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The Effect of Heptachlor on Histology of Seminiferous Tubule and Mice Testis Activity

Nibal Kh. Mousa[1], Labeeb A. Khadim[1], Sarab S. kadhum[1], Alaa Dh. Kadhum[1],

Shahad Sh. Sabbar[1], Abrar N. Mohammad[1], Mohammad Ab. Ayyash[1], Ishrak Ab. Ali[2]

[1]Environmental-Water Research and Technology Directorate, Ministry of Science and Technology

[2]The National Center for Drug Control and Research, Ministry of Health

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Abstract: The study was aimed to illustrate the effect of heptachlor on seminiferous tubule and spermatic cells in the testis of mice .Heptachlor 10 mg / kg was given daily by gulping for five weeks. Mice were sacrificed after injected with colchicines in traperitonially , testis were collected after 7,21,36 days to study the effect of heptachlor on histo-testis and improved heptachlor activity not as chemical mutagen but as a tumor promoter by act directly on DNA which reflected on decreasing testicular Leydig cell and spermatogenic deficiencies when comparison with control and vitamin C.

Key words: Heptachlor; seminiferous tubules, histology; Leydig cell, mice.

تاثير الهبتاكلور على النبيبات المنوية نسجيا وعلى نشاط خصى الفئران

نبال خليل موسى[1]، لبيب احمد كاظم[1]، سراب سلمان كاظم[1] ، آلاء داغر كاظم[1]،

شهد شكري صبار[1]، ابرار ناطق محمد[1]، محمد عبود عياش[1]، اشراق عبد الامير علي[2]

[1]دائرة تكنولوجيا وبحوث البيئة والمياه، وزارة العلوم التكنولوجيا

[2]المركز الوطني للرقابة والبحوث الدوائية، وزارة الصحة

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الخلاصة: تهدف الدراسة لتوضيح تاثير مبيد الهبتاكلورعلى النبيبات المنوية وارومة النطفة في خصية الفئران. كانت جرعة الهبتاكلور 10ملغم / كغم يوميا بالتجريع لمدة خمسة اسابيع. تم تشريح الفئران بعد حقنها بالكولجسين في غشاء الخلب . جمعت انسجة الخصية بعد 7 ،21، 36 يوم لدراسة تاثير الهبتاكلور على النبيبات المنوية نسجيا وكذلك فعالية الخصية و تبين ان الهبتاكلور ليس مطفرا" كيمياويا" وانما محفزا" للورم من تاثيره على الحامض النووي DNA والتي تنعكس على خفض خلايا الخصية والقصور في النبيبات المنوية عند المقارنة مع معاملات السيطرة وفيتامين سي.

Introduction

Gamete production, their transport, reproductive cycle, sexual behavior and fertility may be severely affected by xenobiotics. Large number of chemicals, pollutants, drugs and hormonal imbalances adversely affect the product (1). Involvement of reactive oxygen species (ROS) has been suggested as one of the mechanisms through which most of the xenobiotics exert their deleterious effects on testis (2,3). Toxicity of such agents in testis may lead to testicular dysfunction, arrest of spermatogenesis and production of abnormal sperm (4). Normal gamete formation in testis is akey factor in fertility and development. Oxidation stress seem to play importance role in the etiology of detective sperm formation ,sperm count profile and male infertility (5) presence of high level of(ROS)is linked with lipid peroxidation of the spermatozoa outer membrane, which lead to loss of sperm motility (6), decrease spermatocyte fusion capacity (7) and increased chromatin damage .Once the integrity of spermatozoa becomes affected , they are unable to undergo repair because of deficiency in enzyme system required by ranging male germ cells particularly vulnerable to oxidative stress (8).

Heptachlor-main-trade-names (Drinox; Heptagram; Heptamul). Heptachlor is central nervous system stimulant. The liver is the other organ significantly affected by heptachlor, is applied as a soil treatment, seed treatment (maize, small grains and sorghum) or directly to foliage. It is used to control ants, cutworms, maggots ،termites, thrips, weevils, wireworms and other insect pests in both cultivated and uncultivated soils. Heptachlor also controls household insects and pests of humans and domestic animals (9). In many countries, heptachlor is banned or applied only by subsurface injection. Heptachlor epoxide is not commercially available but is an oxidation product of heptachlor (10). Heptachlor is rapidly absorbed from the gastrointestinal tract of rats following intragastric administration. Heptachlor epoxide is distributed throughout the body of rats and dogs (11). Heptachlor is metabolized by rats to heptachlor epoxide,1-hydroxychlordene and 1-hydroxy-2,3-epoxychlordene, which are the major faecal metabolites. In vitro studies have shown that heptachlor epoxide formation is greater in rats than in humans and that metabolism is, in general, comparable in the two species. Faeces represent the major route of heptachlor elimination by rats (12).

Human body has a strong defense system to combat and counteract the damage caused by free radical (13). In general, antioxidant systems either prevent these reactive species from being formed or remove them before they can damage vital component of cell. However, sometimes the homeostasis gets disturbed due to continuous exposure to toxicant or failure of endogenous antioxidant system. Thus, the balance between ROS production and antioxidant defense lost causing oxidative stress which further deregulates cellular functions leading to various pathological conditions (14). Due to the wide Heptachlor using ranges, the study aimed to understand the residues toxicity and effect on mice testis tissues.

Materials and Methods

Phosphate Buffer Solution (PBS): K2HPO4. 0.87gm was added to distilled water, then adjust the pH to5.5 by using 1N HCl hydrochloride and completed the volume to 100 ml (15), given orally.

Colchicine solution: Colchicine 1mg (one tablet) and sterile distilled water 1ml. The solution was used immediately after preparing 2.5 to 3 hours intraperitonially (16). Vitamin C 180mg and dissolved in 100ml in sterile distilled water (17); given orally .Heptachlor was 10 mg/kg (18), testis collection after 7,21,35 days from gulping mice. Histological study of the seminferous and testis were made according to Bancroft and Steven (19).

Statistical analysis: The statistical analysis has been used to study the effects of treatments in different trails. The least significant difference (LSD) test was used to signify a comparison between the means (20).

Histopathological changes for testis cells when treated with heptachlor showed figures 1,2 and 3 damage in spermatocyte that work on metabolism changes via cells, which related to apoptosis. Most drugs, pesticides and radiation caused sperm cells damage, decrease in fibrosis and inflammation in sperms. The figures 1,2 and 3 showed decreased in numbers of germinal and spermatocyte (primary and secondary) cells when compared with controls figures 4 and 5.

The diameter of seminiferous tubules figures 1,2 and 3 were smaller than in figures 4,5, the number of seminiferous tubules lower than controls. The structure in seminiferous tubules histopathological damage and was illustrated .Testicular structure figures 1, 2 and 3 showed sustainable normal but irregular seminiferous tubules increased in the intercellular space.

Results and Discussion

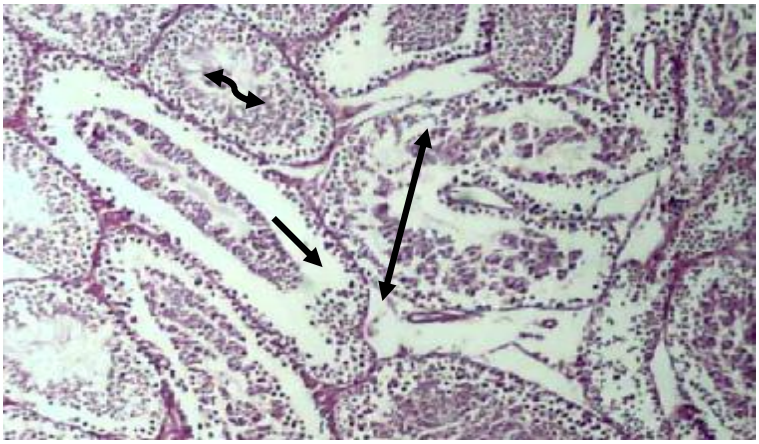

Figure (1): Mice Testes tissue treated with heptachlor for 7 days (↔) seminiferous tubules necrosis, (→) septum , (⟷) Tunica albuginea fibrosis (H+E X200).

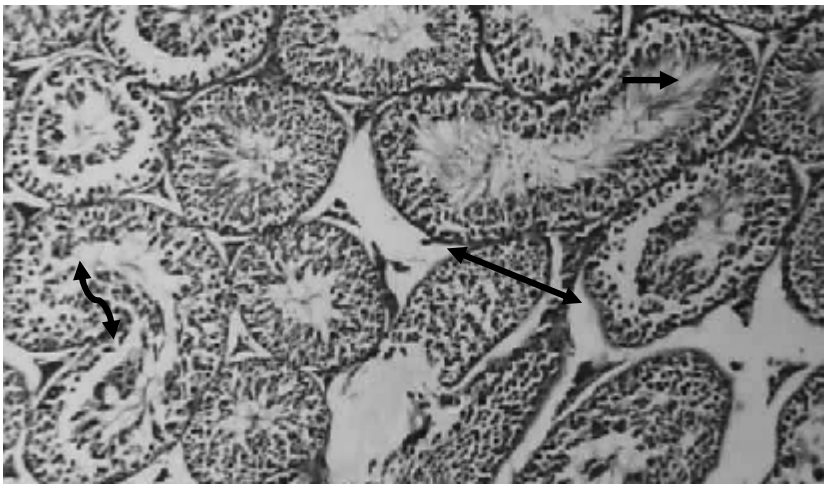

Figure (2): Mice Testes tissue treated with heptachlor for 21 days (↔) seminiferous tubules necrosis, (→) septum , (⟷) Tunica albuginea fibrosis (H+E X200).

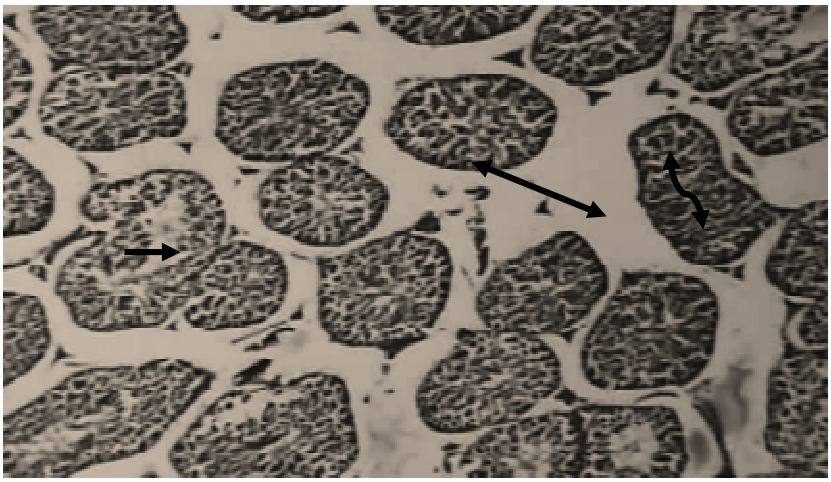

Figure (3): Mice Testes tissue treated with heptachlor for 35 days (↔) seminiferous tubules necrosis (→) septum , (⟷) Tunica albuginea fibrosis (H+E X200)

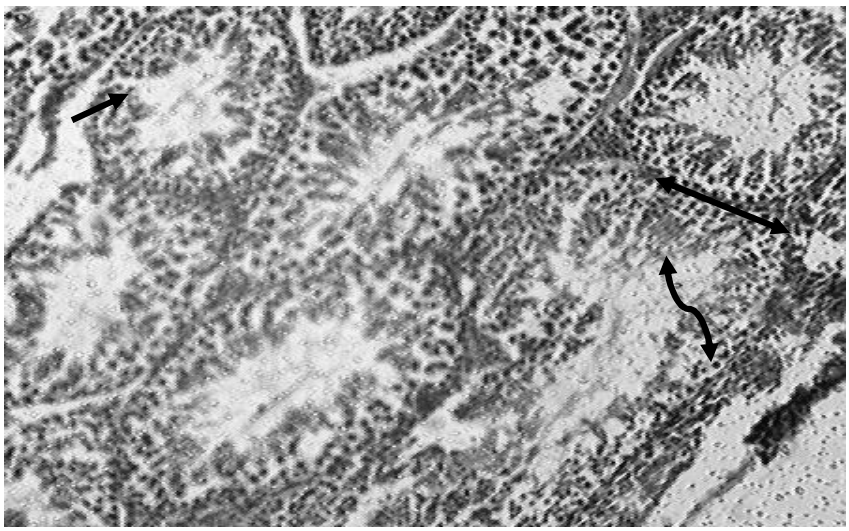

Figure (4): Mice Testes tissue treated with PBS(↝) normal seminiferous tubules , (→) septum , (↔) Tunica albuginea (H+ E X200)

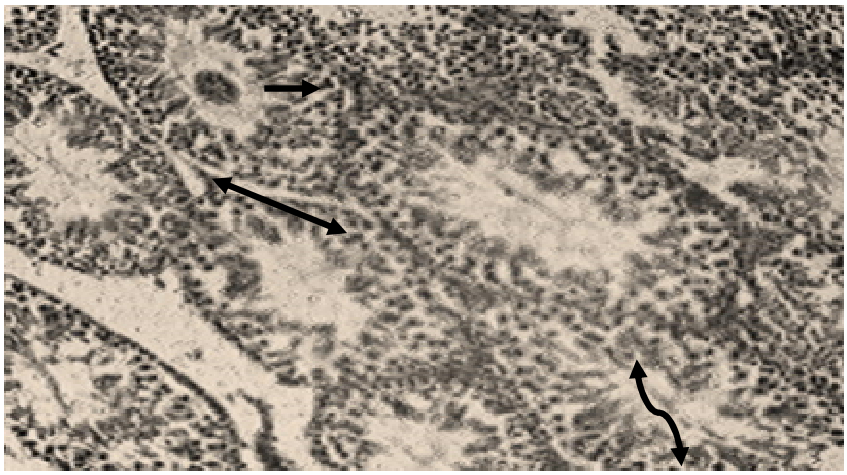

Figure (5): Mice Testes tissue treated with PBS (↝) normal seminiferous tubules , (→) septum , (↔) Tunica albuginea (H+ E X200)

Studies show that female rats exposed to heptachlor were less likely to become pregnant. Those that did become pregnant had smaller litters or the offspring showed developmental problems. Other studies showed that heptachlor fed to animals caused cancer (21).

It is speculated that heptachlor act by a common mode of action and that heptachlor acts not as a chemical mutagen, but as a tumor promoter (22). Most of the evidence from genotoxicity assays indicated that heptachlor act directly on the DNA molecule. The exact mechanism by which these chemicals produce their effects remains unclear, but several lines of investigation are being pursued. Heptachlor have been shown to be potent inducers of protein kinas' C activity in both rat and mouse brain. Several other chlorinated hydrocarbons were also positive for this effect (22).

A decreased testicular Leydig cell count is associated with decreased testosterone production, which may result inspermatogenic deficiencies (23, 24) which in agreement with the present study, heptachlor is achemotherpyid insecticide has toxic effects on male reproduction. Although this reproductive dysfunction is typically characterized by disruptions in spermatogenesis and loss fertility, which heptachlor-inducedinfertility remain unclear. Others explained that heptachlor insecticide led to cell injury by mitotic toxicity, chromatin destruction and DNA disturbance (25). Some research has shown decreased testosterone production by Leydig cells in insecticide treated groups to be one of these mechanisms (24,26 , 27). The Leydig cells produce testosterone needed in the seminiferous tubules to induce differentiation of spermatogonia to spermatozoa (27). Because testicular Leydig cells play a critical role in male reproductive function, alterations in the Leydig cells could be due to many different histopathological or experimental situations associated with spermatogenesis deficiency (28) Reactive oxygenspecies caused by insecticide treatment may be involved in the toxicity of various pesticides (28). Increased ROS may decrease the effective concentration of antioxidant, increasing the harmful effects of ROS to reproductive tissue (29). Other has shown that insecticide treatment caused an increase in lipidperoxidation (LPO) in rat erythrocytes (30). Because spermatozoa have large quantities of poly unsaturated fatty acids (PUFA) in their plasma membranes and cytoplasm contains low concentrations of scavenging antioxidants (29), a cause relationship is suspected. Thus, it's hypothesized that oxidative damage induced by Heptachlor insecticide may be one of these mechanisms which merit future study.

Conclusion

Heptachlor destroy and fibrosis seminiferous tubules from the histology picture that cannot be cure, because heptachlor act directly on DNA molecular that reflect in lowering spermatogonia (primary and secondary) and Leydig cells.

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