Histological and Morphological Study of Cerebrum and Cerebellum of Albino Rats Treated With Antiandrogen Flutamide

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Abstract

The present study was aimed to assess the effects of antiandrogen flutamide on some histological and morphometric cerebrum and cerebellum tissue for white albino rats, Twenty five male rats were divided into five groups each group contains 5 animals, the first and second group regarded as positive and negative control, respectively, while the other groups was treated with flutamide drug orally for 28 days at concentrations of (8, 12, 25 mg/kg/day). Histopathological results revealed that there is a significant increase in cell number of outer granular layers in cerebrum of the groups treated with 25 mg/kg/day compared to positive and negative control group as well as with other treated groups cerebrum, also the results of histopathological pointed that there were no significant differences in cerebellum tissue in all treated groups .

The morphometric results showed that there were no significant differences in thickness of cerebellar layers among different groups , therefore antiandrogen flutamide can be considered as a testosterone modulator. To determine more precise effect of flutamide on the brain, further studies are needed.

Key words : flutamide, antiandrogen, cerebrum, cerebellum

الخلاصة

هدفت هذه الدراسة إلى تقييم تاثيرالمضاد الاندروجيني الفلوتامايد على انسجةالمخ والمخيخ في الجرذان البيض، شملت الدراسه النسجيه والشكليائيه لانسجة المخ والمخيخ .,استخدم في هذه الدراسة خمسة وعشرون جرذا ذكرا قسمت إلى خمس مجموعات (تحتوي كل مجموعة على خمسةحيوانات), المجموعة الأولى والثانية اعتبرت مجموعة السيطرة الموجبة والسالبة على التوالي، والمجاميع الأخرى جرعت بعقار الفلوتامايد flutamide فمويا لمدة 28 يوما بالتراكيز (8و12و 25 ملغم/كغم/يوم).بينت النتائج النسجية أن هناك زيادة كبيرة في عدد الخلايا في الطبقه الحبيبية الخارجيه في نسيج المخ في المجموعه المعامله بتركيز 25 ملغم/كغم/يوم مقارنة مع مجموعتي السيطرة الموجبة والسالبة، وكذلك مقارنة مع مجاميع المعاملة الأخرى التي عوملت بالفلوتامايد ، كما كشفت النتائج النسجية للمخيخ انه لا توجد تغيرات معنويه لجميع المجاميع التي عوملت بالفلوتامايد.

أظهرت نتائج الدراسه الشكليائيه انه لم تحصل أي تغييرات معنويه في سمك طبقات المخيخ فيما بين المجاميع المختلفة،لذلك يمكن اعتبار مضاد الاندروجين الفلوتامايد كتستوستيرون محور. لتحديد تأثير للفلوتامايد على الدماغ بدقه اكبر تحتاج الى دراسات اكثر.

الكلمات المفتاحية: الفلوتامايد ,مضاد الاندروجين,المخ ,المخيخ.

Introduction

Flutamide (FLU) is an antiandrogen drug (a non-steroidal) use for treatment of progressive prostate cancer (Labrie *et al*.,1988). This sign is limited for using to male patients, while FLU is broadly used to women, for the treatment of polycystic ovary syndrome (POCS) related acne and hirsutism (Ehrmann, 2005) ,its supposition is related with a greater occurrence of negative event in women than in male patients. (Giorgetti *et al*., 2017), Nonetheless the flutamide acts as a competitor to

dihydrotestosterone and testosterone receptors, (Labrie *et al*., 1988) . Other androgens, dihydrotestosterone and testosterone exerted most of their effects by binding to specific intracellular receptors of androgen (ARs) (Haendler and Cleve,2012), Anti‐androgens in medical practice doing for the treatment of prostatic carcinoma are used blockers of androgen receptors, Flutamide belongs to a nonsteroidal AR antagonist group owing to blockage of AR in complete form in both central nervous system (CNS) and peripheral tissues(Gao *et al*., 2006).Generally, flutamide is a pure antiandrogen that does not exhibition agonist activity of androgen receptors (Berrevoets *et al*.,2002).

for some nongenomic androgen pathways, antiandrogens may exert AR agonist properties. In this case, antiandrogen bind to AR might be adequate to inducing the activate of AR for specific cell signaling, probably including the neuroprotective (MacLusky *et al*., 2004).It seems that the useful effect of flutamide is prevent the harmful effects and enhance of neuroprotective effects of testosterone (Fanaei *et al*.,2013).

Materials and Methods

1.Preparation of drug concentration: Flutamide drug (Eulexin) used in this study, Each tablet was dissolved in corn oil. The concentration of drug doses was depended on the animal's body weight (Sanches-craido *et al* .,1999).

2.Laboratary animals: were adapted in wire cages under normal condition with 12 hour dark and 12 hour light cycle during the whole period of experiment. Food and tap water provided ad libitum.Male their ages ranged between 8-10 weeks Twenty five male albino rats were divided into five groups (animals per each group) The daily dose of flutamide was administrated orally to each treated animals every day for Twenty eight days.

Animals included :group 1: Considered as a positive control group administrated normal saline where as group 2: considered as negative control group administrated corn oil only but group 3, 4 and 5 where treated with(8 ,12,25) mg/kg/day of flutamide.

Sample collection: The head of treated rats were carefully separated, and the cranial cavity was open very carefully with forceps, scissors and scalp. The brain was washed by saline solution and submerged in a sterile container with fixative solution, in bouin's solution, for approximately 24 hours.

Histological technique: The sectioning and staining of sample were depended on the method by Bancroft and Steven,(1990) .

Results

Histopathological Study:

Cerebrum: Histopathological study revealed that there was significantly increase in cell of outer granular layer in cerebrum of albino rats treated with 25 mg/kg / day in comparson with other group (Fig.1-4), while the other treatments showed no histological change.

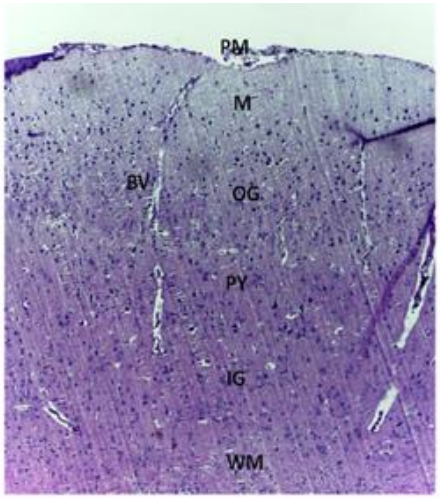


Fig. 1: Brain of control animal (cerebrum). Shows normal histology of the cerebrum; PM, pia mater. M, molecular layer. OG, outer granular layer. PY, pyramidal layer. IG, inner granular layer. WM, whit matter. BV, Cerebral blood vessel. E&H. X100.

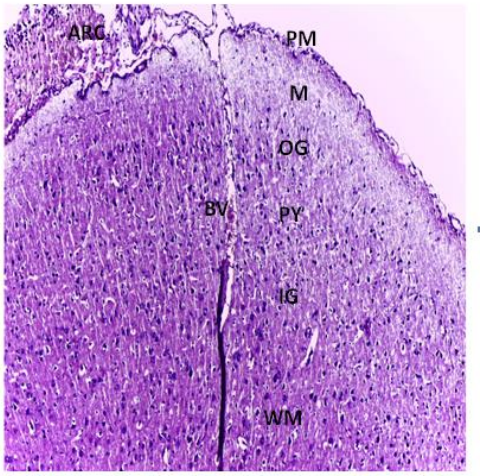


Fig. 2 :Brain(cerebrum) of animal given 8 mg/kg/day flutamide
no significant histological changes can be seen compared to control. ARC, arachnoid, PM, pia mater. M, molecular layer. OG, outer granular layer. PY, pyramidal layer. IG, inner granular layer. WM, whit matter. BV, Cerebral blood vessel. E&H. X100

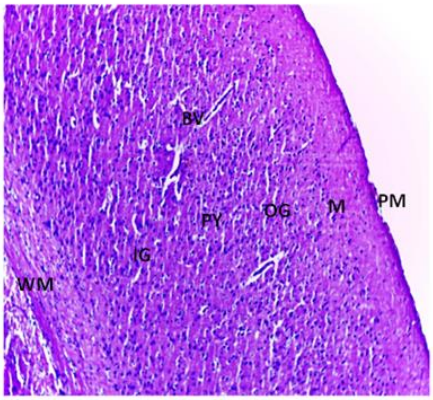


Fig. 3 : Brain(cerebrum) of animal given 12mg/kg/day flutamide : no significant histological changes can be seen compared to control. PM, pia mater. M, molecular layer. OG, outer granular layer. PY, pyramidal layer. IG, inner granular layer. WM, whit matter. BV, Cerebral blood vessel. E&H. X100.

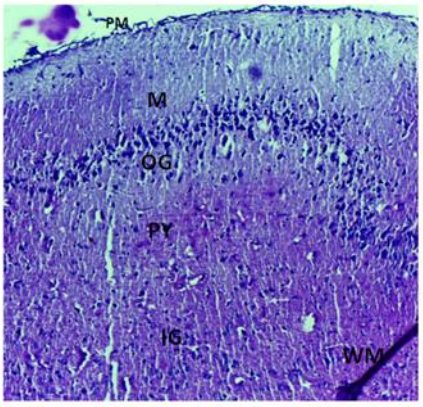


Fig. 4: Brain(cerebrum) of animal given25 mg/kg /day flutamide :In this group there is significantly increase in the cell number in the outer granular layer compared to the previous groups. PM, pia mater. M, molecular layer. OG, outer granular layer. PY, pyramidal layer. IG, inner granular layer. WM, whit matter. BV, Cerebral blood vessel. E&H. X100.

Cerebellum: There were no significant changes in cerebellum tissue of all treated groups as shown in figures (5-11).

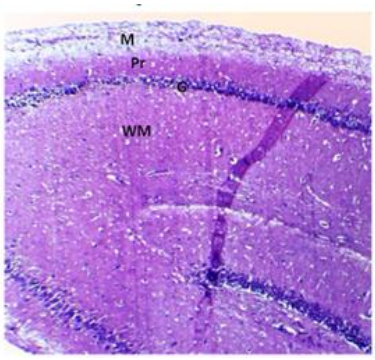


Fig. 5 : Cerebellum of control animal; the cerebellum shows its normal histology. M molecular layer. PR. Perkinj i layer. G, granular layer. WM, white matter. E&H> X100

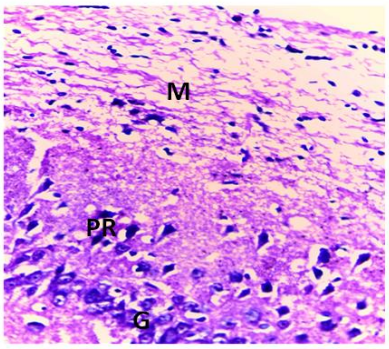


Fig. 6 : Cerebellum of control animal;. the cerebellum shows its normal histology. M molecular layer. PR. Perkinj i layer. G, granular layer. WM, white matter. E&H> X400

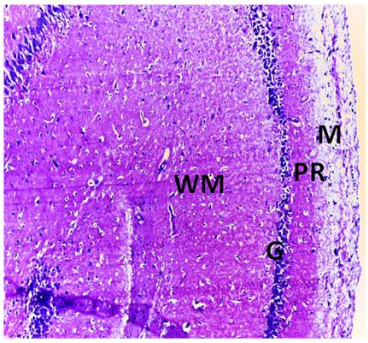


Fig. 7 : Cerebellum of l animal given 8 mg/kg/day flutamide; no significant changes in histology of the cerebellum could be identified compared to control . M molecular layer. PR. Perkinj i layer. G, granular layer. WM, white matter. E&H> X100

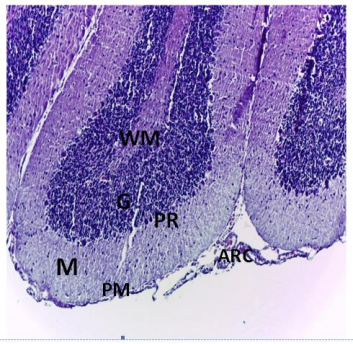


Fig. 8: Cerebellum of a animal given 12 mg/kg/day flutamide; no significant changes in histology of the cerebellum could be identified compared with control. ARC, arachnoid. PM, pia mater. M molecular layer. PR. Perkinj i layer. G, granular layer. WM, white matter. E&H>.X100

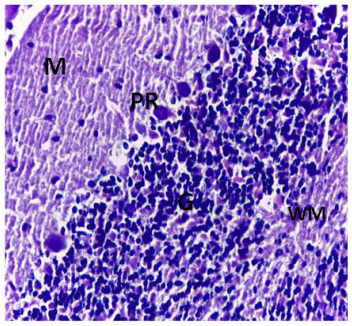


Fig. 9:Cerebellum of a animal given 12 mg/kg/day flutamide; no significant changes in histology of the cerebellum could be identified compared with control. M molecular layer. PR. Perkinj i layer. G, granular layer. WM, white matter. E&H.X400

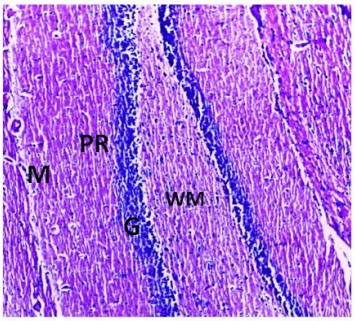
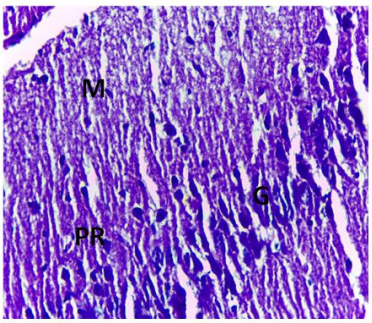


Fig. 10: Cerebellum of animal given 25 mg/kg/day flutamide; no significant changes in histology of the cerebellum could be identified compared with control. M molecular layer. PR. Perkinj i layer. G, granular layer. WM, white matter. E&H.X100



Cerebellum of animal given 25 mg/kg flutamide; no significant changes in histology of the cerebellum could be identified compared with control. M molecular layer. PR. Perkinji layer. G, granular layer. WM, white matter. E&H.X400

Morphometric Study

Cerebellum:

Outer molecular layer I, Inner most granular layer II and **Inner granular layer III**
The results indicated that there were no significant changes in all treated groups when compared with control group as noted in table (1).

Table (1): Thickness (µm) of Cerebellum layers of male albino rats in control and treated groups with antiandrogen Futamide.

Layers / Groups (mg/kg/day)	Thickness (µm)			Significant level
	Outer molecular layer I	inner most granular layer II	inner granular layer III	
Positive Control	**A 182± 15.81**	**A 56±8.49**	**A 352±58.3**	**p≥0.05**
Negative Control	**A 198 ±33.46**	**A 54±15.16**	**A 361±52.16**	
8mg/kg/day	**A 180±24.47**	**A 60±18.70**	**A 370±51.16**	
12 mg/kg/day	**A 180±23.45**	**A 59±11.43**	**A 350±58.17**	
25 mg/kg/day	**A 166±31.49**	**A 61±16.70**	**A 355±62.07**	

Cerebrum:

Morphological study of cerebrum demonstrated that significant increase in the thickness of outer granular layer II in treated group with flutamide 25 mg/kg/day(Fig,13) when compared with positive and negative control groups as well as with other treated groups as shown in figures (12,14,15,16,17).

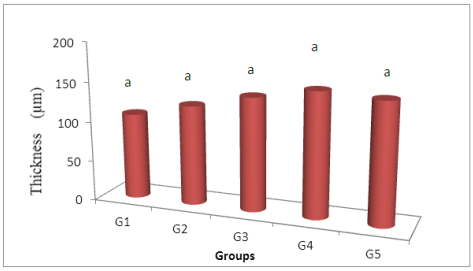


Figure (12): Thickness (µm) of molecular layer (L1) of cerebrum in male albino rats in control group and all treated group with antiandrogen flutamide

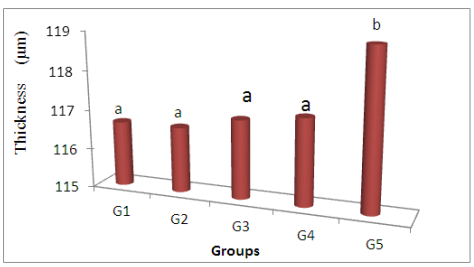


Figure (13): Thickness (µm) the outer granular layer (L2) of cerebrum in male albino rats was a significant increase **p. < 0.05** in G5 treated with antiandrogen flutamide **25 mg/kg/day** compare with other group and control group

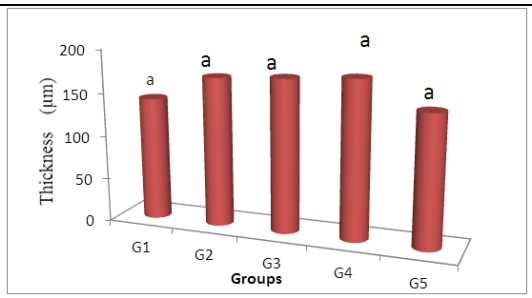


Figure (14): Thickness (µm) of external pyramidal cell (L3) of cerebrum in male albino rats in control group and all treated group with antiandrogen flutamide.

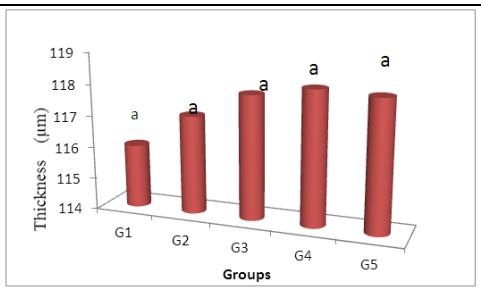


Figure (15): Thickness (µm) of internal granular layer (L4) of cerebrum in male albino rats in control group and all treated group with antiandrogen flutamide. .

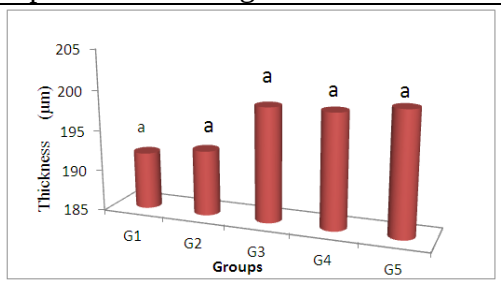


Figure (16): Thickness (µm) of internal pyramidal layer (L5) of cerebrum in male albino rats in control group and all treated group with antiandrogen flutamide. . .

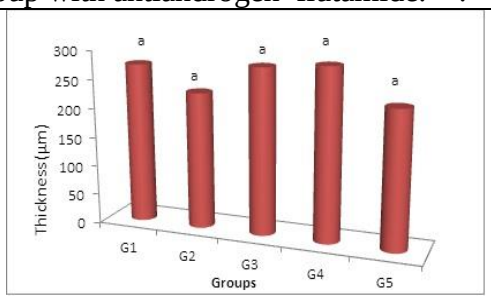


Figure (17): Thickness (µm) of multiform layer (L6) of cerebrum in male albino rats in control group and all treated group with antiandrogen flutamide. . . .

Group 1: Considered as positive control group administrated normal saline, Group 2: Considered as negative control group administrated corn oil, Group 3,Group 4 and Group 5 Rats treated with (8 ,12,25) mg/kg/day of flutamide.

Discussion

The results of current study showed that there were no significant changes in cerebrum tissue, except a slight increase of cells number in the external granular layer of the cerebrum compared with control, also the results showed that there were no significant differences in thickness of the layers of the cerebellum, compared with control. This results was agree with the study of Sajad , *et al*., (2015) who found that nettle root extract (contains anti-androgen compounds) affected structures histological structures of the cerebellar and cerebrum cortices of rats , There was no significant changes in the diameter of Purkinje cells in cerebellum and in the mean number of neuronal cell body in cerebral cortex, also there are no significantly alterations in the thickness of cerebellar layers amongst different groups.

The study of Nguyen *et al*., (2007)noted that the neuronal type cells in cultured the antiandrogen function as classic androgen receptor (AR) agonists by protection against cell death, but as androgen receptor (AR) antagonists by inhibiting classic genomic regulation. Also they observed that cyproterone acetate and flutamide acted as AR antagonists through blocked dihydrotestosterone inducing5α-reductase kind I expression

gene in a concentrated-dependent way and found that flutamide imitated DHT neuroprotection ,Furthermore, the flutamide provides selective protection cells against specific insults, a form of neuroprotective shared by DHT, flutamide similar androgens in this protection (Hammond *et al* ., 2001; Zhang *et al* ., 2004) .Previous data of Pike, (2001) revealed in hippocampal neuron culture that flutamide did not block protection of androgen against Aβ toxics. Instead, flutamide alone is neuroprotective with efficiency equivalent to dihydrotestosterone and testosterone .Also results of Fanaei (2013) showed that flutamide converses protective on the neurologic score and the brain edema and importantly enhanced histological harm of the brain in rat.

Below harmful conditions (e.g., excit, toxicity, serum deprivation, amyloid β and , oxidative stress) the testosterone effects revealed in neuronal and glial cultures ,these effects were stopped by flutamide (Liu *et al.,* 2010; Orlando *et al.,* 2007). One explanation for the protective effects of flutamide was enhancement neuroprotection effects and blockage harmful effects of testosterone, some studies agree with this explanation are observed that flutamide failed to abolish neuroprotective testosterone effect in glial and neuronal cultures (Pike ,2001; Pike *et al* ., 2008).

The interpretation of Uchida *et al*.,(2009) and Liu *et al.,(*2010) found that flutamide is contributes to neuroprotection by function as androgen agonist in inhibiting cell signaling pathways or as androgen antagonist in activating cell signaling ways that are active in harmful testosterone effect, or together of them. The additional possible cause is that flutamide obstructed AR and thus elevated available testosterone by aromatase enzyme is transform to estradiol .Estradiol is recognized as factor for a neuroprotective against cerebral ischemia and for the reason that the presence the aromatase in cerebral tissue can conversion testosterone into estradiol.

Meantime, Pike *et al*.,(2008)found that cyproterone acetate and flutamide like DHT and testosterone in protecting cells specifically against the insults of apoptotic, suggesting a common mechanism of neuroprotection, and also found that the antiandrogens actions that has providing new vision into instruments of androgen receptor-dependent androgen signaling that a shared to neuroprotection. In contrast Ahlbom *et al*.,(2001) observed that the testosterone protective effect against oxidative stress-induced death of cell in granule cells in cerebellar are blocked by flutamide. Similar, Hammod *et al*.,(2001) showed that the inhibition protected of testosterone against apoptosis induced by de-privation of serum in brain neurons of human, that by flutamide

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