THE THERAPEUTIC EFFECT OF COMBINED AQUEOUS EXTRACT OF CORIANDER SATIVUM L. AND ALLIUM SATIVUM L. ON THE MERCURIC CHLORIDE INDUCED REPRODUCTIVE TOXICITY IN ADULT MALE RATS

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Keywords ; antioxidant ,Mercuric chloride ,rats.

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ABSTRACT

The present study was undertaken to investigate the ability of antioxidants Coriander sativum L . (cilantro) and Allium sativum L.(garlic) to protect against mercuric chloride induced reproductive toxicity in male rats .A sub Lethal chronic exposure (0.12mg/kg B.W mercuric chloride)resulted in a decrease of body weight, testicular weight and testosterone hormone with regressed histological properties of testis , epididymis and pre - cancerous changes in epididymis .These changes were ameliorated with the administration of cilantro and garlic .The results of our study suggested that the mentioned antioxidants exert significant protection against mercuric chloride induced male reproductive toxicity and provide a strong evidence for the beneficial role of antioxidant vegetables in prevention of mercury toxicity .

Key words : mercuric chloride ,antioxidant, cilantro, garlic, reproductive toxicity.

INTRODUCTION

Mercury is a highly toxic heavy metal .It causes a variety of adverse health effects including : neurological ,renal , respiratory ,immune ,dermatological ,reproductive and developmental sequelae (1,2,3).The impact of mercury on reproductive functions have been studied at the beginning on the lab animals, which provoked some chromosomal aberrations (4).Reduction in spermatogenesis and fertility (5).And induces alterations in testis along with biochemical changes(6,7) .The exact cellular mechanism by which mercury acts as a reprotoxicant is poorly documented (8).

Antioxidants are found in varying amounts in food such as vegetables ,fruits , grain cereals , eggs , meat , legumes and nuts (9). Many medical plants contain substantial amounts of antioxidants such as vitamins ,flavonoids and cerotenoids (10).Antioxidants have been reported to prevent oxidative damage by reacting with free radicals,chelating ,catalytic metals and also by acting as oxygen scavengers and thus can be utilized to scavenge the excessive free radicals generated from human

body (11)with no side effects and economic viability (12).No study has reported the impact of mercury chloride on local population of rats in Iraq .Therefore ,the objective of the present study is to estimate mercuric chloride intoxication on testis and epididymis in male rats ,on one hand ,and the possible therapeutic role of cilantro and garlic extract supplementation on the other hand .

MATERIALS AND METHODS

Plant collection :

Plants were obtained from local markets in Baghdad. A voucher specimen of the plant was deposited to be identified and authenticated at the national herbarium of Iraq botany directorate in Abu-Ghraib with certificate number (3743)in 6/12/2010 for Garlic and Cilantro .

Preparation of aqueous extract of cilantro and garlic :

The extract was prepared according to the method of (13)as following : Peeled garlic (30 g)was crushed with distilled water in a mortar and further homogenization was occurred, The crushed material was carefully decanted by pressing and 60ml of aqueous extract was extracted. One milliliter of aqueous extract contained 500 mg of garlic materials.

Dose calculation

The toxic dose of mercuric chloride was calculated according to (14).

Experimental design

Sixty male rats aged (8 weeks) and weight (260– 300 gm) were divided equally into 4 groups:-

Group 1 : Rats served as control (C) and received distilled water for two months.

Group 2 : Rats served as experimental and received by gavage mercuric chloride diluted in distilled water at 0.12 mg /kg B.W. /day for two months .

Group 3 : Rats served as experimental and received by gavage mercuric chloride diluted in distilled water at 0.12 mg /kg B.W. /day for one month followed by received by gavage cilantro 25 mg /kg B.W. /day and 50 mg /kg B.W. /day garlic extract diluted in distilled water for one month .

Group 4 : Rats served as control (C) and received distilled water for one month followed by by gavage cilantro 25 mg /kg B.W. /day and 50 mg /kg B.W. /day garlic extract diluted in distilled water for one month .

Histopathology : at the end of each sacrification (every 20 days),five animals from each group were sacrifed by intramuscular injection of high dose of ketamin hydrochloride. Reproductive organs were quickly excised out ,fixed with 10% buffered formalin.The specimens were sectioned (5µ m thickness)and staind with Hematoxyln and eosin according to (15).

Body weight and Testicular weight .

The procedure was done according to (8)

Hormonal assay :

Radioimmunoassay

The method of performing radioimmunoassay is as follows :-

1- An antibody that is highly specific for the hormone to be measured is produced .

2- A small quantity of this antibody is mixed with quantity of fluid from the animal containing the hormone to be measured and a known amount of radioactive iodinated hormone .

3- After binding has reached equilibrium ,the antibody-hormone complex is separated from the unbound iodinated hormone by a variety of the physicochemical means.

4- The amount of hormone present in the plasma can be inferred by comparing with "standard curve (16) .

Statistical analysis.

The calculations were carried out according to program statistical package for social sciences SPSS(version 19)and Microsoft Office Excel (for examination of the specific significant differences among groups).The results (values of PR or IR) were analyzed statistically by analysis of variance (ANOVA) followed by post – hog Tukey Test .P values < 0.05 they were used for the statistical analysis of the results looking for the differences which statistically significant at $P< 0.05$ (17).

RESULTS

1- weight changes :

Body and testicular weight changes :

Table (1)showed the results of the body weight changes .There is significant decrease (*P<0.05)* in the body and testicular weight (284±1.155 , 282±1.414 , 278.75 ±0.957), (1.35±0.016 , 1.27±0.016 , 1.27±0.016) with the time of gavages with mercuric chloride .Cilnatro and garlic supplementation with mercuric chloride showed significant increase (*P<0.05)* in the body and testicular weight (286±2.309 , 284.50 ±0.577 , 282.50 ±1.291),(1.33±0.008 , 1.33±0.008 , 1.39±0.037).while there are no significant difference in body and testicular weight in group gavage with

combined aqueous extract of Cilnatro and garlic when compared with the control group (289.50±0.577 , 291.50±1.732 , 294.25±0.957),(1.47±0.017 , 1.49±0.021 , 1.49± 0.034) respectively .

2- Hormonal changes :

Table (2)showed the results of the hormonal changes (serum testosterone) .There is significant decrease (*P<0.05)* in the serum testosterone (ng/ml) (2.37±0.005 , 1.78±0.012 , 1.17±0.008), with the time of gavages with mercuric chloride .Cilnatro and garlic supplementation with mercuric chloride showed significant increase (*P<0.05)* in the serum testosterone (2.35±0.017, 1.88±0.009 , 1.98±0.008).While there are no significant difference in body and testicular weight in group gavage with combined aqueous extract of Cilnatro and garlic when compared with the control group (2.47±0.016, 2.45±0.040 , 2.50±0.032) respectively .

3-Histopathological findings :-

Testis :Control group :-

The light microscopy examination of the testis of control group (group 1)had normal histological structure .The structural components of the testis are the seminefrous tubules and interstitial tissues .The seminifrous tubules are lined with two general types of cells, spermatogenic cells and sertoli cells (figure1).

Treatment groups :-

Mercuric chloride(0.12mg/kg.B.W.).

At 20 days period : -

In this period the testis exhibited some structural deformities .There is abnormal configuration of seminifrous tubules with thickening of the basal membrane (figure 2).

There is vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium, and abnormal distribution of spermatozoa showed in lumina of seminiferous tubules with thickening of the basement membrane and formation of spermatid gaint cells (figure 3 ,4).

At 40 days period : -

The testis showed much more pronounced changes in their histological structure ,the necrotic changes were noted in spermatogonia ,primary and secondary spermatocytes. In some of the tubules the spermatogenic series of cells appeared to be slightly condensed, dyscohesive and detached at some places leaving only the sertoli cells (figure 5) .Other sections showed moderate fibrous thickening of tunica albuginea and the interstitium which is infiltrated with mononuclear cells .

At 60 days period : -

The testis showed more deformation than at 40 days treatment .The fibrosis of the of tunica albuginea and the interstitium are more extensive which lead to pressure atrophy of the seminifrous tubules in the affected region (figure 6) .

Epididymis :

Control group : -

Sections of epididymis of control group. The epididymal epithelium enclosed a lumen containing spermatozoa .The interstitial space in between the epididymal tubules was filled with sparse stroma. The pseudostratified epithelium was composed of principal cells with nuclei situated at the base (figure 7).

Treated groups:-

Mercuric chloride(0.12mg/kg.B.W.).

At 20 days period : -

The epididymal sections showed a decrease in the number or complete absence of sperms leaving the ductus epididimis empty or containing intraluminal cellular exudates (figure 8).

At 40 days period : -

The main histopathological finding of this period was the formation of spermatic granuloma consisting of large numbers of sperms in the center surrounded by phagocytic cells ,including multinucleated foreign body giant cells and thick band of fibrous connective tissue (figure 9). The interstitium showed moderate fibrosis .

At 60 days period : -

At this period the interstitial fibrosis become more severe accompanied with infiltration of mononuclear cells .The ductal epithelium undergo hyperplasia with formation of papillary projections (figure 10).

Antioxidant supplemented groups:-

The toxic effects of mercuric chloride were reduced in testis of antioxidant supplemented animals . At 20 days the seminiferous tubules showed active developing stage with cellular units(figure 11). At 40 days period the sections exhibited rounded shape seminiferous tubules and many newly formed spermatogenic cells arranged properly inside the tubules (figure 12). At 60 days period all stages of spermatogenesis were clearly visible and spreaded in the lumen of the tubules as were seen in the corresponding control (figure 13). Epididymal sections at the 20 days period showed the presence of few sperms in the lumen of the ductus epididymis with the absence of the cellular debris (figure 14). At 40 and 60 days periods the sections showed nearly normal epididymal structure with presence of

large numbers of sperms in the lumen with return of epithelial lining to its normal thickness (figure 15)

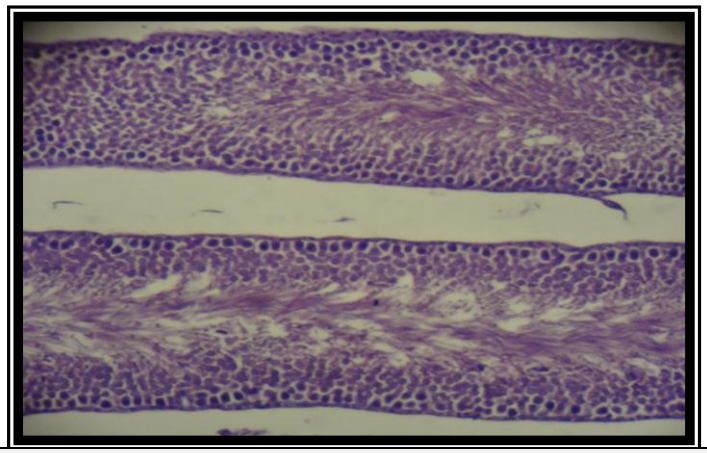


Figure (1): Testis of male rat of (group 1)showing normal histological structure (H&E 100X).

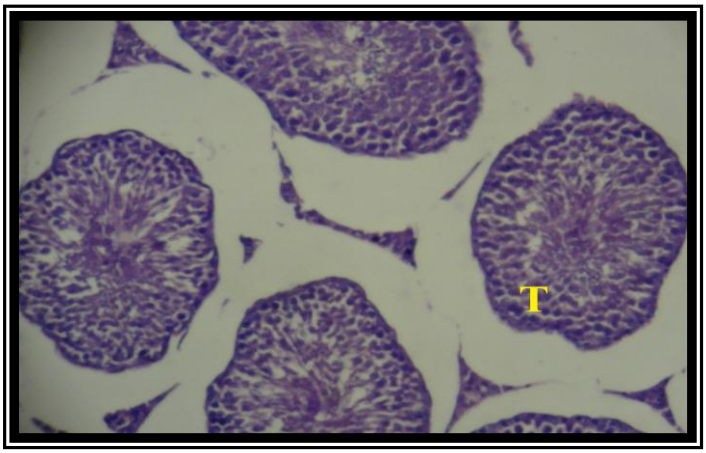


Figure (2): Testis of male rat of (group II) at 20 days period showing abnormal configuration of seminiferous tubules with the thickening of basal membrane (S)(H&E 400X)).

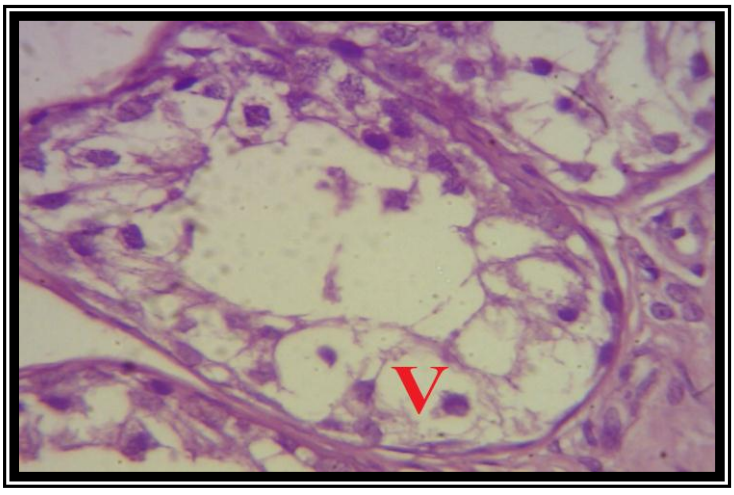


Figure (3): Testis of male rat of (group II) at 20 days period showing vacuolar degenerative changes of the spermatogenic epithelium (V) (H&E 400X).

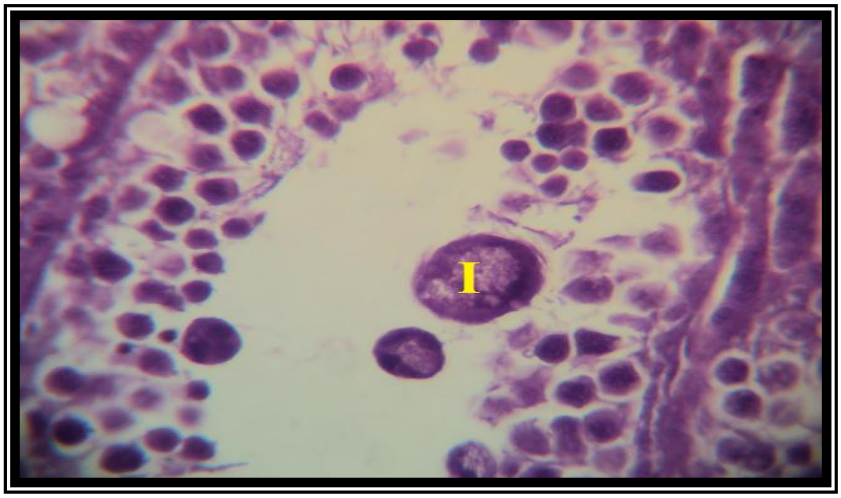


Figure (4): Testis of male rat of (group II) at 20 days period showing formation of intratubular multinucleated spermatid giant cells (I) (H&E 400X).

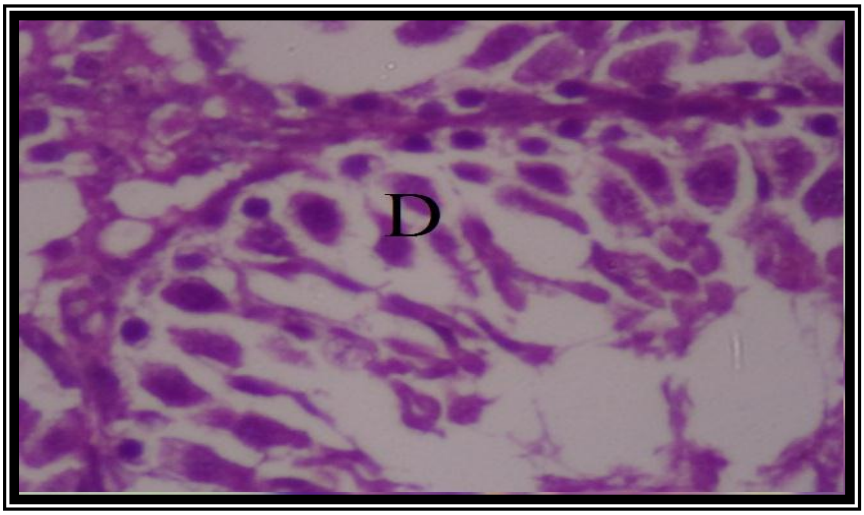


Figure (5): Testis of male rat of (group II) at 40 days period showing detached spermatogenic cells leaving only the sertoli cells (D) (H&E 400X).

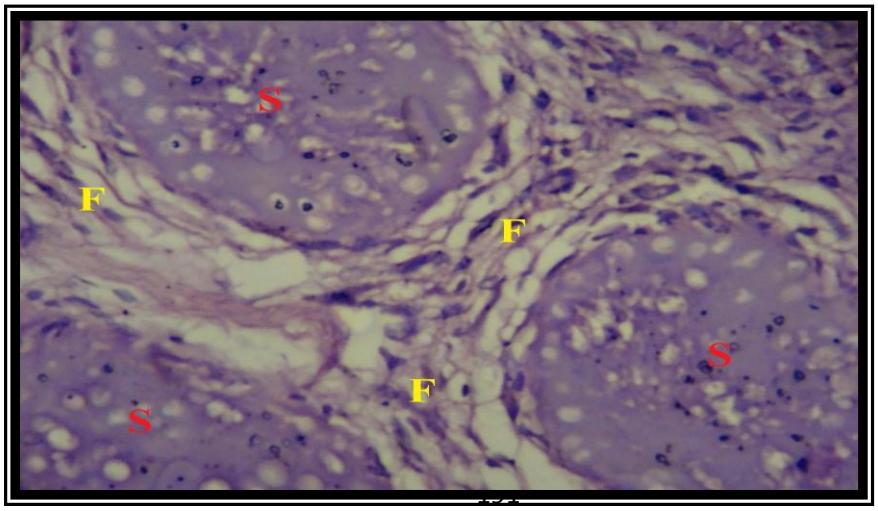


Figure (6): Testis of male rat of (group II) at 60 days period showing extensive fibrosis of the interstitium(F) lead to pressure atrophy of the seminiferous tubules (S) (H&E 400X).

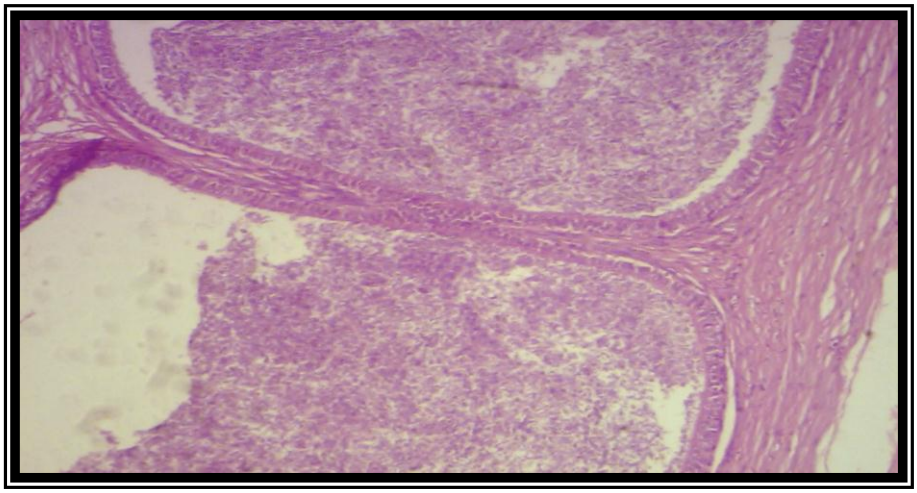


Figure (7): Epididymis of male rat of (group I) showing normal histological structure (H&E 400X).

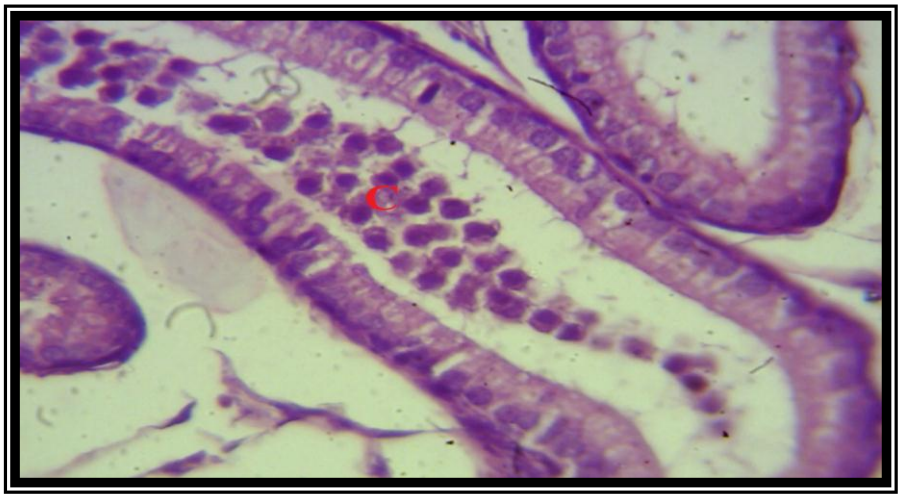


Figure (8): Epididymis of male rat of (group II) at 20 days period showing intra-luminal cellular exudates (C) (H&E 400X).

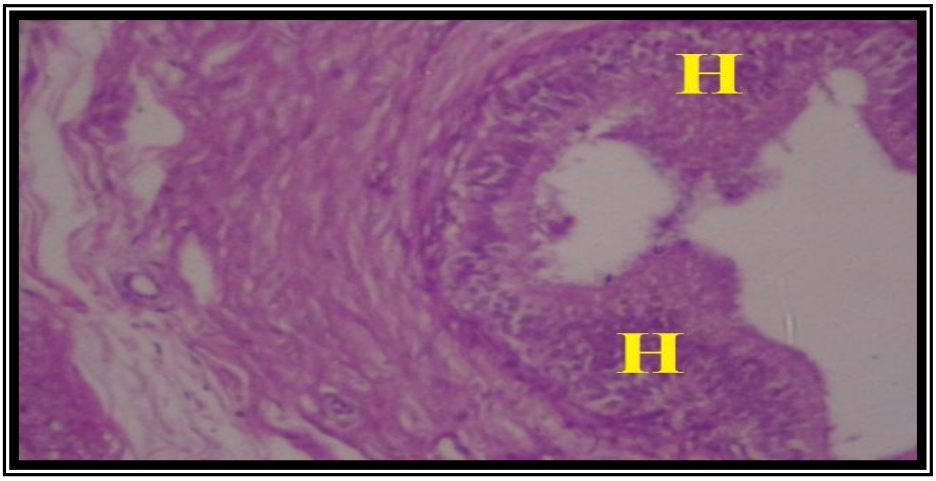


Figure (9): Epididymis of male rat of (group II) at 40 days period showing spermatic granuloma consisting of large numbers of sperms in the center (S),surrounded by phagocytic cells including multinucleated foreign body giant cells (P), thick band of fibrous connective tissue (F) (H&E 400X).

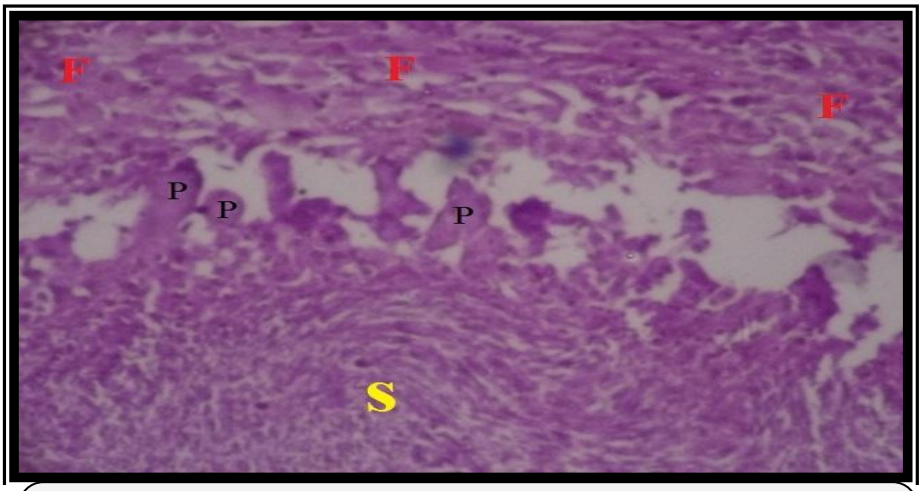
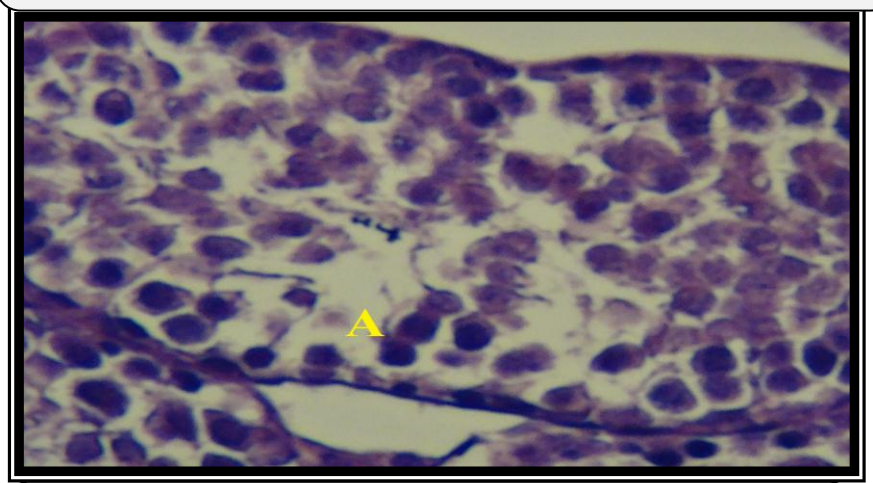
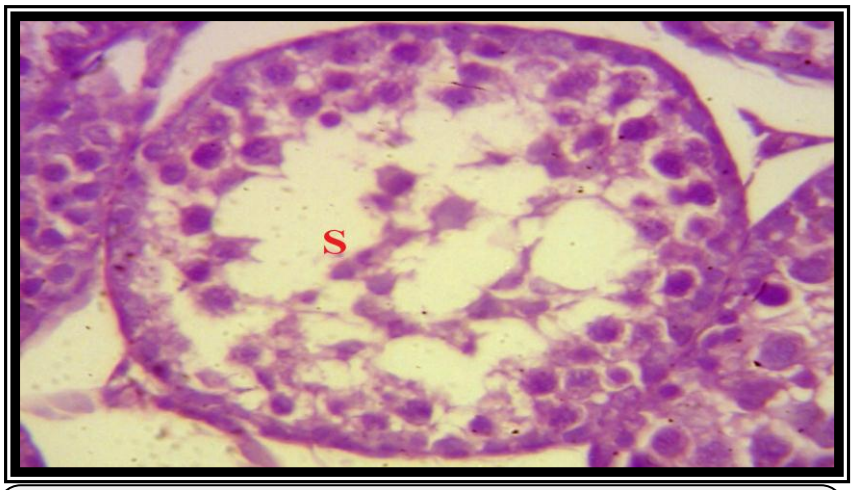


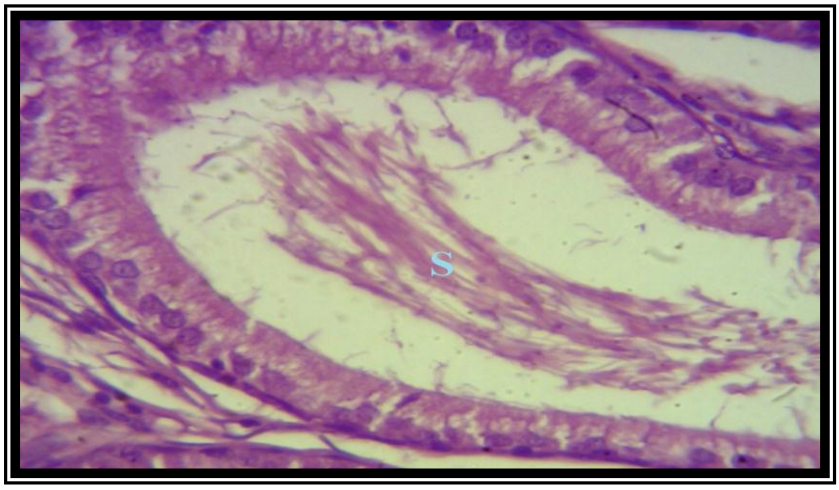
Figure (10): Epididymis of male rat of (group II) at 60 days period showing hyperplasia of ductal epithelium forming papillary projections (H) (H&E 400X).



Figure(11): Testis of male rat of (group III) at 20 days period showing active developing stage with cellular units (A) (H&E 400X).



Figure(12): Testis of male rat of (group III) at 40 days period showing rounded shape seminiferous tubules and many newly formed spermatogenic cells arranged properly (S) (H&E



Figure(13): Testis of male rat of (group III) at 60 days period showing clear all stages of spermatogenesis as seen in the corresponding control (C) (H&E 400X).

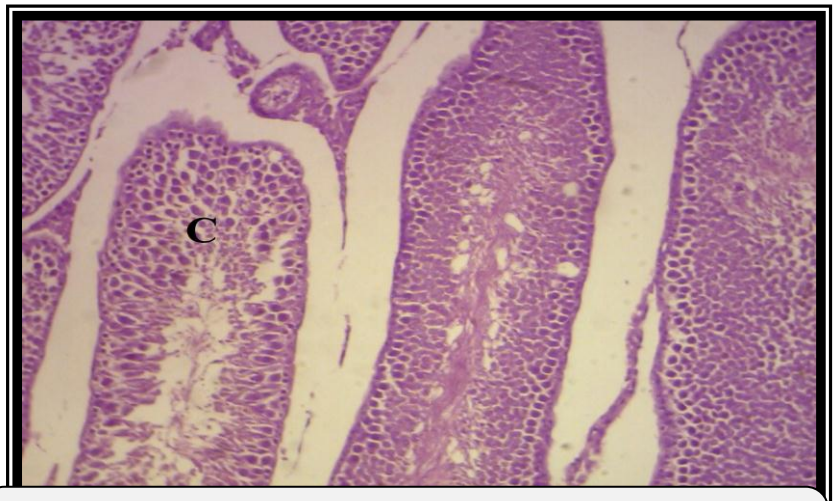


Figure (14): Epididymis of male rat of (group III) at 20 days period showing few sperms in the lumen of ductus epididymis (S) with the absence of cellular debris (H&E 400X).

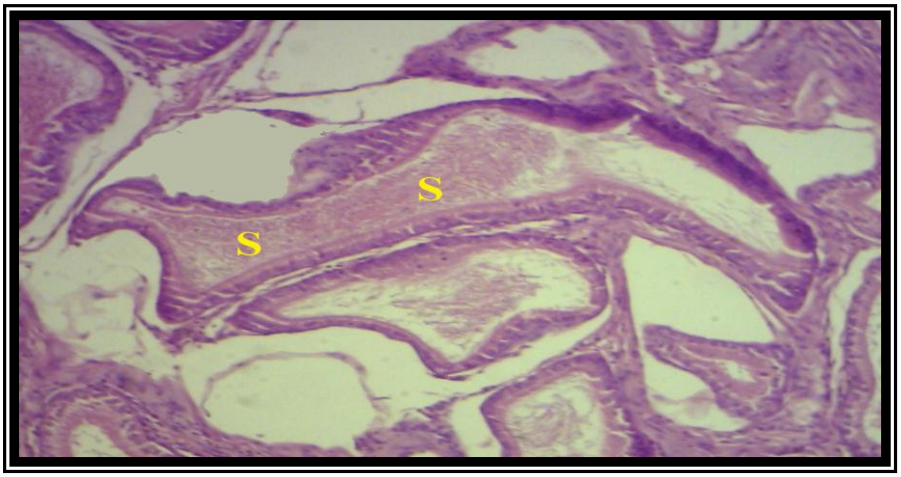


Figure (15): Epididymis of male rat of (group III) at 60 days period showing nearly normal epididymal structure with presence of large numbers of sperms in the lumen (S) (H&E 200X).

Table (1) :Effects of combined aqueous extract of cilantro and garlic on body weight (gm)and reproductive system weight (mg) in the mercuric chloride toxicity in rat .

Parameters	Group 1 (control)	Group 2			Group 3			Group		
		20	40	60	20	40	60	20	40	60
Mean body weight (gm)	288.75 ± 0.957 a	284 ± 1.155 bce	282 ± 1.414 bce	278.75 ± 0.957 bd	286 ± 2.309 ace	284.50 ± 0.577 Bce	282.50 ± 1.291 bce	289.50± 0.577 a	291.50± 1.732 af	294.25± 0.957 Bf
Absolute weight of the testes (gm)	1.47 ± 0.008 a	1.35 ± 0.016 bd	1.30 ± 0.021 bcd	1.27 ± 0.016 bcd	1.33 ± 0.008 bd	1.33 ± 0.008 Bd	1.39 ± 0.037 bde	1.47 ± 0.017 a	1.49 ± 0.021 a	1.49 ± 0.034 A
Gonado-somatic index	0.508 ± 0.008 a	0.477 ± 0.005 bc	0.460 ± 0.002 bd	0.454 ± 0.001 be	0.464 ± 0.004 bd	0.474 ± 0.001 bc	0.491 ± 0.002 bf	0.507 ± 0.001 a	0.513 ± 0.002 b	0.516 ± 0.003 b

***Mean values ± SD followed by different small letter horizontally significantly different**

Gonado-Somatic Index(GSI=)Gonad weight/total body weight) × 100 .

Table (2) :Effects of combined aqueous extract of cilantro and garlic serum testosterone (ng/ ml) in the mercuric chloride toxicity in rat .

parameters	Group 1	Group 2			Group 3			Group		
	(control)	20	40	60	20	40	60	20	40	60
Serum testosterone (ng/ ml)	**2.47 ± 0.005 a**	**2.37 ± 0.005 Bc**	**1.78 ± 0.012 bd**	**1.17 ± 0.008 be**	2.35 ± **0.017 bc**	1.88 ± **0.009 bf**	1.98 ± **0.008 bg**	2.47 ± **0.016 a**	2.45 ± **0.040 a**	**2.50 ± 0.032 B**

*Mean values ± SD followed by different small letter horizontally significantly different

DISCUSSION

The decrease in body weight observed due to mercuric chloride administration may be related to reduced food and water intake this is in accordance with the report of National Toxicology Program Working Group (18) that mercuric chloride treatment to rats resulted in reduced food and water intake leading to weight loss .The decreased testicular weight observed shows the degenerative effect of mercuric chloride .The increased in the body and testicular weight in the supplemented group may be due to increase in the food and water intake and the regenerative process of the spermatogenic cells lining the seminiferous tubules .

Several studies explained the effect of mercuric compounds on biochemical parameters and impaired steroidogenesis which together could affect male fertility ,this could be due to oxidative stress which induce depression of hypothalamo-pituitary-testis system ,mediated by activated hypothalamo –pituitary –adrenocortical system ,resulting in fall in plasma LH and testosterone levels(19,20,21) .

Mercury induces structural and functional damage in several organs, however less information is available concerning the effect of the metal on male reproductive system(21,22).The present investigation try to focus on the testicular and epididymal morphological alterations in male rate . The treated animals with mercuric chloride exhibited distorted arrangement of germ cells ,a decrease spermatogenic cell layer in the seminiferous tubules. These results agreed with other reports which indicated that mercury induce these changes through functional alterations of spermatogenesis with

arrest at spermatocyte stage, hypospermatogenesis and possibly impaired steroidogenesis which together could affect male fertility (7,8,23).

Mercuric chloride exposed group undergo testicular damage at 20 ,40 and 60 days of exposure .The pathological lesions intensity depends on the duration of exposure and that inagreement with(19) they studed that the intensity of damage is directly proportional to the duration of exposure .The presence of spermatid giant cells within the lumen of seminiferous tubules was due to degenerative changes of spermatogonia .(24) reported that spermatid degeneration and giant cell formation were observed after spermatocyte degeneration, spermatid degeneration appeared to be secondary changes resulting from disrupted sertoli - to germ cell association .The fibrous thickening of tunical albugenia and interstitial tissue of both testis and epididymis was due to macrophage as a master regulator of fibrosis .Recent studies have identifies the important role of macrophages in inflammation and fibrosis. Like myofibroblasts these cells are derived from either resident tissue populations ,or from bone marrow immigrants .Studies now suggest the pathogenesis of fibrosis is tightly regulated by distinct macrophage populations that exert unique functional activities throughout the initiation ,maintenance ,and resolution phases of fibrosis (2 5) .The results were coincident with the study conducted by (22) . studied that an increase in free radical formation relative to loss of antioxidant defense system after mercury exposure may render testis and epididymis more susceptible to oxidative damage leading to their functional inactivation .Epididymal papillary hyperplasia was seen in later exposure to mercury,epididymal hyperplasia was considered preneoplasic lesions by some authors (26) .(27)reported that sperms once they have escaped their normal containment within the lumen of excretory system ,incite a foreign body response called spermatic granuloma which may affect testis ,epididymis or vas deferens and that correspond with the present study which showed spermatic granuloma in the interstitial tissue of the epididymis.

In the present study ,the animals which treated simultaneously with mercury and antioxidants (cilantro and garlic) showed protection against mercury induced cytotoxicity alterations .The seminiferous tubules showed less shrinkage and reorganized and malformation of different spermatogenic cells were reduced . Coadministration of combined cilantro and garlic aqueous extract ameliorated the biochemical changes and that attributed to that antioxidants interfere with the oxidation process by reacting with free radicals ,chelating ,heavy metals and also by acting as oxygen scavengers (11) .

Furthermore (28,29) reported that when the cilantro and garlic administered per os simultaneously with mercury compounds to detect the protective effect against heavy metals poisoning accumulation in testis was decreased and histopathological damages were reduced. Cilantro's postulated mechanism of action is to act as a reducing agent changing the charge on the intracellular mercury to a neutral state allowing mercury to diffuse down its concentration gradient into connective tissue

.This is called connective tissue mercury toxicity,the next step is to remove the mercury from the connective tissue (30 Omura et al).The protective effects of garlic has been attributed to prescence of organosulpher compounds which oxidize mercury and make it water soluble to be excreted easily out side the body (31,32Shukla and Taneja,2002:Rudenski,2005).+

Many investigators have reported the significance of the administration of antioxidants in histological damage of male reproductive organs induced by various toxicants .(33,34) reported that supplementation of vitamin E and C along with mercury chloride prevent the mercury induced oxidative damage of germinal cells of male .

On the basis of this study it is concluded that mercury causes severe toxic tissue damage in testis and epididymis .This damage may be caused by reactive oxygen species produced by mercury within the animals body .Antioxidant vegetables interact with mercury ions, neutralize them or bind with transition metals and prevent the ROS mediated oxidative damage in testis and protect the tissue in a manner depends on duration of treatment.

التأثير العلاجي لخليط الخلاصة المائية للكزبرة والثوم على كلوريد الزئبق المستحث للسمية التناسلية في ذكور الجرذان البالغة

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الخلاصة

هدفت الدراسة الحالية الى معرفة التأثير العلاجي للكزبرة والثوم (كمضاد للأكسدة) على السمية التناسلية لذكور الجرذان من خلال التعرض المزمن لكلوريد الزئبق وبجرعة (0.12 ملغم /كغم من وزن الجسم) ,أوضحت النتائج حصول قلة بوزن الجسم ووزن الخصية وهبوط معدل هرمون التستوستيرون مع تغيرات نسجية في كل من الخصية والبربخ وحصول افات مهيأة للتسرطن في البربخ . أدى أعطاء خليط الخلاصة المائية للكزبرة والثوم الى تحسنا في التغيرات المذكورة أعلاه. نستنتج مماسبق ان للكزبرة والثوم دورا مهما في منع تأثير كلوريد الزئبق المستحث للسمية التناسلية في ذكور الجرذان مبرهنا على الدور المهم الذي تلعبه مضادات الاكسدة (الخضروات)في الحماية من التسمم بكلوريد الزئبق .

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