated in alcohol gradient then embedded in paraffin wax. Several 5-µm sections from each sample were cut then stained with hematoxylin and eosin (H&E)and masson(MTS) for histopathological examination

[Saleh and al−Kayat,2017;Carleton et al.,1980] . .

### Statistical analysis

The data presented as mean ± standard deviation(M ±SD). Student T-test was applied for the statistical analysis for the present data of all groups was considered to be significant when P is less than 0.05 (SPSS software). Results thymus weight gain The weight gain exhibited significant.

## 3. Results and discussion

### Thymus gland weight gain

The thymus gland weight gain exhibited significant ($P<0.05$) elevation in two hyperprolactinemia 0.6 mg/kg treated group than control ( Fig. 1).

### Serum biochemical parameter

The results of the current study showed that there is exhibited significant significant($P<0.05$) )in (WBC)and level prolactin elevation in two hyperprolactinemia 0.6 mg/kg treated group than control respectively.And when compared a group of hyperprolactinemia the males and agroup hyperprolactin females.Note a significant difference(increase)in the male group compared to the female group(Fig.2

and Fig.3).

**Histopathology**

Thymus of rat of control group displayed showed that the structure of the tissue of the thymus gland is in the area of the cortex to the area of the medulla in of( 1:1 ),and abundant numbers of epithelial cells were observed in the medulla area, as in figure( 4).with an increase in fiber the collagen in the marrow in medulla area of control group when compared the both group hyperprolactinemia Note the limited collagen fiber deposition in the medulla area as in figure (5).The histologicale study in male and female laboratory rats of the two hyperprolactinemia group also showed clear histological changes compared to the negative control group,reprsented the cortex area increased compared with the medulla area ,where the cortex−to−medulla ratio was about 3:1 and the limited numbers of epithelial cells were observed in the medulla area where the increase in lymphocyte numbers was significantly observed.Also as in figure(6).

Table(1):Showing weights thymus gland for hyperprolactinemia male and female groups.
Data presented as mean ± SD.
Means sigficant difference at P<0.05 as compared to control using student T− test.

| P−value (0.05) | LSD | Thymus gland (M±SD) | Groups |
|---|---|---|---|
| 0.02 | 0.34 | 0.21 ± 1.40 | Control male group |
| | | 0.26 ± 1.05 | Hyperprolactinemia male group |
| 0.04 | 0.21 | 018 ± 124 | Control female group |
| | | 005 ± 121 | Hyperprolactinemia female group |
| 0.21 | 0.15 | 026 ± 105 | Hyperprolactinemia male group |
| | | 005 ± 121 | Hyperprolactinemia female group |

Table(2): Showing white blood cells in all groups.
Data presented as mean ± SD.
Means sigficant difference at P<0.05 as compared to control using student T− test

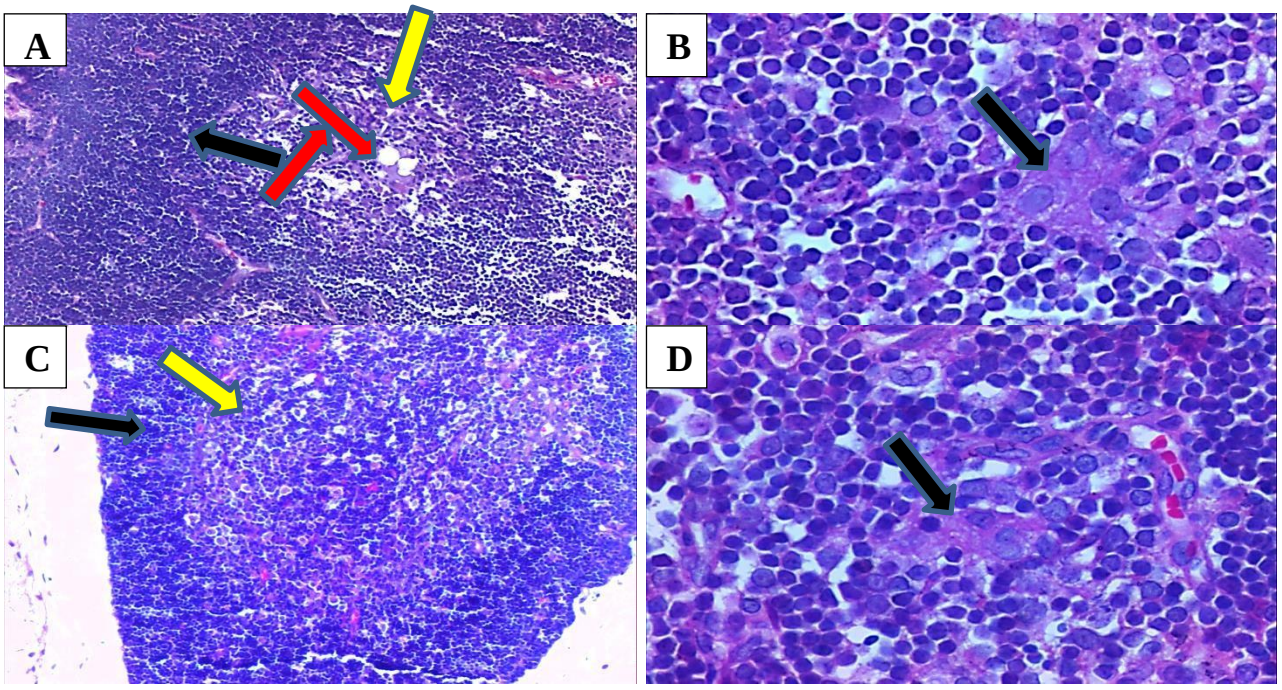
| P−value (0.05) | LSD | White blood cells (M±SD) | Groups |
|---|---|---|---|
| 0.02 | 3.06 | 001 ± 406 | Control male group |
| | | 002 ± 713 | Hyperprolactinemia male group |
| 0.03 | 3.07 | 002 ± 318 | Control female group |
| | | 004 ± 625 | Hyperprolactinemia female group |
| 0.04 | 0.87 | 002 ± 713 | Hyperprolactinemia male group |
| | | 004 ± 625 | Hyperprolactinemia female group |

Table(3): PRLhormone level in all groups.
Data presented as mean ± SD.
Means sigficant difference at P<0.05 as compared to control using student T− test

| P−value (0.05) | LSD | Prolactin hormone (M±SD) | Groups |
|---|---|---|---|
| 0.03 | 14.44 | 0.54 ± 11.78 | Control male group |
| | | 6.25 ± 26.23 | Hyperprolactinemia male group |
| 0.01 | 16.32 | 2.26 ± 17.92 | Control female group |

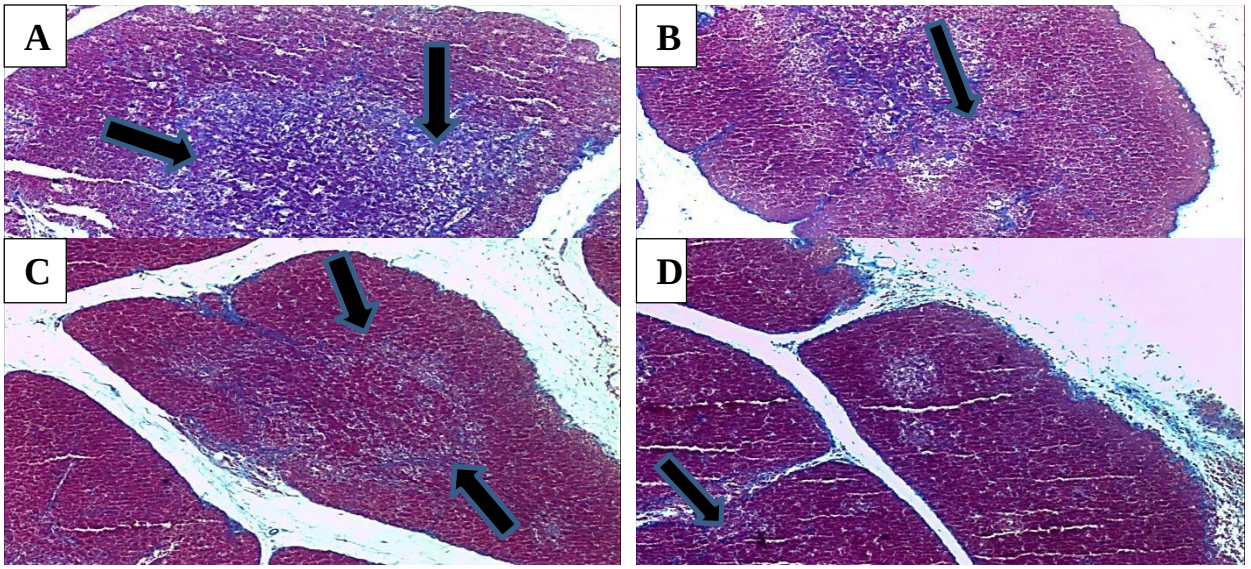
| | | | |
|---|---|---|---|
| | | 11.89 ± 34.25 | Hyperprolactinemia female group |
| 0.06 | 8.02 | 6.25 ± 26.23 | Hyperprolactinemia male group |
| | | 11.89 ± 34.25 | Hyperprolactinemia female group |

**Figure (4):A cross section of the thymus of control rat(A,B males)(C,D femals). A /** Note the cortex area (black arrow) reduced compared with the medulla area (yellow arrow), where the cortex-

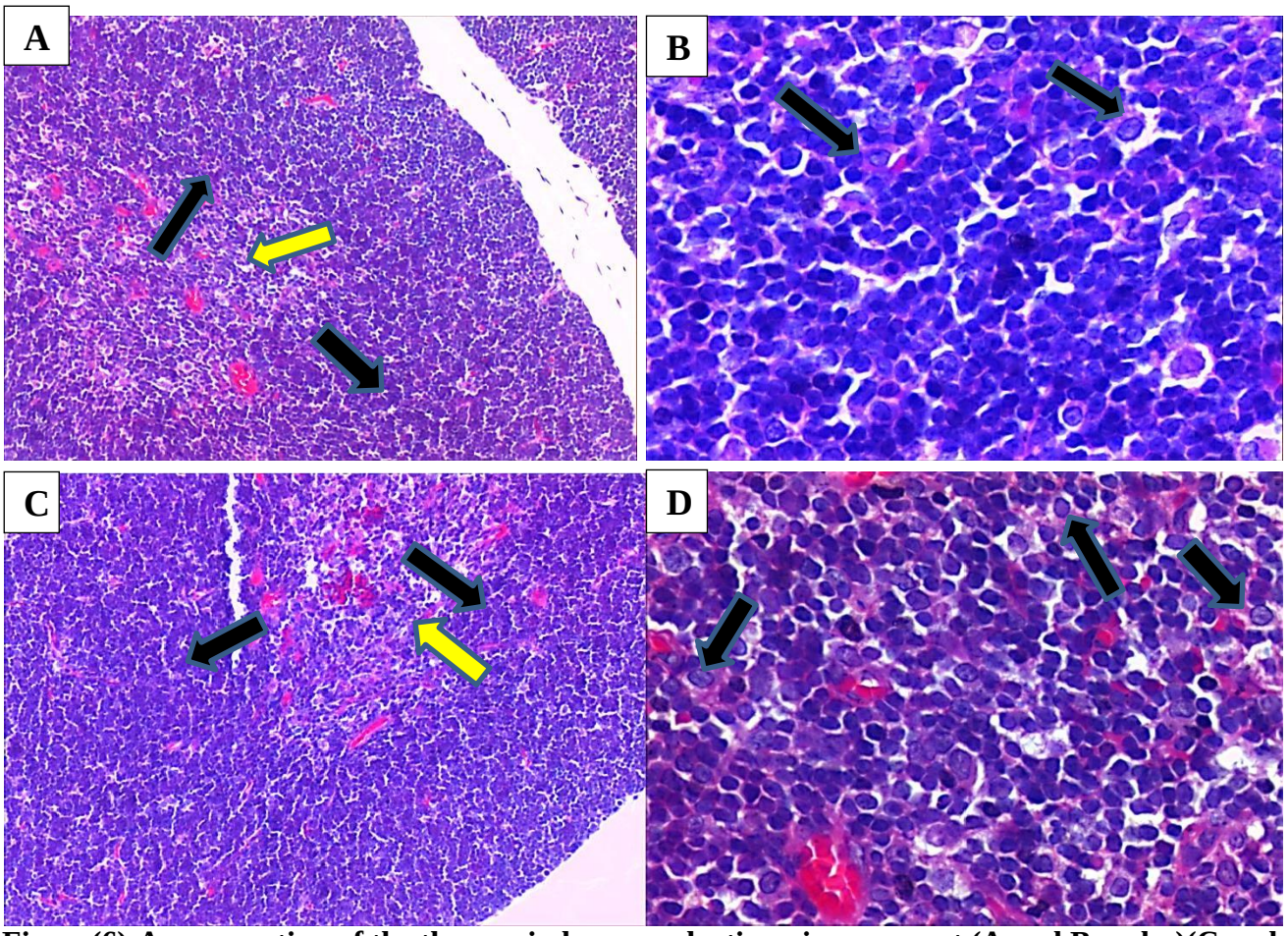



to-medulla ratio was about 1:1.Also, the presence of spaces (red arrow) in the medulla area was observed **B /** Note the presence of abundant numbers of epithelial cells (black arrow) in the medulla area. **C/** Note the cortex area (black arrow) reduced compared with the medulla area (yellow arrow), where the cortex-to-medulla ratio was about 1:1. **D/** Note the presence of abundant numbers of epithelial cells (black arrow) in the medulla area. **H&E. A: 100x and B: 400x.**

**Figure (5): A cross section of the thymus showing collagen fiber .control group**(A male/B femal) Note the increase in collagen fiber (black arrow) deposition in the medulla area. **MTS.40x.**




**hyperprolactinemia group (C male / D femal**) Note the limited collagen fiber (black arrow) deposition in the medulla area. **MTS.40x.**




**Figure(6):Across section of the thymus in hyperprolactinemia group rat.(A and B males)(C and D females) : A**/Note the cortex area (black arrow) increased compared with the medulla area (yellow arrow), where the cortex-to-medulla ratio was about 3:1**. B**/The limited numbers of epithelial cells (black arrow) were observed in the medulla area where the increase in lymphocyte numbers was significantly observed. Also, Hassall's corpuscles were not observed in the medulla area. **H&E. A: 100x and B: 400x.**

The obtained data showed that the thymus Contains three types of epithelial reticular cells; cortical, medullary and Hassall's corpuscles cells and this in agreement with[ Aly et al. 1988]. but disagree with [Suster & Rosai ,1990& Kuper et al. 1995], [DeWaal et al. 1997& Greaves ,2000]. that mentioned that the epithelial cells were divided into distinct four subtypes, however [Gartner& Hiat,2006]. mentioned that there were six types of epithelial reticular cells in which recorded three types of epithelial reticular cells, two of them showed secretory activity. The cortex was darkly stained contains densely packed, small lymphocytes and few epithelial cells and this confirmed the results of [Ham &Cormack 1979].; [Junqueira &Carneiro 2005; Pearse ,2006].. Note the cortex area reduced compared with the medulla area where the cortex-to-medulla ratio was about 1:1. Also, the presence of spaces in the medulla area was observed ,Note the presence of abundant numbers of epithelial cells in the medulla area.The obtained data showed that the medulla was paler staining, and contained more large lymphocytes, prominent epithelial cell, Hassalls corpuscles, as mentioned by [Haley,2003; Pearse 2006]. The results showed three types of Hassalls corpuscles one appeared as group of epithelial cells which undergo degeneration to its nucleus, the

second form appeared as cyst that may had amorphous protein in its center while the third type had onion like appearance in which the epithelial reticular cells concentric arranged with keratinized center this in agreement with [Kandil,1972]. who recorded three types of corpuscles and recorded that the most common type is onion like form. In senile rabbits the thymus enters in stage of involution in which occured progressive cortical lymphocyte depletion and shrinkage of thymic lobule this in agreement with [Contreiras et al. 2004].

## Conclusion

The study concluded that endocrine disruptor had a clear effect on the thymus gland, which led to noticeable changes in the of the thymus gland**.**

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