The Effect of Exposure to Formaldehyde Vapor on the Structure of Testes in the Rabbits

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Summary:

Background: This study has been performed to detect any structural changes in the testes of rabbits that have been exposed to formaldehyde vapor.

Materials and Methods: Eighteen male rabbits has been used in this study, 12 of them were exposed to formalin vapor for 2 month, while the other 6 were exposed to the vapor of distilled water for 2 month too. All animals were killed at the end of experiments, and then pieces of testes were prepared for light microscopic examination. Sections were collected from the paraffin wax blocks and stained by H & E and finally examined by Olympus light microscope.

Results: Most of the seminiferous tubules from the formaldehyde exposed group were atrophied with no evidence of spermatogonia and lined only by Sertoli cells, in addition some sections showed multinucleated giant cells.

Conclusions: This is the first study to report that formaldehyde induces damage to spermatogenesis without a reduction in the number of Sertoli cells and Spermatogenic damage can be seen in most of the formaldehyde exposed rabbits.

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Introduction:

Formaldehyde is a flammable, colorless and readily polymerized gas at ambient temperature, and is considered to be one of the causes of multiple chemical sensitivity (1). It is well known as a preservative, sterilizer and embalming agent, to which about 2 million workers are exposed daily all over the world (2). Formaldehyde has a teratogenic effects on some animals (3), and is probably carcinogenic to humans (4 & 5). There are strong evidence that exposure to formaldehyde vapor is associated with an increased risk of lung injury in rats(6), lung cancer in monkey(7), cancer of the kidney among some workers exposed to its vapor (8), corrosive lesions of the upper part of the gastrointestinal tract by contaminated water (9), and recently in 2006, Al-Saraj has noted that formaldehyde has toxic effects on the haemopoeitic tissues, liver and kidney of rabbits exposed to the vapor of formaldehyde(10). The effects of formaldehyde on the reproductive system have received little attention by some investigators and was concentrated mainly on the rats (11). The author did not find any literature of its effect on the testes of the rabbits; hence the present work has been carried out to record any structural changes in the testes of the male rabbits after exposure to formaldehyde vapor.

Materials and Methods:

18 mature male rabbits have been collected (of about 5-6 month age), who's weight ranges between 850 – 1200 gms. The animals were kept in a stainless steel mesh cages (50 × 50 × 60 cm), being placed as 3 animals per cage at a temperature of 20 – 30 C°, and were allowed for free access to food and water.All animals were given antihelmentic drug subcutaneously (ivermectin in a dose of 2mg/kg body weight) as reported by Al-Saraj (10). Two cages (i.e., 6 animals) were isolated as a control group, while the other 4 cages (12 animals) were exposed to formaldehyde vapor. 10% formaldehyde solution was placed in a 400 – steel container, which is covered by a nylon mesh and filled periodically, and all the treated animals (12 rabbits) were exposed to constant surface area of 10% formalin solution for a period of two month, while the control group (6 rabbits) were exposed to a vapor of distilled water for the same period (i.e two month). The concentration of formaldehyde as part per million (ppm) in the atmosphere of the cages was estimated according to the analytic procedure noted by Hoogenboom et. al. [14], and Al-Saraj[10]. From this analytic procedure, the concentration of formaldehyde in the exposed cages was in the range of 12-14 ppm. All the animals of both groups were killed at the end of the experimental work (i.e 2 month), after being anesthetized by exposing them to chloroform in an air-tighted jar, and the 2 testes from each animal were removed, after that small pieces of about 5mm in thickness were taken from each testes and fixed for 24 hr in neutral buffered formalin solution, then were dehydrated in an ascending strength of ethanol (70%, 80%, 90% and 100%), after being dehydrated, they were immersed in xylene (clearing agent) for 3 changes (for 3 hours each). The specimens were then impregnated in a molten paraffin wax at 60 C° (2 changes) for 3-hours each change, and finally the tissues were embedded in paraffin wax and kept over night in the oven. The paraffin blocks were removed from the oven, then sections were obtained from each block using

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Reichert Rotatory Microtome, after that the sections were collected and being stained by Haematoxylin and Eosin (H & E) stain and finally were examined by Olympus – B x51 light microscope (with a magnification ranges x50 – x1000).

Results:

There are some sloughed off spermatids (SPT), but no evidence of spermatogonia in the lumen of the seminiferous tubules of the formaldehyde exposed group (fig. 1), compared to the control group (saline exposed group) which shows normal architecture with evidence of normal spermatogenesis in the lumen of the tubules with very clear spermatozoa. Some sections of the seminiferous tubules of the formaldehyde exposed group shows little number of Sertoli cells (SC) in the tubules, with infiltration and hyperplasia of leydig cells (LC) in between the tubules (fig. 2) compared to the control group which shows spermatogonia (SPG) and spermatocytes (SPC), while the majority of tubules are lined by Sertoli cells (fig. 3). Some sections of the seminiferous tubules from formaldehyde exposed group show no evidence of germ cells at all, and may show few multinucleated giant cells (fig. 4).

Fig. (1): Micrograph of a testes from the formaldehyde exposed group, showing some sloughed off spermatids (SPT) in the lumen of seminiferous tubules (H & E × 400).

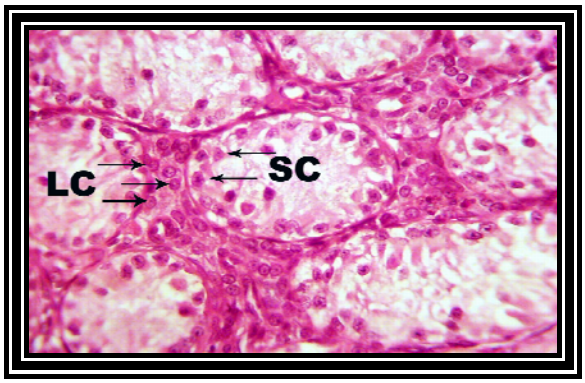

Fig. (2): Micrograph of an atrophied testes from the formaldehyde exposed group, showing little number of Sertoli cells (SC) in the tubules, with infiltration and hyperplasia of leydig cells (LC) in between the seminiferous tubules (H&E×600).

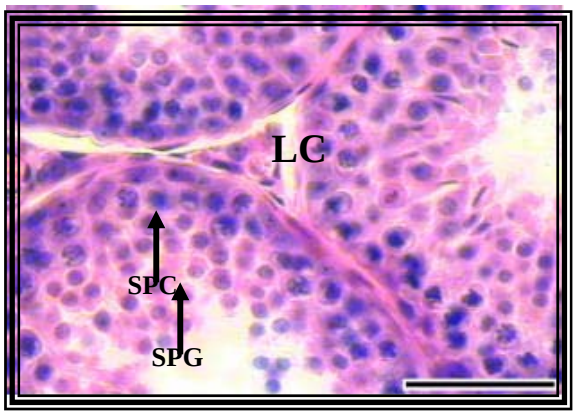

Fig. (3): Micrograph from the saline exposed group (control), showing spermatogonia (SPG), spermatocytes (SPC) in the tubules, with few leydig cells (LC) in between the tubules (H & E × 1000)

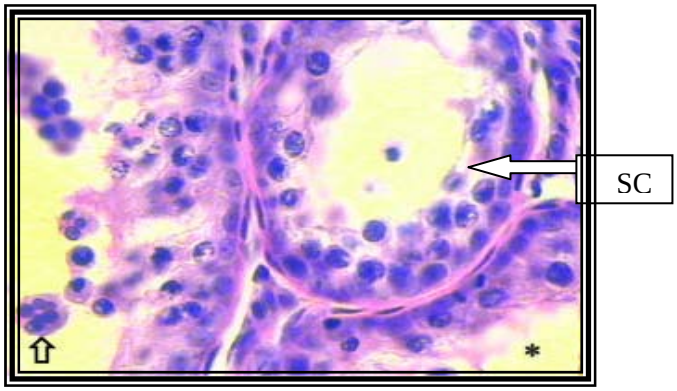

Fig. (4): Micrograph from formaldehyde exposed group showing multinucleated giant cells (⇧), Sertoli cell (Sc). (H & E × 1000

Discussion:

The cycle of the spermatogenic epithelium and testicular maturation in rabbits, are documented in literatures (13 & 14), where the spermatogonia starts to divide at 7 to 8 weeks of age, and spermatids & spermatocytes are first seen at 14 to 15 weeks of age (13). The seminiferous tubules appear histologically mature by 18 week of age and the testes continue to grow and increases sperm production until 6 months of age (14). Exposure to formaldehyde vapor in the rabbits lead to severe damage to spermatogenesis manifests itself as sloughing off the spermatids from the seminiferous tubules with a reduction in the number of advanced germ cells, but without any reduction in the number of Sertoli cells. To the best of my knowledge this is the first study to report that formaldehyde induced germ cells sloughing off in the rabit, and the damage to spermatogenesis was not complicated by a reduction in the number of Sertoli cells. Damage to spermatogenesis has been reported in some animals which were exposed to hydroxyurea as in rats (15), and mice (16), in addition early marked damage to spermatogenesis was reported in the testes of all vasectomized rabbits (17). Unlike our findings, the damage gradually

recovered almost completely few months after vasectomy, while in the case of formaldehyde exposure, the damage seems to be a permenant one. The formaldehyde is a cytotoxic as well as potentially carcinogenic agent which is associated with incremental increase of cellular death (18). The mode of this action is probably similar to the cytotoxic effect of hydroxyurea, which causes a depletion of essential DNA precursors (19). I think that formaldehyde is probably has similar mode of cytotoxity as that exerts by hydroxyurea, thus formaldehyde (in my opinion) probably exerts its action by damaging & killing the dividing cells (as germ cells) by depleting some essential DNA precursors. The most probable explanation (in my opinion) is that testicular dysfunction could be a result of endocrine dysfunction, as testosterone secretion is a function of interstitial cells of Leydig. My opinion can be supported by the findings of Shiraishi et. al., in 2002(20), when they mentioned that interstitial fibrosis in vasectomized animals may affect the paracrine function of the seminiferous tubales which eventually leads to a reduction in spermatogenesis. The multinucleated giant cells which have been seen in the present work are probably formed by fusion of some spermatide due to alteration or absence of the intercellular spaces between some adjacent spermatids. I do not agree with the explanation given by McDonald in 2006(21), who attributed the formation of multinucleated giant cells to pressure changes in the spaces between some spermatids resulting in their approximation & fusion. Thickening of the basement membrance in the seminifirous tubules following formaldehyde exposure has been reported in the guinea pigs (22), and in the rats (23). Such a thickening was also seen in our study on the rabbits, but is limited to some tubules and in some sections, and to a lesser extent (degree) if compared to that reported in guinea pigs and rats, where the thickening form a common and prominent features in most of the seminiferous tubules in guinea pigs and rats.

Conclusions:

Spermatogenic damage could be observed in most of the formaldehyde exposed rabbits, and the damage could be either moderate or progressively severe. Germ Cells loss from the seminiferous epithelium may be caused by disrupting the anchoring junctions at the Sertoli-germ cell interface or contact. This is the first study to report that formaldehyde induced damage to spermatogenesis with out a reduction in the number of Sertoli cells.

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