

Physiological and Histological Study for the Effect of Escalation Doses of Dostinex (Caprigoline) on Male Mice through Some Biochemical Parameters

دراسة فسيولوجية ونسجية لتأثير عقار الـ Dostinex في ذكور الفئران من خلال بعض المعايير الكيموحيوية

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Abstract

Although the history of many drugs proved some beneficial effects, yet uncontrolled use may have serious impact on health especially vital organs as liver and kidney and endocrine glands. Dopamine agonists are the treatment of choice for the majority of patients with hyperprolactinemic disorders. Bromocriptine has been used over the past 30 years, whereas cabergoline has become a first-choice agonist in recent years. The present study was aimed to evaluate the physiological and histological effects of escalated doses of the drug (Caprigoline) in male mice. The experiment includes 40 mice divided on 4 groups. Group 1 treated with PBS represents the control. The groups (2, 3, and 4) treated with the doses (0.5, 5, and 10) mg of caprigoline /Kg body weight respectively. Regarding hormonal assay, the results showed significant ($P<0.05$) differences between the treated groups and the control. There was a significant increase in luteinizing hormone (LH), testosterone, Triiodothyronine (T3) and Throxine (T4) level accompanies the increase in caprigoline dose. In contrast the Follicle stimulating hormone (FSH) and prolactin (PRL) show a decrease in their levels as the dose become higher. Furthermore; there was a significant increase in level of Alanin aminotransaminase (ALT) as an indicator of liver function and a significant increase in level of urea as an indicator of kidney function in the treated groups compared with the control. The histological study reveal an obvious morphological changes compared with the normal state in the animals treated with the high dose. The liver in mice treated with (5 and 10) mg caprigoline showing focal area of necrosis with inflammatory cells. The kidney in mice treated with 10 mg caprigoline shows a degenerative changes and necrosis of epithelial of micro tubules and thyroidization phenomono.

Key words: Caprigoline, prolactin, liver, kidney, mice, tissue

المخلص

على الرغم من أن معظم العقاقير أثبتت فعالية ناعمة إلا أن الاستخدام غير الصحيح لهذه العقاقير قد يؤدي إلى تأثيرات ضارة على الصحة وخاصة في الكبد و الكلى والغدد الصماء. العقاقير المضادة للدوبامين هي الاختيار الأول المستخدم في علاج حالات ارتفاع مستوى هرمون البرولاكتين. استخدم البروموكريبتين لأكثر من 30 عاما وفي الآونة الأخيرة أصبح عقار الكابريكولين هو الاختيار الأول. هدف الدراسة الحالية تقييم التأثيرات الفسلجية والنسجية لجرع مختلفة من عقار الكابريكولين في بعض المعايير الحيوية في ذكور الفئران البيض. شملت التجربة 40 فأرة تراوحت اعمارها بين 8-12 اسبوعاً وبمعدل وزن 20-25 غرام ، قسمت على 4 مجاميع. المجموعة 1 تمثل السيطرة وتم معاماتها بالمحلول الفسيولوجي الداريء والمجاميع 2 و 3 و 4 تم معامتها بالتراكيز (0.5, 5, 10) ملليغرام من الكابريكولين /كغم من وزن الجسم على التوالي. أظهرت النتائج اختلافات معنوية في مستوى الهرمونات الفعالة ما بين المجاميع الثلاثة و المقارنة مع مجموعة السيطرة ، فقد كان هناك زيادة في مستوى هرمونات LH و Testosterone و T3 و T4 مع الزيادة في جرعة العقار، في حين حصل انخفاض في مستوى هرمونات FSH و PRL مع الزيادة في جرعة العقار. وجد من خلال نتائج البحث حصول زيادة في انزيم GPT كمؤشر عن وظيفة الكبد و زيادة مستوى اليوريا كمؤشر عن وظيفة الكلى في المجاميع المعاملة وبالمقارنة مع مجموعة السيطرة. أما بالنسبة للدراسة النسجية فقد أظهرت تغييرات مظهرية واضحة في التركيب النسجي للكبد و الكلى عند معاملة الفئران بالتراكيز العالية. ظهر في نسيج كبد الفئران المعاملة بالتراكيز 5 و 10 ملغم / كغم بؤرة مركزية للتخر النسيجي و تجمع للخلايا التهابية. وفي الكلى ظهر تحلل و تخر للخلايا الطلانية لجدران النبيبات البولية وحدوث ظاهرة تدرق خلايا النبيبات.

الكلمات المفتاحية: الكابريكولين، البرولاكتين، الكبد، الكلى، الفئران، الانسجة

Introduction

Hyperprolactinemia is a common endocrine disorder that can be associated with significant morbidity; it is occur in about 0.4% of the general population [1]. The objective of hyperprolactinemia treatment is to correct the biochemical consequences of the hormonal excess [2]. Several dopamine agonists are currently available for the treatment of hyperprolactinemia, including bromocriptine and cabergoline [3]. Bromocriptine, become out of use because of its short half-life and poor tolerability by a significant proportion of patients accordingly it needs to be administered twice or three times daily. Cabergoline has become a first-choice agonist in recent years because of its enhanced efficacy and tolerability, it has an advantage of once or twice weekly administration with minimal

side-effects [4,5]. Cabergoline is in a class of medications called dopamine receptor agonists and has many uses [6] Cabergoline is used in the treatment of hyperprolactinemia and parkinson disease and some of other disorders [7]. It is also a first-line agent for treating patients with prolactinomas, the major cause of hyperprolactinemia [8]. Drugs are important causes of liver injury and may have a toxic effect on the liver and the kidneys [9]. therefore, this study has been introduced to evaluate the effect of different doses of the drug (Caprigoline) on some vital hormones and examine the function and the histological changes that may occur in the liver and the kidney.

Materials and Methods

Preparation of Caprigoline concentrations

The drug tablets were purchased from local pharmacies then grinded to obtain powder. Three concentrations used in this experiment (0.5, 5, and 10) mg/kg B.W. The dose of the drug are weighted and mixed with 0.2 ml PBS for each mouse. The animals treated with the drug solution orally by a digestive tube daily for 10 days.

Experiment design and Biometry

The experiment carried in animal house of Biotechnology research center, include Forty males of albino Swiss mice (*Mus musculus*) their age ranged from 8 - 12 wk with body weight between (20-25) g, were obtained from biotechnology Research Center, Al-Nahrain University. The animals were used in this study divided mainly into four groups (each one 10 animals) as follows and placed in a separated cage in animal house at room temperature (25C°), the animals were fed a suitable quantity of complete diet in addition to water, and treated as follow:

- **Group 1:** The first main group administrated PBS orally, represent the control group.
- **Group 2:** Administrated with 0.2 ml of the concentration 0.5 mg caprigoline /kg B.W. orally for 10 days, once a day
- **Group 2:** Administrated with 0.2 ml of the concentration 5 mg caprigoline /kg B.W. orally for 10 days, once a day
- **Group 2:** Administrated with 0.2 ml of the concentration 10 mg caprigoline /kg B.W. orally for 10 days, once a day

At the end of the experiment, the blood was obtained by heart puncture, centrifuged at 3000 r.p.m for 10 min. and the animals were sacrificed. The serum was stored at - 80 °C. and then used to determine the levels of the following hormones and biomarkers : LH, FSH, prolactin and testosterone T3 ,T4 , TSH (ELIZA kit /Orgmetric/Germany). (GPT) (ELIZA kit /Orgmetric/Germany) . Urea (Randox kit /England). The liver and kidney were immediately excised and preserved in 10% formalin for histological study, which is conducted according to the method used by [10].

Statistical Analysis

The Statistical Analysis System- SAS (2010) was used to study the effect of different factors in studied parameters. Least significant difference –LSD test was used to significant ($P \leq 0.05$) compare between means in this study [11].

Results and Discussion

The results in this study reveal significant ($P \leq 0.05$) differences in hormone levels between the treated groups in comparison with the control. There was a significant increase in LH, testosterone, T3 and T4 levels accompany the escalation in caprigoline dose. In contrast the levels of FSH and PRL decreased with the escalation of caprigoline dose Table (1).

Table (1): Effect of different doses of Capergoline drug on level of many vital hormones in the male mice.

Groups	Mean \pm SE						
	LH (IU/l)	FSH (IU/l)	rolactin (ng/ml)	estosterone (ng/ml)	T3 (ng/ml)	T4 (μ g/dl)	TSH (mIU/ml)
Control	0.306 \pm 0.02 c	4.60 \pm 0.07 a	3.26 \pm 0.13 a	0.039 \pm 0.003 b	0.600 \pm 0.02 b	5.23 \pm 0.20 b	1.40 \pm 0.5 a
Conc. 0.5	1.70 \pm 0.23 b	1.20 \pm 0.11 c	3.03 \pm 0.12 a	0.486 \pm 0.18 a	0.890 \pm 0.02 b	8.70 \pm 0.28 a	1.65 \pm 0.08 a
Conc. 5	2.80 \pm 0.34 b	1.26 \pm 0.14 c	2.67 \pm 0.16 a	0.470 \pm 0.11 a	2.25 \pm 0.08 a	9.70 \pm 0.23 a	0.400 \pm 0.05 b
Conc. 10	4.36 \pm 0.56 a	2.70 \pm 0.26 b	0.756 \pm 0.05 b	0.580 \pm 0.01 a	2.41 \pm 0.05 a	10.81 \pm 0.02 a	0.270 \pm 0.02 b
LSD value	1.266 *	1.394 *	1.092 *	0.206 *	0.783 *	2.866 *	0.479 *

* ($P < 0.05$)

*Significant, NS : Not Significant

Cabergoline is a long-acting dopamine D2 receptor agonist and *in vivo* rat studies show a direct inhibitory effect on the prolactin secretion in the pituitary's lactotroph cells [12]. Cabergoline is used to treat hyperprolactinemia,

which may cause symptoms such as infertility, sexual problems, and bone loss in women who are not breast-feeding or men [13]. Hyperprolactinaemia may also be the result of disease of other organs such as the liver, kidneys, ovaries and thyroid [14].

Cabergoline inhibits prolactin secretion in both normal subjects and those with hyperprolactinemia, with duration of action of up to 21 days after single oral doses of 0.3 to 1.0 mg. [15]. Many researches recommend a dose of 0.125 to 1.0 mg twice weekly for four weeks decreased prolactin secretion in a dose-dependent fashion, and 95 percent of those receiving 1.0 mg twice weekly had normal serum prolactin concentration [16]. Although cabergoline is usually administered at a median dose of 1 mg/week, treatment may be required for many years. Some patients with hyperprolactinemia may be successfully withdrawn from cabergoline therapy [17]. LH level in the serum accompany the increase in the dose of the drug and this may cause by the low level of prolactin due to drug administration, since there is a strong association between the two hormones as many researches proofed it [18].

Hyperprolactinemia associated with increase and decreased in some hormonal parameter and dopamine agonist agents are effective in correcting the disturbance in those parameter. The highly significant increase in serum prolactin hormone and testosterone hormone levels associated with high significant decrease in serum FSH, LH, and progesterone hormone levels in caprigoline pre-treated patients compared with control group. Furthermore; the high significant decrease in serum prolactin hormone level, and less significant decrease in serum testosterone hormone level associated with high significant increase in serum FSH, LH and progesterone hormone levels in caprigoline post-treatment patients in compared with pre-treated patient's groups [19].

Speroff [20] mention in their study that the decrease in serum levels of FSH and LH hormones in hyperprolactinemic infertile women probably due to the high level of prolactin can work at both central and ovarian sites. In this case the ovulation may be suppressed due to suppression of secretion of gonadotropin releasing hormone (Gn RH) from the pituitary gland. Because the high level of prolactin interferes with hypothalamic-pituitary-gonadal axes through positive feedback effect on dopamine secretion. Increase dopamine reduces Gn RH secretion by suppressing arcuate nucleus function and this will lead to reduction pulsatile secretion of LH and FSH hormone [21]. Also high level of prolactin hormone interfere with action gonadotropin at ovarian level and impairing normal gonadal steroid secretion, which in turn alter positive feedback effect at the hypothalamus and pituitary level, this lead to lack of gonadotropins cyclic and to gonadal dysfunction [22].

As showed in table 1, TSH level decreased as the dose of caprigoline become higher, in return levels of T3 increased according to feedback mechanism. It is found that the cabergoline decrease the release of TSH by stimulating the D2-dopaminergic receptor existing in thyrotropic cell and increase of T3 concentration with low dose of drug [7]. Therefore, cabrigoline have different physiological effect, in different amount, and alter the function of pituitary-thyroid axis.

Table (2): Effect of different doses of Capergoline drug on level of some biochemical tests.

Group	Mean ± SE	
	ALT (IU/L)	Urea (mg/dl)
Control	14.67 ± 1.76 c	20.67 ± 1.76 c
Conc. 0.5	14.00 ± 1.15 c	31.00 ± 1.73 b
Conc. 5	36.67 ± 4.09 b	36.33 ± 5.89 b
Conc. 10	44.06 ± 0.52 a	46.60 ± 0.28 a
LSD value* (P<0.05).	7.837 *	8.941 *

*Significant, NS : Not Significant

Table (2) show level of Alanin aminotransaminase as an indicator of liver function and the level of urea as an indicator of kidney function. The results reveal significant differences in the level of both indicators in the treated groups compared with the control. As there was no significant difference in the level of the main indicator of liver function, in case of low dose but a highly significant differences do exist between the groups of high doses compared with the control, it could be concluded that caprigoline has no side effect on liver and kidney in low doses but has an impact effect in high doses. The urea as an indicator of kidney function shows a significant increase in its level as the dose of administrated caprigoline become higher. This refer to a sever effect of a high dose of caprigoline on the kidney and more study seems to be needed.

The histological studies confirm those facts as the figures of both liver and kidney show normal structure and morphology of both organs in the low dose Figures (2,5), but in the higher doses (5 and 10) mg cause an obvious morphological changes represented by focal inflammation and necrosis Figures (3,4) in liver and degenerative changes and necrosis of epithelial tubules Figure (7) and thyroidization phenomenon Figure (8) in kidney tissue.

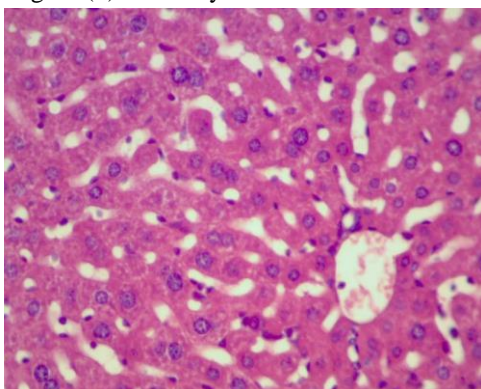


Fig. (1): Section of liver in mice in the control groups showing normal structure (X400) (H &E)

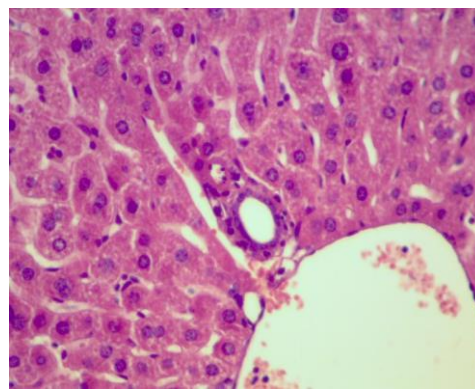


Fig. (2): Section of liver in mice treated with 0.5 mg caprigoline showing normal like structure appearance of hepatocyte cells with mild congestion in the control vein with mild sinusoidal dilatation around portal area (X400) (H &E).

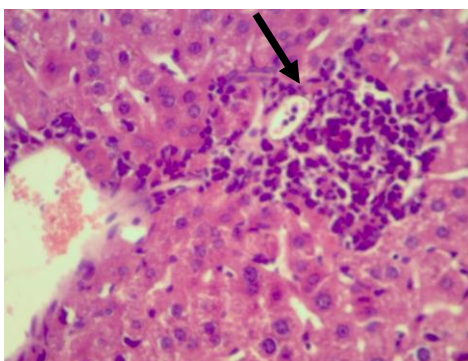


Fig. (3): Section of liver in mice treated with 5 mg Ca prigoline showing focal area of necrosis with in flammatory cells (X400) (H &E).

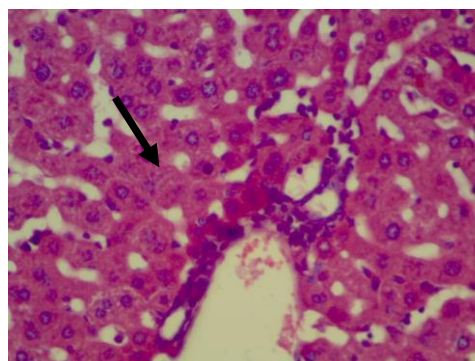


Fig. (4): Section of liver in mice treated with 10 mg caprigoline showing focal inflammation and necrosis(X400) (H &E).

The liver has an impact role in the metabolism of chemical drugs and plasma protein synthesis [7], the doses used by the later should be taken in consider, since the maximum dose used is 1 mg, while in our study the maximum dose reach 10 mg . This doses and higher used to treat some cases that show tolerance to this drug [23]. Therefore, Caprigoline drug has no side effect on liver and kidney in low doses but high dose cause functional and histological disturbances in both organs.

Long term, low dose of caprigoline significantly reduced tumor volume and normalizes serum prolactin level in a great majority of patients bearing hyperprolactinemia [24]. So, using high dose to treat such cases needed more study. Besides, the rapid buildup of caprigoline doses increases its efficacy in terms of clinical improvement of gonadal functions without compromising its safety [25].

Another point revealed by this research, that the high levels of caprigoline cause changes in kidney tissue describes as (Thyroidization phenomenon) which characterized by atrophic lining epithelium with dilated tubules contain colloid-like casts; this made the tissue appeared like thyroid tissue, hence, this made caprigoline one of the drugs that induced Nephrotoxicity [26] , and this definitely need more future studies.

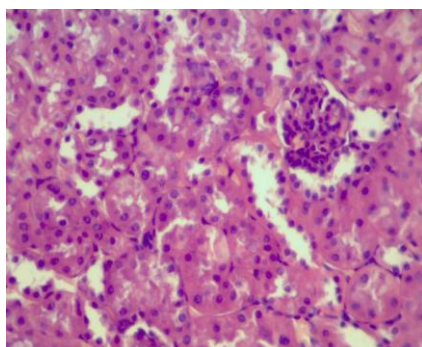


Fig. (5): Section of kidney in mice treated with 0.5 mg caprigoline showing normal like structure appearance (X400) (H &E).

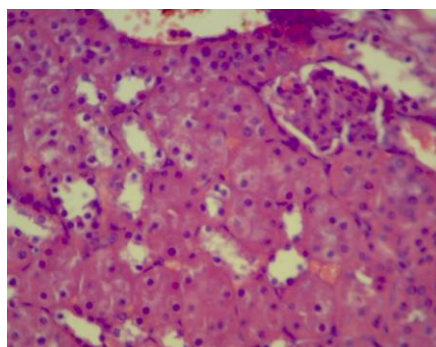


Fig. (6): Section of kidney in mice treated with 5 mg caprigoline showing normal looking appearance of renal tissue which consist of glomeruli and renal tubules (X400) (H &E).

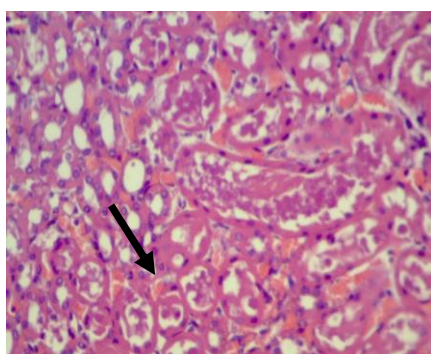


Fig.(7): Section of kidney in mice treated with 10 mg caprigoline showing degenerative changes and necrosis of epithelial tubules (X400) (H &E).

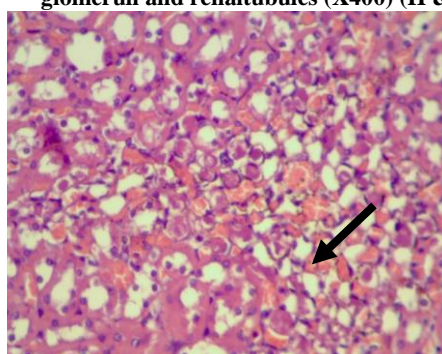


Fig.(8): Section of kidney in mice treated with 10 mg caprigoline showing thyroidization phenomenon which occur in the presence of chronic inflammation (X400) (H &E).

In conclusion, in male, the treatment with low doses of caprigoline cause an improvement in reproductive hormones levels, since there was a significant increase in LH and testosterone. On the other hand high doses may cause functional and histopathological disturbances in liver and kidney.

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