ENDOCRINAL DISRUPTION EFFECTS OF ATRAZINE ON THE PUBERTY OF JUVENILE ALBINO RATS: A SUB-CHRONIC STUDY

By

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ABSTRACT

Background: Atrazine (ATR) is one of the most commonly used triazine herbicides in the world. It was found that ATR can cause adverse effects on reproductive function in both genders of several mammalian and non-mammalian species. **The aim of this work** was to evaluate the pubertal hormonal disruption effects of atrazine herbicide during and after its sub-chronic administration in juvenile albino rats of both sexes. **Material and methods:** One hundred twenty six juvenile albino rats of both sexes were used. They were divided into 3 equal groups as follow: Group I (negative control group), group II (positive control group), group III (ATR group). Each group was subdivided equally into two subgroups; male rats (subgroup a) and female rats (subgroup b). Female rats were monitored daily for vaginal opening. At the end of the 3rd and the 6th week of the study, 7 rats of each subgroup were submitted to estimate the serum levels of estradiol and luteinizing (LH) hormones in all rats of both sexes in addition testosterone in male rats. Then the rats were sacrificed. The testis and epididiymis in males, uterus and ovary in females were dissected and subjected to histopathological examination. The remaining rats were left without intervention for another 3 weeks served as follow up group. **Results:** Atrazine was found to delay puberty in male rat presented by significantly decreased levels of testosterone level and increased in the estradiol levels, impaired spermatogenesis and decrease in number and size of Leydig cells with disorganization. It also was found to delay puberty in females denoted by delayed vaginal opening, underdevelopement of the uterus, impaired folliculogenesis and ovulation in the ovaries. Three weeks of follow up resulted in partial improvement. **Recommendation:** more efforts are needed to limit exposure to atrazine especially in ground and drinking water.

Key words: herbicide, atrazine, puberty, endocrine disruption.

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INTRODUCTION

Herbicides are a class of pesticides that are marketed specifically for the purpose of killing or inhibiting the growth of weeds. Because of widespread use and easy accessibility, poisoning with herbicides has become a global environmental and health problem ***(Bhatti et al., 2011).***

Atrazine, 2-chloro-4-(ethyl amino)-6-(isopropyl amino)- s-triazine, is a widely used herbicide for the control of grass and broadleaf weeds in crops such as maize, sugarcane, and pineapple plantations. Although it has been banned in the European Union in 2004 because of its persistent ground water contamination, it is still one of the most widely used herbicides in the world ***(Ackerman, 2007).***

As a result of its indiscriminate use, residues of ATR are found not only in plants, soil, water, and cultivated ground but also in agricultural products such as fruits, milk, butter, and sugar beet ***(Purcell et al., 2001).***

Human exposure can occur through ingestion of these products, drinking contaminated water, consumption of contaminated fish, also through both inhalation and dermal absorption during its manufacture, its formulation and its application by spraying ***(Singh et al., 2011).***

Atrazine was considered as an endocrinal disruptor as it was found that ATR can cause adverse effects on reproductive function in both genders of several mammalian and non-mammalian species. Moreover, it has been reported to inhibit androgen receptor function *in vitro.* On the other hand, it can affect thyroid gland function in mammalian animals and lead to delayed sexual maturation ***(Hayes et al., 2002; Betancourt et al., 2006; Oka et al., 2008 and Rey et al., 2009).***

The aim of this work was to evaluate the pubertal hormonal disruption effects of ATR herbicide during and after its sub-chronic oral administration in juvenile albino rats of both sexes through daily examination for vaginal opening, assay of testosterone, estradiol and luteinizing (LH) hormones. Histopathological examination of testis, epididymis, uterus and ovary were also evaluated.

MATERIAL AND METHODS

(I)Material:

a) Chemical:

Atrazine in the commercial product gesaprim 80WP (an 80% wettable powder) was obtained from Syngenta Crop Protection / Basel, Switzerland, import and refilling by El-Nasr company for intermediate chemicals, in the form of white powder. Corn oil was obtained in the

form of oily solution as solvent agent from commercial sources.

b) Animals:

Juvenile albino rats of both sexes with average age of 3-4 weeks ***(Stoker et al., 2000b)***, each weighed about 50-75 gm were obtained from animal's house, Faculty of Medicine, Zagazig University.

(II)Methods:

1- Experimental Design:

This study was carried on 126 juvenile albino rats of both sexes (63 male and 63 female albino rats separated from each others). They were divided into 3 equal groups as follow:

Group I (negative control group): These rats were left without intervention to measure the basic parameters.

Group II (positive control group): These rats were gavaged orally with a daily dose 0.5 ml of corn oil for 6 weeks. **Group III (ATR group):** These rats were gavaged orally with a daily dose of ATR (300mg/kg) in corn oil which represents 1/10 of the oral LD_{50} of ATR (3000mg/kg) ***(Hauswirth and Wetzel, 1998)*****,** for 6 weeks.

N.B) each group was subdivided into two equal subgroups: male rats (subgroup a) and female rats (subgroup b).

Female rats were monitored daily for vaginal opening. The age at complete vaginal opening was recorded.

At the end of the 3rd and the 6th week of the study, blood samples were obtained from the retro-orbital plexus as described by ***Joslin (2009)*** from each subgroup to estimate the serum levels of estradiol and luteinizing (LH) hormones in all rats of both sexes in addition testosterone in male rats by ELISA according to ***Tsang et al. (1980); Chen et al. (1991) and Morimoto and Inouye(1997).*** Then the rats were sacrificed.

The testis and epididiymis were dissected from male rats and fixed in Bouin's solution, while the uterus and ovary were dissected from female rats and fixed in 10% formalin saline then subjected to histopathological examination by light microscope according to the method described by ***Horobin and Bancroft (1998).***

The remaining rats from each group were left without intervention for another 3 weeks for follow up. At the end of the 9th week they were submitted to the same investigations.

2- Methods of Statistical Analysis:

SPSS Software program was used. Mean values ± standard deviations (SD) were calculated, t test, ANOVA (F) test followed by least significant difference test (LSD test) were performed. P value of less than 0.05 was considered to be significant.

RESULTS

No statistically significant differences were observed in the studied parameters between negative and positive control groups of both sexes ***(Tables 1, 2).***

Biochemical findings:

As regard male rats in this study, ATR male group showed a significant gradual decrease in the mean values of testosterone, luteinizing hormone (LH) and a significant gradual increase in the mean value of estradiol compared to those of positive control male group ***(Table 3).***

These effects were more prominent after 6 weeks of ATR administration. In the follow up group, although the mean values of testosterone and LH showed significant decreases in comparison with those of positive control male group, these mean values showed significant increases when compared to those after 6 weeks of ATR administration. Also, the mean value of estradiol showed a significant increase after the follow up in comparison to that of positive control male group, but this mean value showed a significant decrease when compared to that after 6 weeks of ATR administration ***(Tables 3, 4).***

regarding female rats, ATR female group showed a significant gradual decrease in the mean value of LH in comparison with that of positive control female group. This effect was more prominent after 6 weeks of ATR administration. In the follow up group, although the mean value of LH showed a significant decrease compared to that of positive control female group, this mean value showed a significant increase when compared to that after 6 weeks of ATR administration ***(Tables 5, 6).***

Moreover, ATR female group showed non-significant changes in the mean values of estradiol compared to that of positive control female group after 3, 6 weeks of ATR administration and after the follow up period ***(Tables 5,6).***

Daily examination for complete vaginal opening:

In this study ATR female group showed a highly significant delay in the mean value of the age at complete vaginal opening when compared to the mean value of the age at complete vaginal opening of positive control female group ***(Table 7).***

Histopathological results:

Microscopic examination of the testicular specimens of the male rats revealed reduction in layering of the germinal epithelial lining, maturation arrest, hypospermia, dissociation and

separation of germ cells, intraluminal sloughing of germ cells and apoptosis in germ cells. The interstitial tissue showed edema with few disorganized small vacuolated Leydig cells with strip like configuration.

The appearance of these histopathological changes was time dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery noticed in follow up group ***(Table 8) & (Plate I , Figs B,C,D,E).***

Microscopic examination of the epididymal specimens of the male rats showed no sperms and hypospermia. The appearance of these histopathological changes was time dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery had occurred after the follow up ***(Table 9) & (Plate* II *, Figs B,C,D).***

Microscopic examination of the uterus showed underdeveloped structure in the form of absence of apparent endometrial glands which was regressive in the follow up group ***(Table 11) & (Plate* III*, Figs B,C, D).***

By the end of the 3rd week of ATR administration, **microscopic examination of the ovarian sections** showed numerous primary follicles separated by cellular stroma with no mature graafian follicles and no histopathological changes ***(Table 11) & (Plate* IV *, Fig B).***

By the end of the 6th week of ATR administration, the same histo-pathological changes as those described by the end of the 3rd week of ATR administration were noticed. In addition, appearance of secondary follicle, atretic follicles were observed ***(Table 11) & (Plate* IV *, Fig C).***

After the follow up, the ovarian sections showed mature graafian follicles and corpus luteum were formed with decrease in numbers of atretic follicles ***(Table 11) & (plate* IV *, fig D).***

Table (1): Student (t) test for comparison of the mean values of serum testosterone (ng/ml), estradiol (pg/ml) and LH (mIU/ml) between –ve control male group and +ve control male group (daily gavaged with 0.5ml of corn oil) after 3, 6 and 9 weeks of the study in male albino rats.

Parameter	Duration	-ve control male "Ia" Mean ± SD	+ve control male "IIa" Mean ± SD	t	p
Testosterone (ng/ml)	3 weeks	0.96±0.04	0.94±0.2	0.13	0.89
	6 weeks	2.1±0.2	2.03±0.3	1.109	0.28
	9 weeks	2.77±0.23	2.67±0.9	0.37	0.71
Estradiol (pg/ml)	3 weeks	9.14±0.7	9.35±1.0	0.45	0.6
	6 weeks	9.20±0.63	9.3±1.97	0.09	0.92
	9 weeks	10.7±0.76	10.6±1.3	0.25	0.8
LH (mIU/ml)	3 weeks	0.21±0.04	0.24±0.03	1.58	0.13
	6 weeks	0.32±0.11	0.33±0.06	0.11	0.9
	9 weeks	0.77±0.1	0.73±0.17	0.8	0.58

Number of sacrificed rats for each group was 7 rats.
SD : Standard Deviation. p>0.05 : non-significant

Table (2): Student (t) test for comparison of the mean values of serum estradiol (pg/ml) and LH (mIU/ml) between -ve control female group and +ve control female group (daily gavaged with 0.5ml of corn oil) after 3, 6 and 9 weeks of the study in female albino rats.

Parameter	Duration	-ve control female "Ib" Mean ± SD	+ve control female "IIb" Mean ± SD	t	p
Estradiol (pg/ml)	3 weeks	12.29±1.5	12.8±2.4	0.47	0.6
	6 weeks	14.6±2.4	14.3±2.7	0.1	0.91
	9 weeks	19.16±3	19.8±3.7	0.23	0.81
LH (mIU/ml)	3 weeks	0.21±0.02	0.24±0.04	1.77	0.10
	6 weeks	0.32±0.04	0.3±0.07	0.64	0.52
	9 weeks	0.6±0.11	0.55±0.13	0.78	0.44

Number of sacrificed rats for each group was 7 rats.
SD : Standard Deviation. p>0.05 : non significant.

Table (3): Student (t) test for comparison of the mean values of serum testosterone (ng/mL), estradiol (pg/mL) and LH (mIU/mL) between +ve control male group "IIa" and ATR male group "IIIa" after 3, 6 weeks of ATR administration and after the follow up in male albino rats.

	Duration	+ve control male group "IIa" Mean ± SD	ATR male group "IIIa" Mean ± SD	t	p
Testosterone (ng/mL)	**After 3 weeks of ATR administration**	0.94 ±0.2	0.24 ±0.1	7.35	<0.001**
	After 6 weeks of ATR administration	2.03±0.3	0.15±0.02	16.54	<0.001**
	After the follow up	2.67±0.9	1.06±0.5	4.07	0.0015*
Estradiol (pg/mL)	**After 3 weeks of ATR administration**	9.35 ±1.0	12.6 ±1.4	4.57	<0.001**
	After 6 weeks of ATR administration	9.3±1.97	19.4±3.3	6.99	<0.001**
	After the follow up	10.6±1.3	13.4±3.1	2.21	0.047*
LH (mIU/mL)	**After 3 weeks of ATR administration**	0.24 ±0.03	0.18 ±0.06	2.36	0.03*
	After 6 weeks of ATR administration	0.33±0.06	0.12±0.04	7.7	<0.001**
	After the follow up	0.73±0.17	0.28±0.04	6.82	<0.001**

Number of sacrificed rats for each group was 7 rats.
ATR: Atrazine SD : Standard Deviation.
*: significant (p<0.05) **: highly significant (p<0.001).

Table (4): A statistical comparison between the mean values of serum testosterone (ng/ml), estradiol (pg/ml) and LH (mIU/ml) in ATR male albino rats "IIIa" after 3 and 6 weeks of oral ATR administration (300 mg/kg) and after the follow up by ANOVA and LSD tests:

Duration / parameter	After 3weeks of ATR administration Mean± SD	After 6 weeks of ATR administration Mean± SD	After the follow up Mean± SD	F	p
Testosterone(ng/ml)	0.242±0.1	0.148±0.02 **a**	1.065±0.54 **b c**	17.4	<0.001**
Estradiol(pg/ml)	12.6±1.4	19.4±3.3 **a**	13.4±3.1 **c**	12.93	<0.001**
LH(mIU/ml)	0.18±0.06	0.12±0.04 **a**	0.28±0.04 **b c**	20.1	<0.001**

Number of sacrificed rats for each group was 7 rats. ATR: Atrazine
SD : Standard Deviation. **: highly significant (p<0.001).
a: significance between 3 & 6 weeks of ATR administration (P< 0.05)
b: significance between 3 weeks of ATR administration & follow up group (P< 0.05)
c: significance between 6 weeks of ATR administration & follow up group (P< 0.05)

Table (5): Student (t) test for comparison of the mean values of serum estradiol (pg/mL) and LH (mIU/mL) between +ve control female group "IIb" and ATR female group "IIIb" after 3, 6 weeks of ATR administration and after the follow up in female albino rats.

	Duration	+ve control female group "IIb" Mean ± SD	ATR female group "IIIb" Mean ± SD	t	p
Estradiol (pg/mL)	**After 3 weeks of ATR administration**	12.8±2.4	12.4±2.4	0.3	0.76
	After 6 weeks of ATR administration	19.8±3.7	15.72±4.2	1.92	0.07
	After the follow up	20.8 ± 3.1	18.6±4.2	1.11	0.28
LH (mIU/mL)	**After 3 weeks of ATR administration**	0.24±0.04	0.19±0.03	2.64	0.02*
	After 6 weeks of ATR administration	0.3±0.07	0.12±0.06	5.16	<0.001**
	After the follow up	0.55±0.13	0.33±0.07	3.76	0.002*

Number of sacrificed rats for each group was 7 rats.
SD : Standard Deviation. ATR: Atrazine
*: significant (p<0.05) **: highly significant (p<0.001).

Table (6): A statistical comparison between the mean values estradiol (pg/ml) and LH (mIU/ml) in ATR female albino rats "IIIb" after 3 and 6 weeks of oral ATR administration (300 mg/kg) and after the follow up by ANOVA and LSD tests.

Duration parameter	After 3 weeks of ATR administration Mean± SD	After 6 weeks of ATR administration Mean± SD	After the follow up Mean± SD	F	p
Estradiol(pg/ml)	12.4±2.4	15.72±4.2	18.6±4.2 **a**	4.9	0.02*
LH(mIU/ml)	0.19±0.03	0.12±0.06 **b**	0.333±0.07 **a c**	26.33	<0.001**

Number of sacrificed rats for each group was 7 rats.
ATR: Atrazine SD : Standard Deviation.
*: significant (p<0.05) **: highly significant (p<0.001).
a: significance between 3 weeks of ATR administration and follow up group (P< 0.05)
b: significance between 3 and 6 weeks of ATR administration (P< 0.05)
c: significance between 6weeks of ATR administration & follow up group (P< 0.05)

Table (7): Student (t) test for comparison of the mean value of the age at complete vaginal opening between +ve control female group "IIb" and ATR female group "IIIb" along duration of the study in peripubertal female albino rats.

	+ve control female "IIb" Mean ± SD	ATR female group "IIIb" Mean ± SD	t	p
Age at complete vaginal opening / days	34.7±2.56	45.4±2.7	7.6	<0.001**

Number of rats = 21 rats for each group SD : Standard Deviation.
** : highly significant (P<0.001) ATR: Atrazine

Table (8): Frequency of histopathological changes in the testis specimens after 3 and 6 weeks of atrazine administration and after the follow up.

Histopathological parameter		After 3 weeks of ATR administration		After 6 weeks of ATR administration		After the follow up	
		N	%	N	%	N	%
Seminiferous tubules	Maturation arrest	6	85.7	1	14.3	0	0
	Hypospermia	1	14.3	6	85.7	4	57.1
	Dissociation and separation of germ cells	3	42.9	4	57.1	2	28.5
	Intra luminal sloughing of germ cells	0	0	3	42.9	1	14.3
	Apoptosis in germ cells	0	0	4	57.1	2	28.5
Interstitial tissue	Edema & widening	2	28.5	5	71.5	3	42.9
	Congested blood vessels	0	0	2	28.5	1	14.3
Leydig cells	Decrease in size & number	7	100	7	100	3	42.9
	Vacuolation	1	14.3	5	71.5	2	28.5
	Strip like configuration	0	0	4	57.1	2	28.5

N= number of rats % = percentage
ATR: Atrazine

Table (9): Frequency of histopathological changes in the epididymal specimens after 3 and 6 weeks of atrazine administration and after the follow up.

Histopathological parameter	After 3 weeks of ATR administration		After 6 weeks of ATR administration		After the follow up	
	N	%	N	%	N	%
No sperm	6	85.7	2	28.5	0	0
Hypospermia	1	14.3	5	71.5	3	42.9

N= number of rats %= percentage
ATR: Atrazine

Table (10): Frequency of histopathological changes in the uterus after 3 and 6 weeks of atrazine administration and after the follow up.

Histopathological parameter	After 3 weeks of ATR administration		After 6 weeks of ATR administration		After the follow up	
	N	%	N	%	N	%
Immature uterus (absence of endometrial glands)	5	71.5	4	57.1	0	0
Mature uterus	2	28.5	3	42.9	7	100

N= number of rats %= percentage
ATR: Atrazine

Table (11): Frequency of histopathological changes in the ovarian specimens after 3 and 6 weeks of atrazine administration and after the follow up.

Histopathological parameter	After 3 weeks of ATR administration		After 6 weeks of ATR administration		After the follow up	
	N	%	N	%	N	%
No mature graafian follicle	5	71.5	4	57.1	2	28.5
Mature graafian follicle	2	28.5	3	42.9	5	71.5
Atretic follicle	0	0	4	57.1	2	28.5

N= number of examined tissues % = percentage
ATR: Atrazine

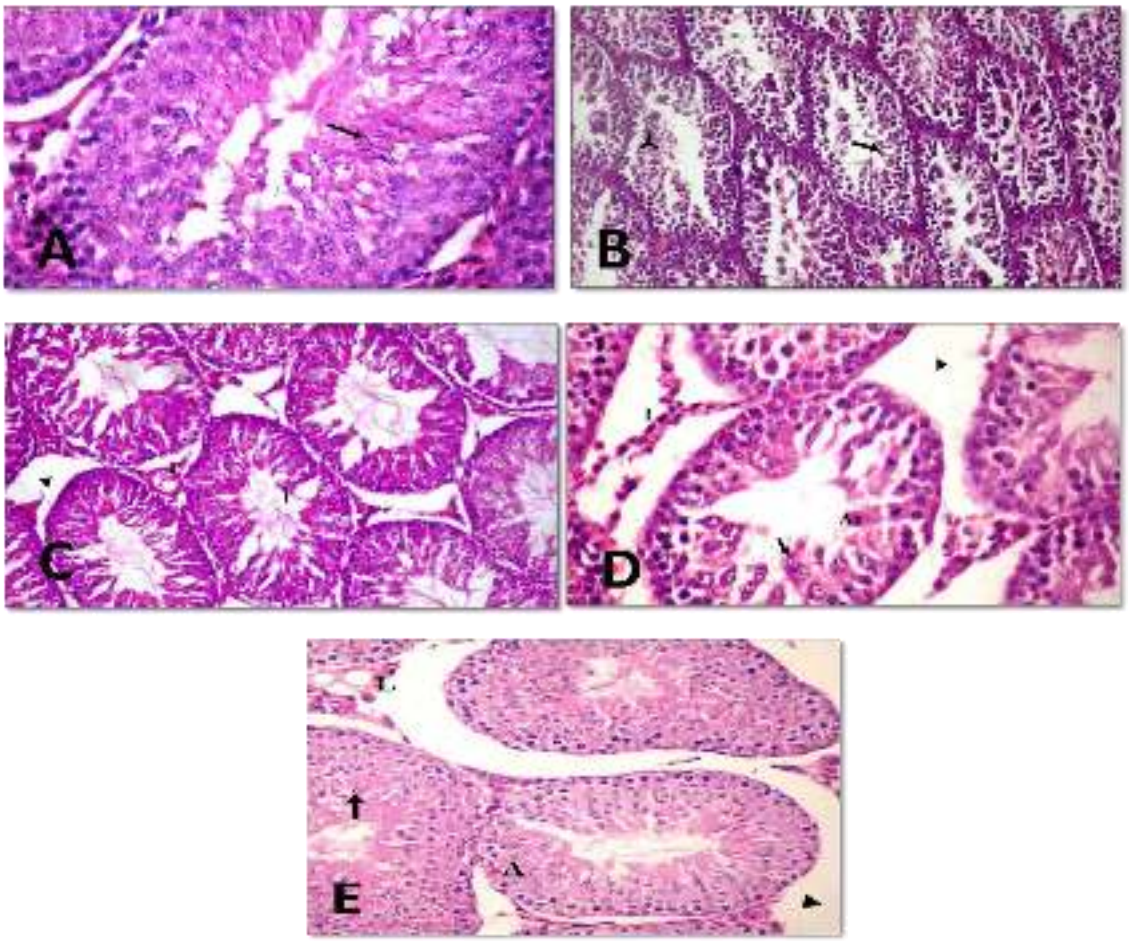

Plate (I) : sections in the testes of male albino rats showing:

Fig. (A) : normal mature testis (control group) **(H&E x400).**

Figs. (B,C,D,E): testicular tubules with reduction in layering of the germinal epithelial lining

Fig. (B) : maturation arrest(→), marked dissociation and separation of germ cells(⅄), and few and small Leydig cells (**L**) (atrazine administration for 3 weeks) **(H&E x200).**

Fig. (C) : hypospermia(→), edema in the interstitial tissue(▶) and degenerated and vacuolated Leydig cells(**L**) (atrazine administration for 6 weeks)**(H&E x200).**

Fig. (D) : maturation arrest(→), apoptosis(**A**) widening and edema in the interstitial tissue (▶)and decrease in number of Leydig cells with strip- like configuration(**L**) (atrazine administration for 6 weeks) **(H&E x400).**

Fig. (E) : hypospermia(→), apoptosis(**A**), wide interstitial space(▶), and vacuolation with degeneration of Leydig cells(**L**) (follow up group) **(H&E x200).**

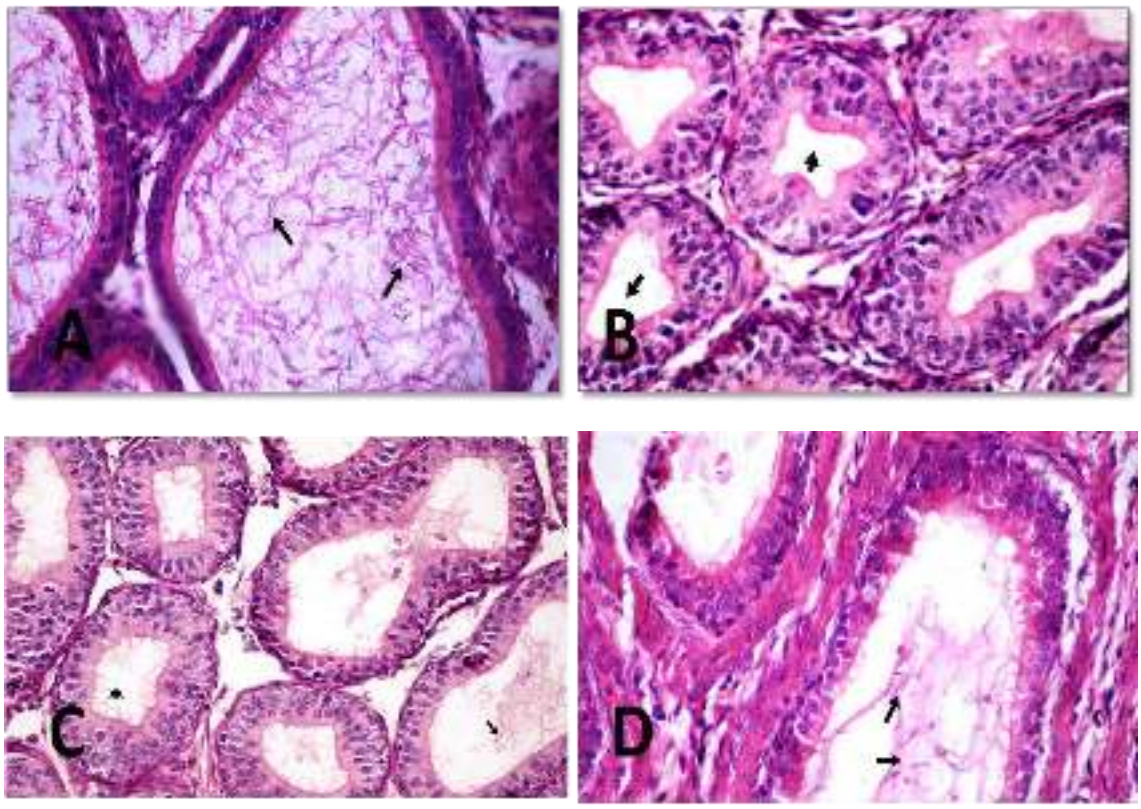

Plate (II) : sections in the epididymis of of male albino rats showing:
Fig. (A) : normal epididymal ducts distended by seminal fluid containing numerous mature sperms (→) (control group) **(H&E x400).**
Fig. (B) : epididymal ducts containing secretion devoid of sperms (atrazine administration for 3 weeks) **(H&E x400).**
Fig. (C) : Some epididymal tubules are distended by seminal fluid containing few sperms (→). Other tubules are rounded and contain no sperms(✸) (atrazine administration for 6 weeks) **(H&E x200).**
Fig. (D) : epididymal ducts distended by seminal fluid containing few sperms (follow up group) **(H&E x400).**

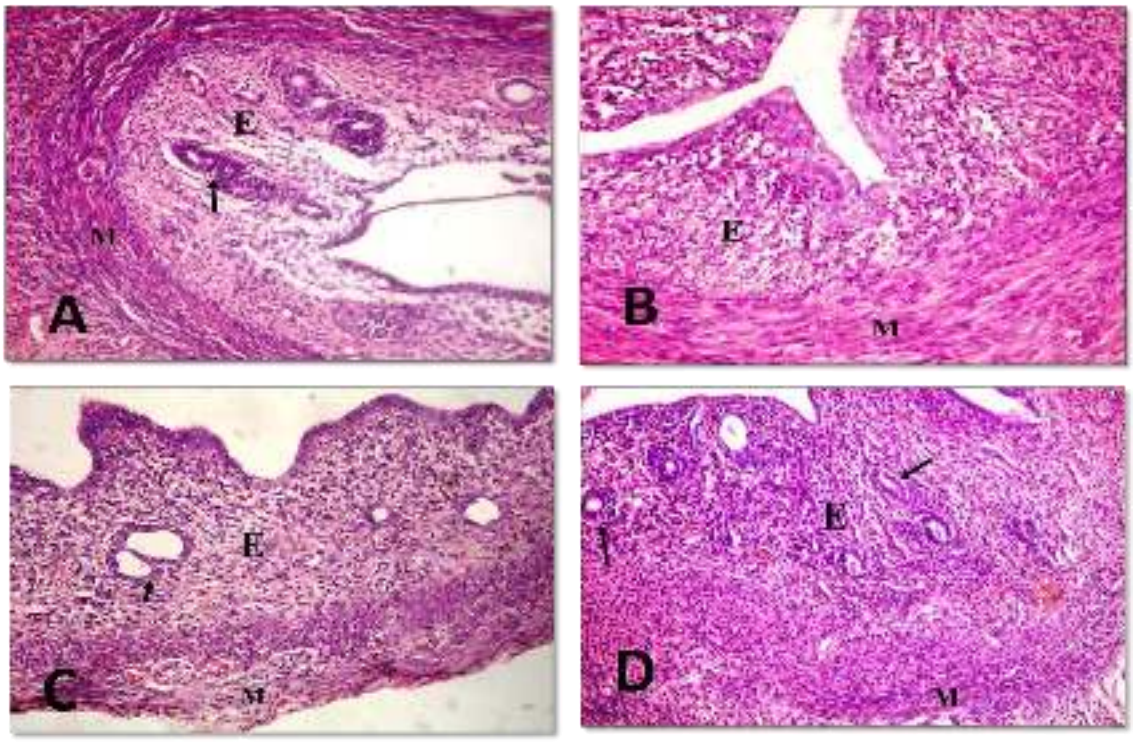

Plate (III) : sections in the uterus of female albino rats showing:
Fig. (A) : normal mature uterus. The endometrium (E) lined by surface columnar epithelium with the underlying endometrial glands (→) and stroma. Outer to the endometrium is the myometrium (M) formed of bundles of smooth muscle fiber and vascular stroma (positive control female group) **(H&E x200).**
Fig. (B) : the endometrium (E) has no apparent glandular differentiation (atrazine administration for 3 weeks) **(H&E x200).**
Fig. (C) : the endometrium (E) with the underlying endometrial glands (→) (atrazine administration for 6 weeks) **(H&E x200).**
Fig. (D) : the endometrium (E) with the underlying endometrial glands (→)(follow up group) **(H&E x200).**

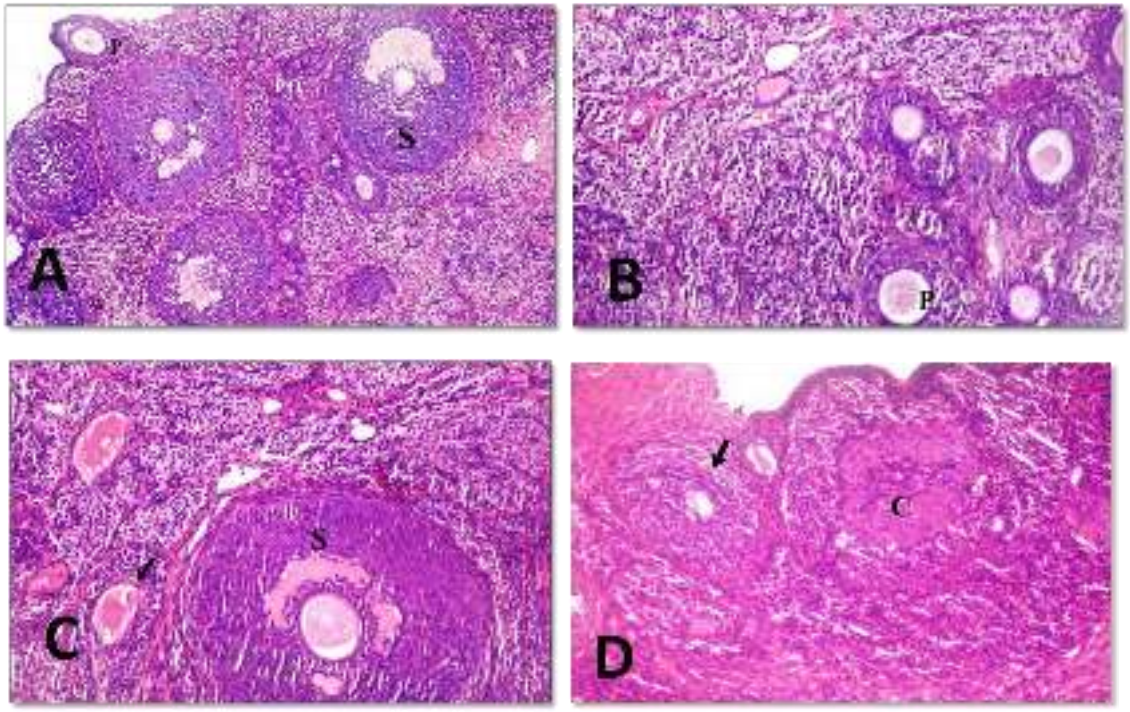

Plate (IV) : sections in the ovary of female albino rats showing:
Fig. (A) : normal mature ovary (control group) **(H&E x100).**
Fig. (B) : numerous primary follicles separated by cellular stroma (atrazine administration for 3 weeks) **(H &E x 200).**
Fig. (C) : secondary follicle (S) and atretic follicles (→), separated by cellular stroma (atrazine administration for 6 weeks) **(H&E x200).**
Fig. (D): normal corpus luteum (C) and atretic follicles (→), separated by cellular stroma (follow up group) **(H&E x 200).**

DISCUSSION

Atrazine is an agricultural pesticide used widely all over the world to control grasses and weeds. Its extensive use and its detection in surface and ground water have made ATR the subject of a number of studies that are designed to determine its possible adverse effects on endocrine parameters and reproductive function in both females and males ***(Rosenberg et al., 2008).***

Puberty is an important milestone in reproductive life as it is a transition between childhood and the adult reproductive stage. Puberty development is a multifaceted process that is under control of different hormonal regulatory mechanisms. It is a vulnerable stage of life and changes in the timing of puberty have been associated with health and psychosocial problems. So, it is considered as an area of great research interest ***(Maranghi and Mantovani, 2012).***

As regard male rats in this study, ATR male rats showed significant progressive and time dependent decreases in the serum levels of testosterone and LH hormones in comparison with those of the positive control male rats. At the same time, these rats showed a significant progressive and time dependent increase in the serum level of estradiol in comparison with that of the positive control male rats. The follow up group showed partial recovery in these hormones.

These biochemical results coincided with those of ***Stoker et al. (2000a)***; ***Victor-Costa et al. (2010) and Jin et al.(2013)*** who demonstrated that ATR exposure in male rats caused significant decreases in serum testosterone and LH levels and significant increases in serum estradiol levels.

Also, the results of the present study were in a harmony with the studies performed by ***Feyzi-Dehkhargani et al. (2011)and (2012)*** on mature male rats received ATR orally for 12, 24 and 48 days. The serum levels of testosterone and LH declined dramatically in time dependent manner.

The partial improvement present in the current study after ATR cessation could be attributed to ATR deposition in adipose tissue as described by ***Revised et al. (1996).***

Stoker et al.(2000a) stated that decreased testosterone synthesis by the Leydig cell after exposure to ATR was secondary to suppression of LH release.

Hayes et al. (2002) hypothesized that decreases in testosterone associated with ATR exposure could be driven by an atrazine-induced increase in aromatase enzyme activity. This enzyme is responsible for catalyzing the conversion of testosterone to estradiol, so leading to depleted testosterone levels via increased estradiol synthesis.

Pogrmic et al. (2009) and Jin et al. (2013) reported that when juvenile male rats and mice respectively exposed to ATR, the transcription of several genes responsible for steroidogenesis such as 17 β hydroxysteroid dehydrogenase *(17β-HSD)* and cytochrome p450 (CYP)17A1 were reduced significantly in the Leydig cells which is accompanied by decreased testosterone concentration.

Normally, testosterone levels rise gradually from postnatal day (PND) 20 to 40, and abruptly double by PND 50 in rats ***(Monosson et al., 1999).*** So, the present research suggested that ATR can delay puberty in male rats.

Cooper et al. (2007) demonstrated that the concentration of the GnRH decapeptide was increased in the median eminence of the atrazine-received rat. This finding suggested that ATR works at the level of the hypothalamus to inhibit GnRH release decreasing LH hormone level.

Dooley et al. (2013) suggested that ATR metabolites forms adducts on proteins involved in GnRH-induced calcium signaling and subsequent decreased release of LH.

Estrogens appear to play an important role in the regulation of puberty in both girls and boys ***(Delemarre-van de Waal et al., 2001).*** Estradiol is the most potent, biologically prevalent and active compound of estrogens ***(McCarthy, 2008).***

Saksena and Lau (1979) showed that, prior to PND 22, serum estradiol and estrone concentrations were high in the male rats. However, there is a dramatic decrease in the serum levels of these two hormones between PND 22 and PND 32. ***Anderson et al. (1995)*** and ***Chapin et al. (1997)*** found that natural and environmental estrogens typically delay puberty in male rats if administered prior to puberty. Thus, one might expect that a persistent elevation in serum estradiol contributed to the atrazine-induced pubertal delay ***(Stoker et al., 2000a).***

As regard female rats in the present study, ATR produced a significant progressive and time dependent decrease in the mean values of LH hormone in comparison with that of positive control female rats. After the follow up period, there was a partial recovery in the serum level of LH.

These results were in a harmony with that of ***Gojmerac et al. (2004).*** They found that administration of ATR slows progression of the estrous cycle and suppresses circulating concentrations of LH in female pigs.

This can be supported by the study of ***Cooper et al. (2000).*** They demonstrated that ATR, like aging, caused a dose- and time-dependent decrease in the amplitude of the LH release in female rats.

Foradori et al. (2009a,b and 2011) found that the inhibition of LH pulses in female rats received ATR are likely to be mediated by interference with central mechanisms controlling GnRH release from the hypothalamus.

The results of ***Foradori et al. (2013)*** study suggest that ATR acts to inhibit the secretory dynamics of GnRH pulses without interfering with GnRH mRNA and protein synthesis.

In this study, there were non-significant changes in the mean values of estradiol after 3 and 6 weeks of ATR administration and after the follow up in comparison with that of the positive control female group.

Wilhelms et al. (2006) did not support the hypothesis that ATR possesses overt estrogenic activity or can enhance endogenous estrogen production in sexually immature quail.

Tennant et al. (1994) reported that administration of very high doses of ATR expressed anti estrogenic activity in uteri of female rats without expressing intrinsic estrogenic activity.

Moreover, ***Zorrilla et al. (2010)*** studied the effects of a 21- and 41-day exposure to simazine, an example of chlorotriazine herbicides, on pubertal development in juvenile/peripubertal female rat. In both the 21-day and 41-day exposure, delayed vaginal opening and non significant changes in serum estradiol levels were reported.

The onset of puberty in the female rats is a transitional process and encompasses the period of vaginal opening (VO) and first ovulation ***(Rivest, 1991)***. Vaginal opening is an external marker of puberty onset in the female rats ***(Mayer et al., 2010)***.

In the present study, female rats gavaged with ATR 300 mg/kg/day showed a highly significant delay in the age of complete VO by 9.2 days when compared to the positive control female group. This delay in the age at complete VO indicates that ATR delays puberty in female rats.

This result agreed with the results of ***Laws et al. (2000) and Ashby et al. (2002).*** The authors found that atrazine significantly delay VO in juvenile female rats.

Laws et al. (2000) demonstrated that the cause of delay in female rat sexual maturation induced by ATR, was due to inhibition of hypothalamic GnRH release and subsequently decreased LH release.

Testicular specimens of the male rats in this study revealed reduction in layering of the germinal epithelial lining, maturation arrest, hypospermia, dissociation and separation of germ cells, intraluminal sloughing of germ cells and apoptosis in germ cells. The interstitial tissue showed edema with few disorganized small vacuolated Leydig cells with strip like configuration.

The appearance of these histo- pathological changes was duration dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery had occurred after the follow up.

These histopathological lesions were described by ***Luangpirom and Junsanjuk (2008); Feyzi-Dehkhargani et al. (2011)*; *Milena et al. (2012) and Severi-Aguiar and Silva-Zacarin (2012)*** who support our findings.

Atrazine altered sex organs affecting spermatogenesis process. This is attributed to its oxidative stress and increased reactive oxygen species ***(Abarikwu et al.,2010; Adesiyan et al.,2011; Feyzi-Dehkhargani et al., 2012* and *Abarikwu et al., 2013)***.

In the present study, epididymal specimens of the male rats showed no sperms and hypospermia. The appearance of these histopathological changes was time dependant as they increase in the severity and number of affected rats along the duration of the study with partial recovery had occurred after the follow up

These results coincided with the results of ***Stoker et al. (2000a)* and *(2002)*** who reported hypospermia in the histopathological evalutation of peripubertal male rats gavaged with ATR and ATR metabolites respectively. This observation was probably a result of the delay in puberty in ATR group.

Uterine tissues of the female rats in the present study revealed underdeveloped structure in the form of absence of apparent endometrial glands which was regressive after in the follow up group.

This result was consistent with the findings of ***Laws et al. (2000).*** The authors found underdevelopment of the uterine structure in the form of decreased myometrial development, absent endometrial glands, and immature endometrial stroma.

McMullin et al. (2004) reported that ATR does have anti-estrogenic properties *in vitro* and *in vivo* and inhibited binding of estrogen to its receptors. So, the presence of underdeveloped uterus in spite of non significant changes in estradiol levels in the present research, may be attributable to ATR's inhibition binding of estrogen to its receptors.

By the end of the 3rd week of ATR administration, **Ovarian sections** in this study showed numerous primary follicles separated by cellular stroma with no mature graafian follicles and no histopathological changes.

By the end of the 6th week of ATR administration, the same histo-pathological changes as those described by the end of the 3rd week of ATR administration were noticed. In addition, appearance of secondary follicle, atretic follicles were observed.

In the follow up group, the ovarian sections showed mature graafian follicles and corpus luteum were formed with a decrease in the number of atretic follicles. This means that there was an improvement in the ovary and ovulation had occurred.

These results were consistent with the results of ***Laws et al. (2000) and (2003).*** They observed that juvenile female rats exposed to ATR had absence or decrease in corpus luteum development.

The histopathological results of ovaries of the present study coincide with those of ***Shibayama et al. (2009)***. They suggesting that ATR had an anovulatory effect through suppression of the luteinizing hormone.

Atrazine is known to suppress the secretion of LH from the pituitary gland ***(McMullin et al.,2004).*** Luteinizing hormone is known to stimulate the development of ovarian follicles in cooperation with follicle- stimulating hormone from the pituitary gland ***(Shibayama et al., 2009)***. So, immature ovaries and increased numbers of atretic follicles observed in this study may have been induced by lower LH secretion caused by ATR.

CONCLUSION

From the above mentioned results, it can be concluded that:

- Atrazine administration at a dose of 300 mg/kg/day (1/10 LD_{50}) for 6 weeks had resulted in a delayed puberty in the juvenile male albino rats that represented by: significantly decreased levels of testosterone and increased in the estradiol, impaired spermatogenesis and decrease in number and size of Leydig cells with disorganization. These previous effects may be due to central and peripheral effects of atrazine.

- Atrazine administration by the same dose for 6 weeks had resulted in a delayed puberty in the juvenile female albino rats that represented by delayed vaginal opening, underdevelopement of the uterus, impaired folliculogenesis and ovulation in the ovaries. This endocrine disruption effect of atrazine may be due to central effect as LH hormone was decreased.
- Toxic effects induced by atrazine were time dependent.
- Follow up periods for 3 weeks resulted in partial improvement of the reproductive effects of atrazine in both sexes.

RECOMMRNDATIONS

More attention should be given to sources, environmental impact of atrazine and its metabolites in ground and drinking water together with more efforts to limit exposure to atrazine which may be a significant contributory factor to the delay puberty that leading to health and psychosocial troubles.

REFERENCES

Abarikwu, S.O.; Adesiyan, A.C.; Oyeloja, T.O.; Oyeyemi, M.O. and Farombi, E.O. (2010): Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine. Arch. Environ. Contam. Toxicol., 58:874-882.

Abarikwu, S.O.; Pant, A.B. and Farombi, E.O. (2013): Quercetin decreases steroidogenic enzyme activity, NF-κB expression, and oxidative stress in cultured Leydig cells exposed to atrazine. Mol. Cell. Biochem., 373(1-2):19-28.

Ackerman, F. (2007): The Economics of Atrazine. Int. J. Occup. Environ. Health,13:441–449.

Adesiyan, A.C.; Oyeloja, T.O.; Abarikwu, S.O.; Oyeyemi, M.O. and Farombi, E.O.(2011): Selenium provides protection to the liver but not the reproductive organs in an atrazine-model of experimental toxicity. Exp. Toxicol. Pathol., 63(3): 201-207.

Anderson, S. A.; Pearce, S. W.; Fail, P. A.; McTaggart, B. T.; Tyle, R. W. and Gray, L. E. (1995): Validation of the alternative reproductive test protocol to assess toxicity of methoxchlor in rats. Toxicol., 15: 164.

Ashby, J.; Tinwell, H.; Stevens, J.; Pastoor, T. and Breckenridge, C.B. (2002): "The effects of atrazine on the sexual maturation of female rats." Regul. Toxicol. Pharmacol., 35(3):468-473.

Betancourt, M.; Resendiz, A. and Fierro, E.C. (2006): Effect of two insecticides and two herbicides on the porcine sperm motility patterns using computer-assisted semen analysis (CASA) in vitro. Reprod. Toxicol., 22: 508–512.

Bhatti, J.S.; Sidhu, I.P.S. and Bhatti, G.K.(2011): Ameliorative action of melatonin on oxidative damage induced by atrazine toxicity in rat erythrocytes. Mol. Cell. Biochem. 353(1-2):139-49.

Chapin, R. E.; Harris, M. W.; Davis, B. J.; Ward, S. M.; Wilson, R. E.; Mauney, M. A.; Lockhart, A. C.; Smialowicz, R. J.; Moser, V. C.; Burka, L. T. and Collins, B. J. (1997): The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system functions. Fundam. Appl. Toxicol., 40:138-157.

Chen, A.; Bookstein, J.J. and Meldrum, D.R. (1991): Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. Fertil. Steril., 55: 1202-1203.

Cooper R.L.; Laws, S.C.; Das, P.C.; Narotsky, M.G.; Goldman, J.M.; Lee, Tyrey. E. and Stoker, T.E. (2007): Atrazine and reproductive function: mode and mechanism of action studies. Birth Defects. Res. B. Dev. Reprod. Toxicol., 80:98-112.

Cooper, R.L.; Stoker, T.E.; Tyrey, L.; Goldman, J.M. and McElroy, W.K. (2000): Atrazine Disrupts the Hypothalamic Control of Pituitary-Ovarian Function. Toxicol. Sci., 53 (2): 297-307.

Delemarre-van de Waal, H.A.; van Coeverden, S.C. and Rotteveel, J.(2001): Hormonal determinants of pubertal growth. J. Pediatr. Endocrinol. Metab.,14 (6):1521-1526.

Dooley,G.P.; Tjalkens, R.B. and William H. Hanneman, W. H. (2013): The atrazine metabolite diaminochlorotriazine suppresses LH release from murine LβT2 cells by suppressing GnRH-induced intracellular calcium transients. Toxicol. Res., 2: 180-186.

Feyzi-Dehkhargani, S.; Malekinejad, H.; Shahrooz, R. and Ali-Sarkhanloo, R.(2011): Detrimental Effect of Atrazine on Testicular Tissue and Sperm Quality: Implication for Oxidative Stress and Hormonal Alterations. Int.J.Toxicol. 5 (12) :426-435.

Feyzi-Dehkhargani, S.; Shahrooz, R.; Malekinejad, H. and Sadrkhanloo, R.A. (2012): Atrazine in sub-acute exposure results in sperm DNA disintegrity and nuclear immaturity in rats . Vet. Res. Forum.,3 (1): 19-26.

Foradori, C.D.; Handa, R.J.; Hinds, L.R., Hanneman, W.H.; Legare, M.E. and Clay, C.M. (2009a): Atrazine Inhibits Pulsatile Luteinizing Hormone Release Without Altering Pituitary Sensitivity to a Gonadotropin-Releasing Hormone Receptor Agonist in Female Wistar Rats. Biol. Reprod., 81: 40–45.

Foradori, C.D.; Hinds, L.R.; Hanneman, W.H. and Handa, R.J. (2009b): Effects of Atrazine and Its Withdrawal on Gonadotropin-Releasing Hormone Neuroendocrine Function in the Adult Female Wistar Rat. Biol. Reprod., 81: 1099-1105.

Foradori, C.D.; Hinds, L.R.; Quihuis, A.M.; Lacagnina, A.F.; Breckenridge, C.B. and Handa, R.J. (2011): The differential effect of atrazine on luteinizing hormone release in adrenalectomized adult female Wistar rats. Biol. Reprod., 85(4):684-689.

Foradori, C.D.; Zimmerman, A.D.; Hinds, L.R.; Zuloaga, K.L.; Breckenridge, C.B. and Handa, R.J. (2013): Atrazine inhibits pulsatile gonadotropin-releasing hormone (GnRH) release without altering GnRH messenger RNA or protein levels in the female rat. Biol. Reprod., 88(1):9.

Gojmerac, T.; Pleadin, J.; Zuric, M.; Rajkovic-Janje, R. and Korsic, M. (2004): Serum luteinizing hormone response to administration of gonadotropin-releasing hormone to atrazine-treated gilts. Vet. Hum. Toxicol., 46: 245-247.

Hauswirth, J.W. and Wetzel, L.T. (1998): Toxicity characteristics of the 2-chlorotriazines, atrazine, and simazine. In: Triazine Herbicides Risk Assessment. (Ballentine, L.G.; McFarland, J.E. and Hackett, D.S., Eds.) Oxford University Press. Washington, DC, pp. 370–383.

Hayes, T.B.; Collins, A.; Lee, M.; Mendoza, M.; Noriega, N. and Stuart, A.A. (2002): Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc. Natl. Acad. Sci. USA., 99(8): 5476-5480.

Horobin, R. W. and Bancroft, J. D. (1998) : Hematoxylin and Eosin as an oversight stain In: Trouble Shooting Histology Stains. 1st ed., Chapter (22), Churchill living stone, Pearson Professional Limited press, New York, London and Madrid, 88 - 93.

Jin, Y.; Wang, L. and Fu, Z. (2013): Oral exposure to atrazine modulates hormone synthesis and the transcription of steroidogenic genes in male peripubertal mice. Gen. Comp. Endocrinol., 184: 120-127.

Joslin J (2009): Blood collection: Techniques in exotic small mammals. J. Exotic. Pet. Med., 18(2): 117-139.

Laws, S.C.; Ferrell, J.M.; Stoker, T.E. and Cooper, R.L. (2003): Pubertal development in female Wistar rats following exposure to propazine and atrazine biotransformation byproducts, diamino-S-chlorotriazine and hydroxyatrazine. Toxicol. Sci., 76:190-200.

Laws, S.C.; Ferrell, J.M.; Stoker, T.E.; Schmid, J. and Cooper, R.L. (2000): The effects of atrazine on female wistar rats: an evaluation of the protocol for assessing pubertal development and thyroid function. Toxicol. Sci.,58(2):366-376.

Luangpirom, A. and Junsanjuk, T. (2008): Effect of Atrazine on Spermatogenesis of Mice. KKU Sci. J., 36(2551) :44-50.

Maranghi, F. and Mantovani, A. (2012): Targeted toxicological testing to investigate the role of endocrine disrupters in puberty disorders. Reprod. Toxicol., 33(3): 290-296.

Mayer, C.; Acosta-Martinez, M.; Dubois, S.L.; Wolfe, A.; Radovick, S.; Boehm, U. and Levine, J.E. (2010): Timing and completion of puberty in female mice depend on estrogen receptor α-signaling in kisspeptin neurons. Proc. Natl. Acad. Sci., 107(52): 22693-22698.

McCarthy, M.M.(2008): Estradiol and the Developing Brain., Physiol. Rev ., 88(1): 91-134.

McMullin, T.S.; Andersen, M.E. ; Nagahara, A.; Lund, T.D.; Pak, T.; Handa, R.J. and Hanneman, W.H.(2004): Evidence that atrazine and diaminochlorotriazine inhibit the estrogen/progesterone induced surge of luteinizing hormone in female sprague-dawley rats without changing estrogen receptor action. Toxicol. Sci., 79: 278-286.

Milena, S.; Sanja, V.; Vojinovi, S.M.; Mirjana, M.; Mirjana, M.; Dragin, S. and Milica, M. (2012): Is Atrazine A potential Risk on Mammalian Diversity? Acta Veterinaria (Beograd)., 62(2-3): 193-205.

Monosson, E.; Kelce, W. R.; Lambright, C.; Ostby, J. and Gray, L. E., Jr. (1999): Peripubertal exposure to the antiandrogenic fungicide, vinclozolin, delays puberty, inhibits the development of androgen-dependent tissues, and alters androgen receptor function in the male rat. Toxicol. Ind. Health, 15: 65-79.

Morimoto, K. and Inouye K.A. (1997): sensitive enzyme immunoassay of human thyroid-stimulating hormone (LH) using bispecific F(ab')2 fragments recognizing polymerized alkaline phosphatase and LH. J. Immunol. Methods, 205(1):81-90.

Oka, T.; Tooi, O.; Mitsui, N.; Miyahara, M.; Ohnishi, Y.; and Takase, M. (2008): Effect of atrazine on metamorphosis and sexual differentiation in Xenopus laevis. Aqua. Toxicol., 87 : 215–226.

Pogrmic, K.; Fa, S.; Dakic, V.; Kaisarevic, S. and Kovacevic, R. (2009): Atrazine oral exposure of peripubertal male rats downregulates steroidogenesis gene expression in Leydig cells. Toxicol. Sci., 111(1): 189-197.

Purcell, M.; Neault, J.F.; Malonga, H.; Arakawa, H.; Carpentier, R. and Tajmir-Riahi, H.A. (2001): Interactions of atrazine and 2, 4-D with human serum albumin studied by gel and capillary electrophoresis and FTIR spectroscopy. Biochem. Biophys. Acta., 1548:129–138.

Revised, A.; Blair, A. and Fraumeni, J. (1996): Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. J. Amer. Med. Association, 256: 1141-1147.

Rey, F.; Gonzalez, M.; Zayas, M.A.; Stoker, C.; Durando, M. and Luque, E.H. (2009): Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male Caiman latirostris. Gen. Comp. Endocrinol., 162 : 286–292.

Rivest, R.W. (1991): Sexual maturation in female rats: Hereditary, developmental and environmental aspects. Experientia., 47: 1027-1038.

Rosenberg, B.G.; Chen, H.; Folmer, J.; Liu, J.; Papadopoulos, V. and Zirkin, B.R. (2008): Gestational exposure to atrazine: effects on the postnatal development of male offspring. J. Androl., 29: 304-311.

Saksena, S. K. and Lau, I. F. (1979): Variations in serum androgens, estrogens, progestins, gonadotropins and prolactin levels in male rats from prepubertal to advanced age. Exper. Ageing Res., 5: 179-194.

Severi-Aguiar, G.D.C. and Silva-Zacarin, E.C.M. (2012): Effects of Herbicide Atrazine in Experimental Animal Models, Herbicides - Properties, Synthesis and Control of Weeds, Dr. Mohammed Nagib Hasaneen (Ed.). Available from: http://www.intechopen.com/books/herbicides-properties-synthesis-and-control-of-weeds/effects-of-herbicideatrazine-in-experimental-animal-models.

Shibayama, H.; Kotera, T.; Shindoda, Y.; Hanada, T.; Kajihara, T.; Ueda, M.; Tamura, H.; Ishibashi, S.; Yamashita, Y. and Ochi, S. (2009): Collaboration work on evaluation of ovarian toxicity. 14) two- or

four – week repeated –dose studies and fertility study of atrazine in female rats. J. Toxicol. Sci., 34(1) :147-155.

Singh, M.; Sandhir, R. and Kiran, R. (2011): Effects on antioxidant status of liver following atrazine exposure and its attenuation by vitamin E. Exp. Toxicol. Pathol., 63(3):269-276.

Stoker, T.E.; Guidici, D.L.; Laws, S.C. and Cooper, R.L. (2002): The effects of atrazine metabolites on puberty and thyroid function in the male Wistar rat. Toxicol. Sci., 67(2):198-206.

Stoker, T. E.; Laws, S. C.; Guidici, D. L. and Cooper, R. L. (2000a): The Effect of Atrazine on Puberty in Male Wistar Rats: An Evaluation in the Protocol for the Assessment of Pubertal Development and Thyroid Function. Toxicol. Sci., 58 (1): 50-59.

Stoker, T.E.; Parks, L.G.; Gray, E. and Cooper, R.L. (2000b): Endocrine-Disrupting Chemicals: Prepubertal Exposures and Effects on Sexual Maturation and Thyroid Function in the Male Rat. A Focus on the EDSTAC Recommendations. Crit. Rev. Toxicol., 30(2):197-252.

Tennant, M.K.; Hill, D.S.; Eldridge, J.C.; Wetzel, L.T.; Breckenridge, C.B. and Stevens, J.T. (1994): Chloro-s-triazine antagonism of estrogen action: limited interaction with estrogen receptor binding. J. Toxicol. Environ. Health.,43(2):197-211.

Tsang, B.K.; Armstrong, D.T. and Whitfield, J.F. (1980): Steroid biosynthesis by isolated human ovarian follicular cells in vitro, J. Clin. Endocrinol. Metab., 51: 1407-1411.

Victor-Costa, A.B.; Simone Miranda Carozzi Bandeira, S.M.C.; Oliveira, A.G.; Mahecha, G.A. B and Oliveira, C.A. (2010): Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. Reprod. Toxicol., 29(3): 323-331.

Wilhelms, K.W.; Cutler, S.A.; Proudman, J.A.; Carsia, R.V.; Anderson, L.L. and Scanes, B.G. (2006): lack of effects of atrazine on estrogen-responsive organs and circulating hormone concentrations in sexually immature female Japanese quail (Coturnix coturnix japonica). Chemosphere. 65: 674-681.

Zorrilla, L.M.; Gibson, E.K. and Stoker , T.E. (2010): The effects of simazine, a chlorotriazine herbicide, on pubertal development in the female Wistar rat. Reprod. Toxicol., 29(4): 393-400.

تأثيرات الاضطرابات الهرمونية للأترازين على البلوغ فى الجرذان البيضاء الغير بالغة: دراسة مزمنة قصيرة المدى

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مقدمة البحث: يعتبر الأترازين واحدا من مبيدات الأعشاب تريازين الأكثر إستخداما في العالم. وقد وجد أن الأترازين يمكن أن يسبب آثارا ضارة على الوظيفة التناسلية في كلا الجنسين في العديد من أنواع الثدييات وغير الثدييات. **الهدف من البحث**: كان الهدف من هذا البحث تقييم آثار الاضطراب الهرموني لسن البلوغ أثناء وبعد تناول الأترازين لمدة قصيرة المدى في الجرذان البيضاء الصغيرة من كلا الجنسين. **طريقة البحث**: تم استخدام مائة ستة وعشرون من صغار الجرذان البيضاء من كلا الجنسين وقد تم تقسيمهم إلى ٣ مجموعات متساوية على النحو التالي:المجموعة الاولى (المجموعة الضابطة السالبة)، المجموعة الثانية (المجموعة الضابطة الموجبة)، المجموعة الثالثة (مجموعة الأترازين). ثم تم تقسيم كل مجموعة بالتساوي إلى مجموعات فرعية من الذكور (مجموعه أ) و الاناث(مجموعة ب). تم فحص فتحة المهبل لإناث الجرذان يوميا وتسجيل عمرهن عند اكتمال فتحة المهبل. فى نهاية الأسبوع الثالث و السادس تم أخذ عينات دم من ٧ جرذان من كل مجموعة وقياس مستوى هرمونات الاستراديول والليوتينيزنج فى كل الجزدان من كلا الجنسين بالاضافه الى التستوستيرون فى ذكور الجرذان . وبعد ذبح الجرذان تم إستئصال الخصية ، البربخ، الرحم والمبيض وفحصها بالميكروسكوب الضوئي لتحديد التغيرات الباثولوجية التى حدثت بها. الجرذان المتبقية في كل مجموعة تم متابعتها بدون علاج لمدة ثلاث أسابيع. **النتائج** : من نتائج البحث وجد أن الأترازين قد أدي الى تأخر سن البلوغ فى ذكور الجرذان ممثله فى إنخفاض بشكل ملحوظ في مستويات مستوى هرمون التستوستيرون وزيادة في مستويات استراديول، ضعف فى تكوين الحيوانات المنوية، مع وجود عدد غير منظم و قليل من خلايا ايديغ. كما وجد أيضا أن الأترازين قد أدى الى تأخر البلوغ في إناث الجرذان ممثلة فى تأخر فتحة المهبل و عدم نضوج أنسجه الرحم، مع ضعف الإباضة في المبيض. المتابعة لمدة ٣ أسابيع بعد توقف إعطاء الأترازين أسفرت عن تحسن جزئي. **التوصيات:** توصى هذه الدراسة ببذل المزيد من الجهود للحد من التعرض للأترازين خصوصا في الأرض ومياه الشرب.