Effect of Diazinon on Reproductive System of Adult Male Mice

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Abstract

This study was designed to investigate the effect of diazinon on reproductive system of adult male mice reproductive performance. Forty adult male albino mice were randomly divided into two equal groups. Collars made to the mice that were caged alone. The animals of the first group were dipped in tap water without of diazinon and served as control group, while the animals of the second group were dipped with diazinon 60% in dilution (1-1000) and used as treated group. Five animals from each group were sacrificed on the days 1, 14, 28 and42 after the end of treated period. The results revealed that there were significant decrease in sperm count, sperm motility, serum testosterone concentration and diameter and thickness of seminiferous tubules. These results associated with significant increase in dead and abnormal sperm. The activity of acetylcholinesterase was significantly decreased at the first day of post treated period. Histopathological examination of the testes also revealed degenerative changes in the cell lining the seminiferous tubules. In conclusion, diazinon had adverse effect on the reproductive system of adult male mice may be occurrence of oxidative stress.

Key word: diazinon , sperm parameters ,testosterone, testes, epididymis , acetylcholine esterase .

تأثير الديازنيون على الجهاز التناسلي لذكور الفئران البالغة

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المستخلص

صممت الدراسة لمعرفة تاثير الديازنيون على الكفاءة التناسلية لذكور الفئران البالغة. 40 من ذكور الفئران البالغة قسمت عشوائيا الى مجموعتين متساويتين. عملت ياقات (Collors) للفئران التي وضعت بشكل منفرد في كل فقص. غطست حيوانات المجموعة الاولى (السيطرة) بالماء العادي الدافئ في حين غطست حيوانات المجموعة الثانية (المعاملة) بحملول الديازنيون (60%) وبتخفيف (1 – 1000) وهو ذات التخفيف المستخدم في تغطيس او رش الحيوانات الحقلية ولمدة (21 يوما) (بين يوم واخر). تمت التضحية (5)

حيوانات من كل مجموعة وللايام (42,28,14,1) بعد نهاية التغطيس. حيث تم دراسة المعايير التالية: وزن الجسم ، وزن الخصى ، تركيز وحركة النطف ، النسبة المئوية للنطف الميتة الى النطف الحية ، تشوهات النطف ، وقياس تركيز هرمون التستوستيرون ونشاط انزيم الاستيل كولين استريز اضافة الى التغيرات النسيجية للخصى والبربخ. أظهرت النتائج وجود انخفاض معنوي (P>0.05) في تركيز النطف وحركتها وتركيز التستوستيرون اضافة الى انخفاض سمك اقطار الانابيب المنوية، كما اظهرت النتائج الى وجود زيادة معنوية (P>0.05) بنسب النطف الميتة والنطف المشوهة. كما لوحظ انخفاض معنوي (P>0.05) بنشاط انزيم الاستيل كولين استريز لليوم الاول بعد انتهاء فترة التغطيس. كما اظهرت المقاطع النسيجية حدوث تغيرات تنكسية في الخلايا المبطنة للانابيب المنوية مع قلتها تفجي هيولي لخلايا سرتولي. يستنتج من ذلك ان هنالك تاثير سلبي للديازنيون على الكفاءة التناسلية لذكور الفئران البالغة بدلالة انخفاض معايير تقييم النطف وانسجة الخصية والبربخ اضافة الى انخفاض مستوى هرمون التستوستيرون وقد يكون ذلك بسبب حدوث الأجهاد التأكسدي .

الكلمات المفتاحيه : دايازينون ، معايير النطف ،التستيستيرون، الخصى ،البربخ ، اسيتيل كولين استريز .

Introduction

Organophosphorus insecticides are most commonly used in the world in eradication programs of insect in human, animals and plants, as well as in other sectors of farming industry. One of these organophosphorus insecticides is the diazinon which is used widely as insecticide for eradication of insect in animals and even in houses (31and 20). It has also been widely used for treating sheep from ectoparasites (25). Diazinon's chemical name is (O,O-diethyl-O-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl) phosphorothioate (9). Diazinon is converted inside in the body to active substance called diazoxon(12), in the latter which is then antagonized with function of central nervous system through the inhibition of acetylcholinestrase (Ach E)(4).Exposure to diazinon cause headache, nausea, vomiting, burning eyes, difficulty in breathing and lethargy(11), and (34) reported severe histological alterations in the cardiac tissue in diazinon-treated animals, in addition, increased the oxidative stress and oxidative modifications in the genomic DNA content of the cardiac tissues. Other studies recorded the effect of diazinon on normal function of both male and female reproductive system in mammals, birds and fishes due to the damage of gonads (1,15, 18 and 32). The present study was designed to investigate the effect of dipping with diazinon (60%) at dilution (1-1000) in tap water for 21 consecutive days (as used in field) and examine the histological and physiological changes in reproductive organs of mice.

Materials and Methods

Forty mature male mice (8-10 weeks old, average weight 26 gm.) had ad labium access to tap water and fed with standard commercial pellets and kept under a photoperiod of 12 dark :12 light hour. The animals were randomly divided into two equal

groups. Collars were made to each one and caged alone in plastic boxes (29x15x12 cm), and left for 14 days for adaptation to the experiment condition. The animals of the first group were dipped in warm tap water (36-37'c) and served as a control, while the animals of the second group were dipped with warm tap water containing diazinon 60% in dilution (1-1000) for a period of 21 days (as used in field) and considered as treated group. Each five animals were sacrificed on the days 1, 14, 28, and 42 after the end of treated and blood samples were collected directly from the heart of anesthetized animals with diethyl ether and the serum was then separated for measuring serum concentration of the following : a-testosterone by using Radioimmunoassay kits(Bekman,France),b- acetyl cholinesterase activity as described by (19).

The activity of acetyl cholinesterase was estimated by using the potentiometer method as the following equation:

$$\% \text{ of inhibition} = \frac{\text{Enzyme activity of control group\_ Enzyme activity of treated group}}{\text{Enzyme activity of control}}$$

In addition the left testis of each animal was isolated for histopathological examination (5).

To preparation of sperm right epididymial tail was isolated and mix with 1ml of normal saline at (37-39'C), then cutted by micro surgical scissors to almost 200 pieces to release the sperm as described by (37), and the following parameters were studied as follow: the percentage of dead and viable sperm were calculated according to (33), sperm motility according to (22), sperm concentration as described by (14) and Sperm abnormalities according to (28). Data was expressed as mean ± SE. Statistical significance differences was determined using two way analysis of variance(ANOVA) and least significant differences (LSD) . The significant level was set at $P< 0.05$ (29).

Results and Discussion

The results reveled that a significant decrease ($P<0.05$) in sperm count at day 1 and 14 of the treatment period, while there was no significant differences ($P>0.05$) at day 28 and 42 post the treatment. Within the time the results showed a significant increase ($p<0.05$) in sperm count in treated group at days 14,28 and 42 of treatment as compared with day 1 of treatment (table -1) .

The results also showed a significant decrease ($P<0.05$) in the percentage of sperm motility in treated group at days 1 and 14 of treatment compared to control group. While there is no significant differences ($P>0.05$) in the percentage of sperm motility at day 28 and 42 post the treatment between two experimental groups . Within the time, there was a significant increase ($P<0.05$) in the percentage of sperm motility at days 28 and 42 post treatment of treated group as compared with days 1and 14(table 2) .

Exposure of males mice to diazinon caused a significant increase (P<0.05) in the percentage of dead sperm after 1 and 14 days of treatment compared to control group, while there was no significant differences (P>0.05) in this parameter at days 28 and 42 of treatment as compared with control group (table -3). During the experimental period the results showed a significant decrease (P<0.05) in the percentage of dead sperm at days 14,28 and 42 post treatment as compared with percentage at days 1 of treatment. Besides, analysis of the spermatozoa of male mice exposed to diazinon reveled that there is a significant increase (P<0.05) in the percentage of teratozoospermic spermatozoa (abnormal sperm) at day 1 of the treatment as compared with control (table-4).

Serum testosterone concentration in both experimental groups illustrated in table-5. The results reveled that a significant decrease (P<0.05) in the concentration of blood testosterone in treated group at days 1 and 14 of the treatment as compared with control. Besides, serum testosterone concentration increased significantly (P<0.05) in treated group at days 28 and 42 of the experiment as compared with days 1 and 14 of the treatment. On the other hand, exposure of male mice with diazinon caused a significant decrease (P<0.05) in the activity of acetylcholinestrase after day 1 of treatment as compared with control group (table -6).

Spermatogenesis is the process under the control of hypothalamic-hypophysial-gonadal axis and the interaction between Sertoli cells and Lydig cells (24), the alteration in testosterone biosynthesis affect mainly the spermatogenesis and this will explain the decrease in sperm count at day 1and 14 post dipping probably due to the direct effect of diazinon on spermatogenesis (23) or indirectly on the hypothalamus-hypophysial axis (13). The decrease in motility of sperm and increased in percentage of dead sperm at day 1and 14 post dipping may be due to the alteration of sperm chromatin structure by diazinon (23) which is lead to changes in sperm viability (10) or due to the effect of diazinon on mitochondria (10), or due to the increased in germinal cells apoptosis (7). The increased in teratozoospermia percentage also was recorded in our experiment at day 1 and 14 post dipping may be due to the alteration in germinal DNA during spermiogenesis (23 and 30) . These changes may be due to diazinon suppresses reproductive function with endogenous hormonal disruption (17) which affect the pathway of testosterone biosynthesis (7). Diazinon induces the production of oxidative stress (by alteration of antioxidant enzyme activity) and increasing lipid peroxidation in the testis is associated with implications for male fertility (3).So the significant decrease in serum testosterone concentration at day 1 and 14 post dipping may be due to the direct effect of diazinon on Leydig cells, or indirectly through the alteration of the hypothalamus-hypophysial axis by these organophosphorus compound that lead to decrease in prolactin level which effect on testosterone secretion via increasing in LH receptors on Leydig cells (8 and 32), or due to the effect of these organophosphorus on dopamine which affect the prolactin secretion (6). The estimation of the plasma acetylcholinesterase activity is a useful method to evaluate the exposure to organophosphorus (21), the result showed a significant decrease in acetylcholinesterase activity at day 1 post dipping this implies the acute

effect of diazinon .It can be concluded that acetylcholine in the cholinergic neurons has a potential threshold to perform a crucial part in the complex circuitry of neuro-endocrine regulatory mechanisms (6) .Besides , disappearance the effect of diazinon at days 14, 28 and 42 post dipping, may be duo to the low sensitivity of mature mice to the inhibition of this enzyme (16).

Table (1) Sperm Concentration in the epididymal tail (Sperm / ml x 10^6) of mice treated with diazinon.

Groups / Days after Dipping	Control	Treatment
1	24 ± 0.82 Aa	12.6 ± 1.02 Ba
14	25.6 ± 1.15 Aa	15.92 ± 1.15 Bb
28	23.37 ± 1.7 Aa	17.3 ± 1.22 Ac
42	23.42 ± 0.58 Aa	22.34 ± 0.28 Ac

Values are expressed as mean ± SE . n= 5 / group .
Small letters denote significant differences within groups P < 0.05.
Capital letters denote significant differences between groups P < 0.05.

Table (2) Sperm Motility (%) of mice treated with diazinon.

Groups / Days after Dipping	Control	Treatment
1	81 ± 4.58 Aa	59 ± 4 Ba
14	85 ± 2.74 Aa	62 ± 4.06 Ba
28	81.4 ± 2.42 Aa	81 ± 4 Ab
42	85 ± 2.23 Aa	81 ± 2.91 Ab

Values are expressed as mean ± SE . n= 5 / group .

Small letters denote significant differences within groups P < 0.05

Capital letters denote significant differences between groups P < 0.05.

Table (3) Dead to life sperms (%) of mice treated with diazinon.

Groups / Days after Dipping	Control	Treatment
1	19.2 ± 3.4 Aa	52.6 ± 3.4 Ba
14	16.8 ± 1.7 Aa	35 ± 2.7 Bb
28	18.6 ± 3.6 Aa	20 ± 1.4 Ac
42	17 ± 1.6 Aa	21.6 ± 1.9 Ac

Values are expressed as mean ± SE . n= 5 / group .

Small letters denote significant differences within groups P < 0.05.

Capital letters denote significant differences between groups P < 0.05.

Table (4) Teratoazoospermia in the epididymal tail (%) of mice treated with diazinon.

Days after Dipping	Control	Treatment
1	22.2 ± 1.01 Aa	43.4 ± 1.9 Bb
14	24.8 ± 1.7 Aa	26 ± 1.3 Aa
28	22.8 ± 1.5 Aa	23 ± 1.8 Aa
42	25 ± 1.9 Aa	23 ± 2.21 Aa

Values are expressed as mean ± SE . n= 5 / group .Small letters denote significant differences within group P < 0.05

Capital letters denote significant differences between groups P < 0.05.

Table (5) Serum testosterone concentration (ng/ml) of mice treated with diazinon.

Groups / Days after Dipping	Control	Treatment
1	3.05 ± 0.63 Aa	0.42 ± 0.14 Bb
14	2.78 ± 0.2 Aa	0.52 ± 0.15 Bb
28	2.91 ± 0.23 Aa	2.63 ± 0.24 Aa
42	3.02 ± 0.03 Aa	2.98 ± 0.32 Aa

Values are expressed as mean ± SE . n= 5 / group .Small letters denote significant differences within groups P < 0.05

Capital letters denote significant differences between groups P < 0.05.

Table (6) Plasma acetylcholine esterase of mice treated with diazinon.

Groups	Enzyme Activity: Change in pH / min. (1%) Control		Treatment	
Days after Dipping	ENZ. activity	Inhibition %	ENZ. activity	Inhibition %
1	1.33 ± 0.04 Aa	Zero %	0.88 ± 0.02 Bb	33.8 %
14	1.30 ± 0.01 Aa	Zero %	1.23 ± 0.02 Aa	5.3 %
28	1.34 ± 0.02 Aa	Zero %	1.24 ± 0.04 Aa	7.4 %
42	1.44 ± 0.06 Aa	Zero %	1.34 ± 0.04 Aa	6.9 %

Values are expressed as mean ± SE . n= 5 / group .Small letters denote significant differences within group P < 0.05

Capital letters denote significant differences between groups P < 0.05.

Histopathological examination:

The results showed a significant decrease (P<0.05) in the diameter and thickness of the lining cells of seminiferous tubules of treated group at days 1, 14 of treatment as compared with control group. Within the time , there is significant decrease (p<0.05) in the diameter of the seminiferous tubules of treated group at days 1 and 14 of the treatment as compared with days 28 and 42 of the treatment (tables -7,8).

Table (7) Mean of diameter of seminiferous tubules (µm) of mice treated with diazinon.

	Control	
Days after Dipping		
1	**187 ± 1.5 Aa**	**165.2 ± 2.7 Ba**
14	**185.7 ± 2.3 Aa**	**172.2 ± 2.6 Bb**
28	**182.2 ± 2.9 Aa**	**180.5 ± 1.6 Ac**
42	**184 ± 2.6 Aa**	**181 ± 3.1 Ac**

Values are expressed as mean ± SE . n= 5 / group .
Small letters denote significant differences within group P < 0.05
Capital letters denote significant differences between group P < 0.05

Table (8) Mean of Lining Cells of the seminiferous tubules of mice treated with Diazinon.

Groups	Control	Treatment
Days after Dipping		
1	77 ± 0.7 Aa	57.6 ± 1.2 Ba
14	77.9 ± 2.1 Aa	64.28 ± 1.3 Bb
28	80 ± 1.3 Aa	76.95 ± 0.9 Ac
42	79.6 ± 1.3 Aa	78.08 ± 1.4 Ac

Values are expressed as mean ± SE . n= 5 / group.
Small letters denote significant differences within group P < 0.05
Capital letters denote significant differences between group P < 0.05

Furthermore, testicular histopathological examination of treated group at days 1 and 14 showed a significant decrease in the activity of spermatogenesis due to the decrease in the lining cells and thickness of the basement membrane of the seminiferous tubules, which associated with necrotic changes in Sertoli cells, while other sections showed that the seminiferous tubules lining only with Sertoli cells or basement membrane (figures-3,5) compared to control (figure-1). The sections of epididymis showed there are a hyperplasia in the lining cells of the epididymis, also the epididymial duct cells appeared cuboidal with presence of monocyte in the lumen (figures-4) compared to control (figure-2). While the histological features of the testes and epididymis of treated group appeared normal at days 28 and 42 of treatment (figures 6,7,8, and 9) . The changes in germinal cell lining the seminiferous tubules and the

presence of vacuolation in the cytoplasm of Sertoli cells this due to the effect of diazinon on interaction between Leydig and Sertoli cells which is necessary for normal intratesticular testosterone production and their effect to induce the differentiation of spermatogenesis to spermatozoa in the seminiferous tubules (2,7,26 and 32).

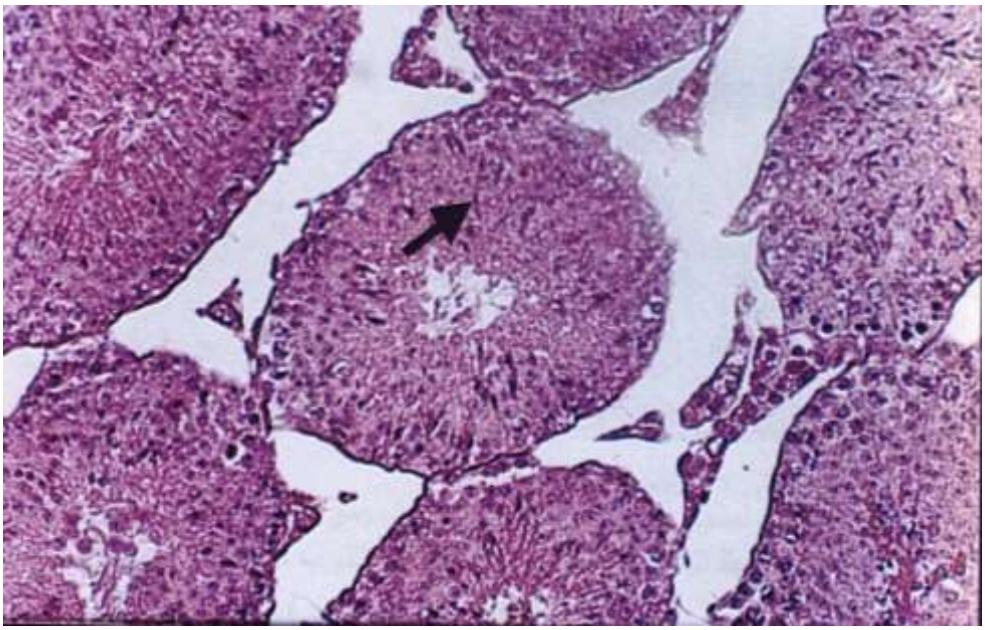

Figure (1) Section in the testis of the mice in control group, note normal structure of seminiferous tubules and spermatogenesis. (H & E X200).

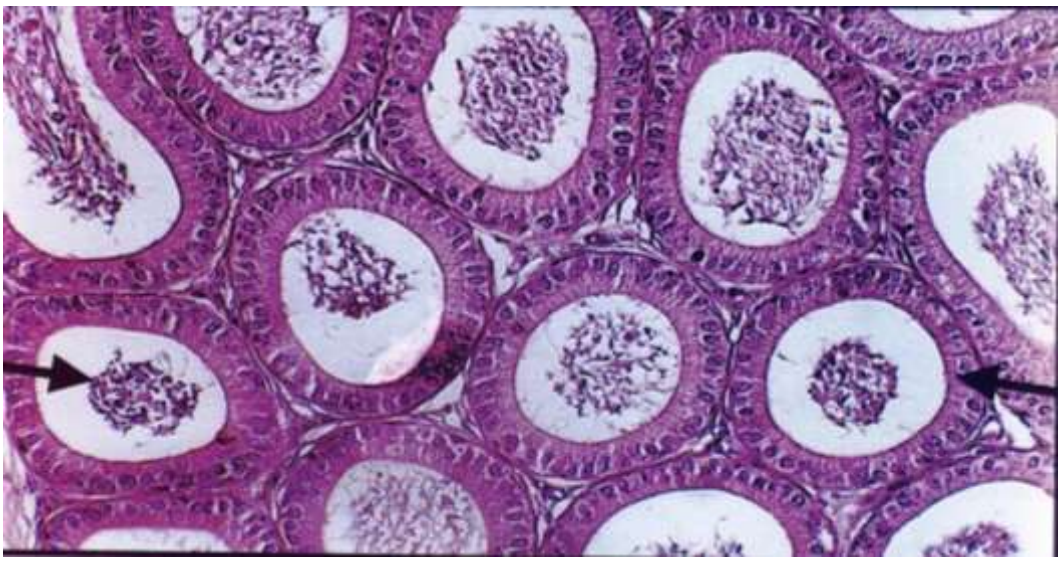

Figure (2): Section in the epididymis of the mice in control group, note normal epididmyal tissue (H & Ex 200).

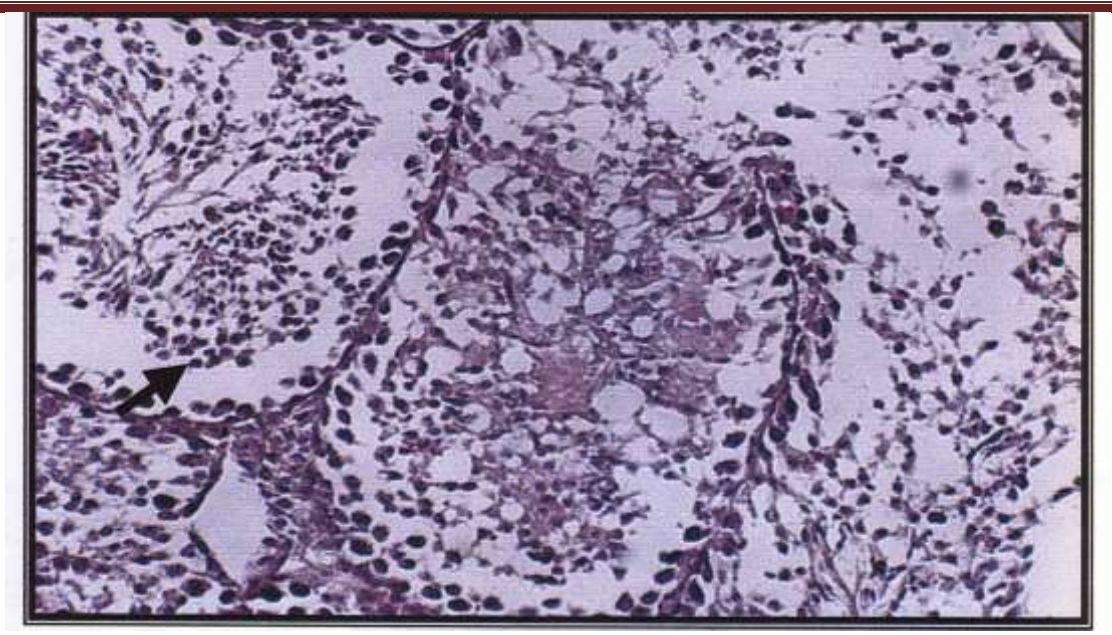

Figure (3): Section in testes of treated group at 1day after dipping, note the degenerative changes in the lining cells of the seminiferous tubules. (H & Ex 200).

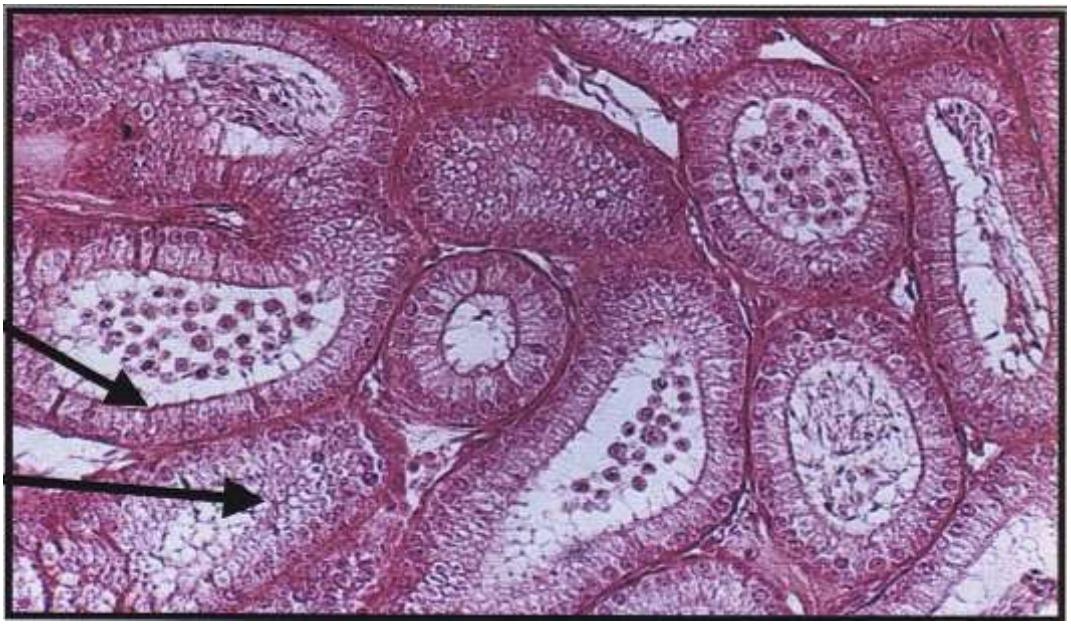

Figure (4) Section in epididymis of treated group at 1day after dipping, note vacuolation of lining cells. (H & E x 200).

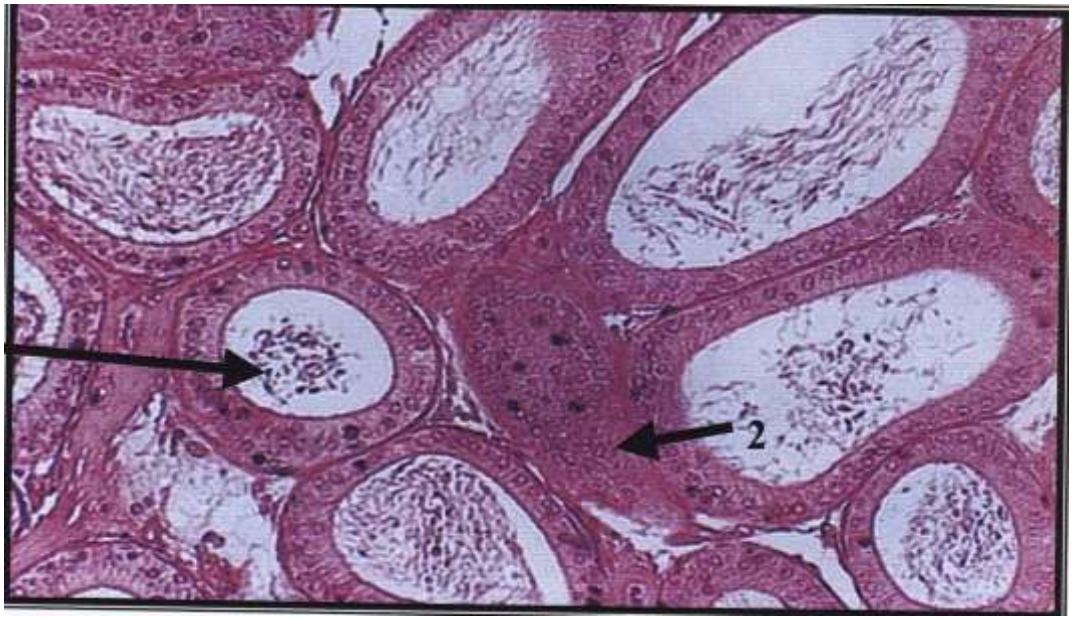

Figure (5) Section in testes of treated group at 14days after dipping, note the degenerative changes in the lining cells of the seminiferous tubules and presence of monocytes in the lumen (H & E x 200).

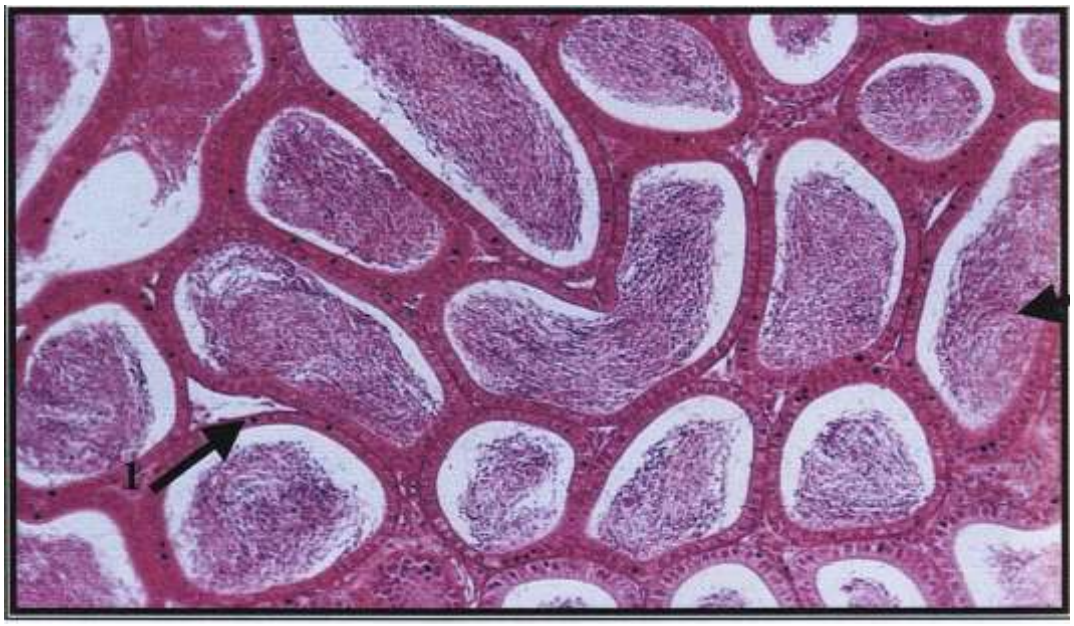

Figure (6) Section in testis of treated group at 28days after dipping, note repair of the seminiferous tubules with little vacuolation of some cells. (H & E x 200).

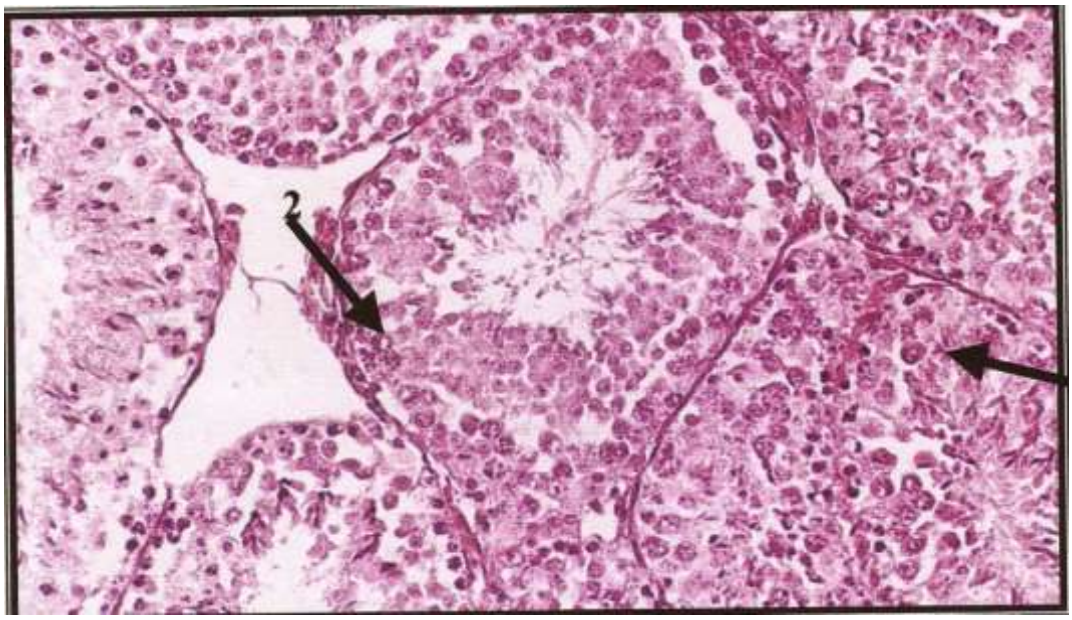

Figure (7) Section in epididymis of treated group at 28days after dipping, note normal cells and the lumen occupied with sperms. (H & E x 200).

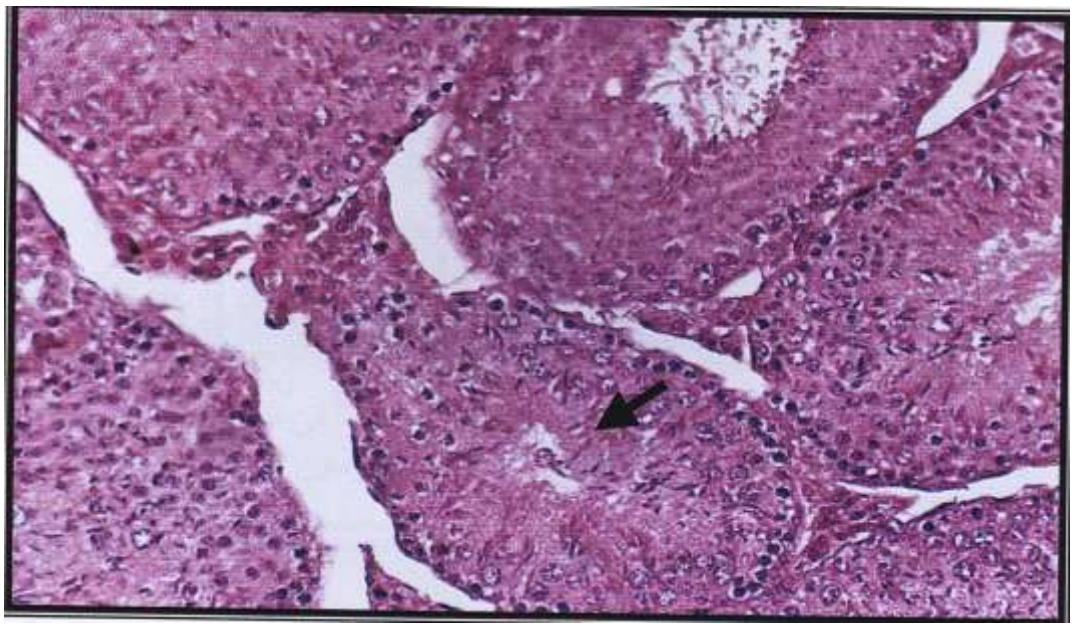

Figure (8) Section in testis of treated group, at 42days after dipping, note normal seminiferous tubules and spermatogenesis (H & E x 200).

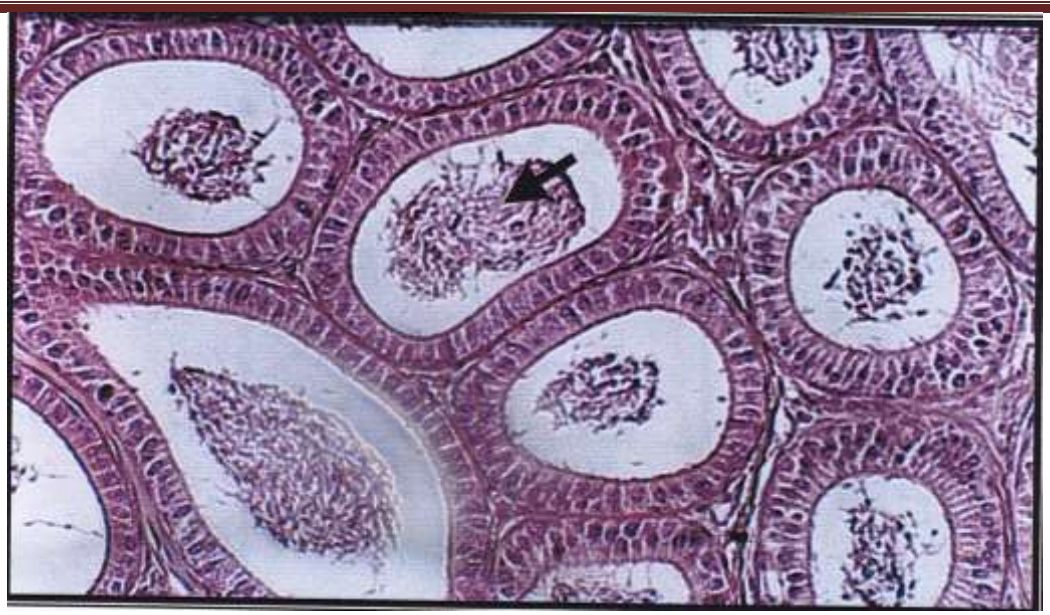

Figure (9) Section in epididymis of treated group, at 42days after dipping, note the lumen occupied with sperms and normal lining cells (H & E x 200).

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