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Formaldehyde exposure induces histopathological and morphometric changes in the rat testis

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Formaldehyde is a chemical which is traditionally used for fixing cadavers and routine histopathology techniques. It is vaporised during the dissection and practical study of a cadaver. Previous studies have shown that this vapour may cause clinical symptoms such as throat, eye, skin and nasal irritation. This study was designed to determine the histopathology and morphometrics of the rat testis when all the experimental animals were exposed to formaldehyde for 18 weeks. The study was performed in 2004 on 28 albino Wistar rats of 6–7 postnatal weeks. The rats were divided into three case groups (E1: 4 h/d, 4 d/w; E2: 2 h/d, 4 d/w; E3: 2 h/d, 2 d/w) and one control group. The testes specimens were sectioned at 5 µm and stained with the haematoxylin and eosin staining technique for histological and morphometrical studies. We found a severe decrease in germ cells associated with spermatogenesis arrest in the E1 group. A decrease in germ cells and a thickening of the basal membrane of the seminiferous tubules were seen in E2. Displacement of Sertoli and germinal cells were also found in the E3 group. The mean seminiferous tubular diameter and seminiferous epithelial height in the experimental groups were decreased in comparison with the control group and the differences were statistically significant ($p < 0.05$).
The findings of this study revealed that chronic formaldehyde exposure can cause histopathological and morphometric changes to the seminiferous epithelium in rats and that these changes depend on the duration of the formaldehyde exposure.

Key words: spermatogenesis, seminiferous epithelium diameter, seminiferous epithelial height, Sertoli, germ cell, duration

INTRODUCTION

Formaldehyde (CH_2O) is a flammable, colourless, reactive gas, readily polymerised at normal room temperature and pressure, with a relative molecular mass of 30.03 and a pungent odour. Formaldehyde is soluble in water, ethanol and diethyl ether. It is also used in polymerised form as paraformaldehyde [23]. Under atmospheric conditions formaldehyde is readily photo-oxidised by sunlight to carbon dioxide. In the absence of nitrogen dioxide the half-life of formaldehyde is approximately 50 minutes during the daytime; in the presence of nitrogen dioxide, it drops to 35 minutes [23].

There are various sources of formaldehyde, but the major anthropogenic sources which affect humans are present in indoor environments. Other anthropogenic sources include direct emissions, especially from the production and use of formaldehyde [23].

Its potential to act as an electrophile and act with macromolecules such as DNA, RNA and protein to

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form reversible adducts or irreversible cross-links [10] makes it useful as a conventional tissue fixative, particularly for cadaver fixation.

Acute formaldehyde exposure produces mainly mucosal irritation of the eye and upper respiratory tract in humans [26], and long-term exposure leads to the production of nasal tumours in rodents [15]. Formaldehyde also causes pulmonary function impairment [1] and asthmatic reactions in sensitised individuals [2, 6]. Some studies have indicated teratogenic and cytotoxic effects of formaldehyde. In one study administration of a high intraperitoneal dosage of formaldehyde caused changes in the seminiferous epithelial cells [21]. Furthermore, in another study, administration of formaldehyde caused a decrease in the motility and number of spermatozoids [14].

The present study was performed on albino Wistar rats with the aim of analysing the histopathological and morphometric changes in the seminiferous epithelium resulting from formaldehyde exposure in the dissection laboratory and to determine its relationship with the duration of exposure.

MATERIAL AND METHODS

The study was performed in 2004 on 28 albino Wistar rats of 6–7 postnatal weeks (provided from the Iranian Pasteur Institute) in the Faculty of Medicine, Gorgan University of Medical Sciences. The rats were randomly divided into three equal case groups on the basis of the differences between the periods of exposure to formaldehyde:

— E1 — 4 hours/day, 4 days/week for 18 weeks (4 h/d, 4 d/w);
— E2 — 2 hours/day, 4 days/week for 18 weeks (2 h/d, 4 d/w);
— E3 — 2 hours/day for 2 days/week for 18 weeks (2 h/d, 2 d/w).

There was also a control group without any exposure.

Approval for this study was gained from the Gorgan University of Medical Sciences animal Care and Ethics Committee.

It was found by means of a digital scale that the mean weights for each group were 252 g (E1), 209 g (E2), 222 g (E3) and 195 g (control group). The concentration of formaldehyde vapour was measured at the beginning, during and at the end of the study by means of a detector tube and dragger pump (model 31, made in Germany) after the covers of the cadavers had been removed. The mean vapour concentration of the dissection room was 1.5 ppm. The temperature of the dissection room was 20–26°C and the air pressure was 760–763 atm.

At non-exposure times all groups were kept in the laboratory animal quarters, which were far from the place of exposure and where no formaldehyde was detected. The animal quarters were ventilated and the temperature kept at about 21°C; air conditioning and adequate light were supplied. All groups were fed with a similar standard diet (provided by the Iranian Pasteur Institute) twice a day (morning and afternoon) with water available *ad libitum*.

The cages of the case groups were placed at the height of a cadaver, separated by a distance of 15 cm, for 18 weeks, corresponding to the time protocols mentioned above. During each period of exposure the control group was kept in the animal quarters.

When the exposure period had expired, each of the rats of the three experiments and the control group were anaesthetised with ether. After cervical dislocation the left testis of each experimental rat was extracted. Specimens with dimensions of 4 × 4 × 2 mm were then taken from each. After tissue processing and paraffin embedding, 30 sections from each specimen, taken from the left testis, were cut at 4 μm and stained with haematoxylin and eosin (H & E). Twenty seminiferous tubules in stage VI–X [9] were measured in each section. All of the sections were studied by means of an OLYMPUS light microscope with multiple magnifications (400 ×, 1000 ×). A morphometric study of the diameter and height of the seminiferous tubules (STD and SHE respectively) was made with the use of Olysia Autobioreport software. The data were analysed with Student's t test and the χ^2 test ($\alpha = 0.05$).

RESULTS

A severe decrease in germ cells was seen in the more than 85% of the seminiferous tubules in animals of group E1. In addition, spermatozoids were rarely seen in tubules and the spermatogenesis process was arrested (Fig. 1).

In group E2 a decrease in germ cells and an increase in thickness in the basal membrane of 75% of the tubules were found (Fig. 2).

In group E3 an increase in the spaces between germ cells were seen in tubules, and the association between Sertoli and germinal cells was also disrupted (Fig. 3). No histopathological changes were seen in the control specimens (Fig. 4).

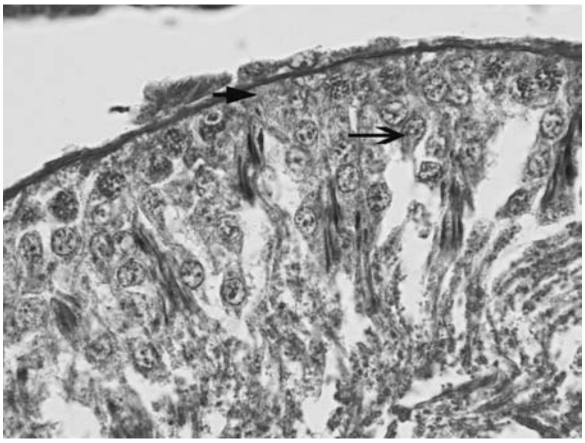

Figure 1. Histological architecture of the testis in group E1 (stage VII). Decreased germ cells (>) and arrested spermatogenesis (—>). H & E staining, 1000 ×.

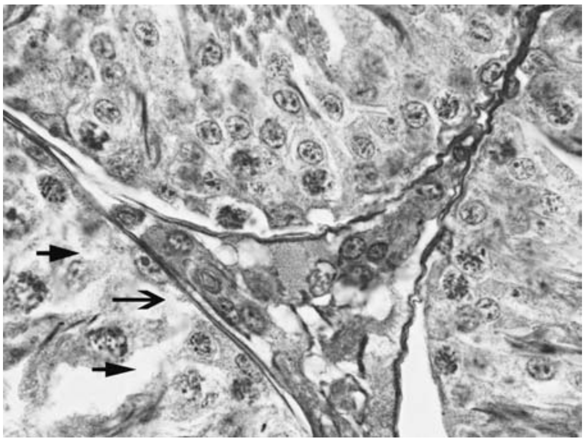

Figure 3. Histological architecture of the testis in group E3 (stage VI). Disruption to the arrangement of Sertoli cells and germinal cells (>). Increasing space between germ cells (—>). H & E staining, 1000 ×.

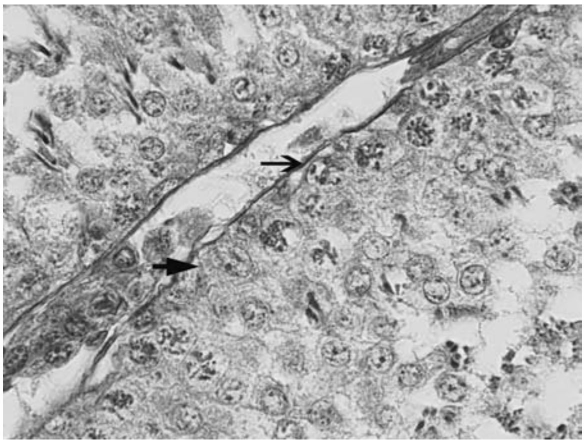

Figure 2. Histological architecture of the testis in group E2 (stage VII). Decreased germ cells (>) and increased thickness of basement membrane (—>). H & E staining, 1000 ×.

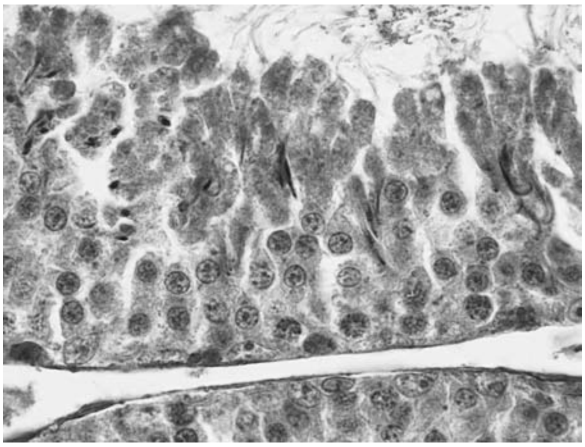

Figure 4. Histological architecture of the testis in the control group (stage VIII). H & E staining, 1000 ×.

Table 1. Mean ± SE of seminiferous tubular diameter (STD) and seminiferous epithelial height (SEH) in experimental groups and control

	Control	E1 (4 h/d, 4 d/w)	E2 (2 h/d, 4 h/w)	E3 (2 h/d, 2 d/w)
STD [μm]	252.12 ± 4.82*	204.55 ± 3.29*	232.45 ± 2.42*	238.94 ± 4.37*
SEH [μm]	82.77 ± 2.00**	65.26 ± 1.43**	69.46 ± 1.78**	72.80 ± 2.03**

*, **control with E1, E2, E3 were significant

The morphometric findings, including those for STD and SEH, are outlined in Table 1. In comparison to those of the control group the mean STD and SEH had decreased significantly ($p < 0.05$). In addition, a relationship was found between changes in SEH and the duration of exposure.

DISCUSSION

This study showed that formaldehyde exposure can cause a severe decrease in germ cells, spermatogenesis arrest and a thickening of the basement membrane of seminiferous tubules in the testis of rats. The result of this study is similar to that of other research [14, 20, 21, 24, 25], although the dosage, duration and administration of formaldehyde were different in these studies.

According to the study by Tang et al. [21], intraperitoneal administration of formaldehyde with dosages of 0.2, 2 and 20 mg/kg can cause degeneration and necrosis of the secondary spermatocytes, spermatogenic cells and spermatozoids. Furthermore, Chowdhury et al. [3] found disruption of the Leydig cells and spermatogenesis arrest as a result of intraperitoneal injection of formaldehyde at dosages of 5, 10 and 15 mg/kg in rats.

Zhou et al. [24] the experimental animals to formaldehyde vapour (10 mg/m^3 for two weeks and observed atrophy of the seminiferous tubules, a decrease in the number of spermatogenic cells and disorganisation of the seminiferous epithelial cells.

The Majumder and Kumar study [14] showed that formaldehyde, administered intraperitoneally at 10 mg/kg for 30 days, caused a decrease in the motility, viability and number of spermatozoid cells in rats.

The results of the Sarsilmaz et al. study [19] showed that exposure to formaldehyde (10 and 20 ppm) caused a severe decrease in Leydig cells in the mouse testis. The experimental study of Shah et al. [20] also showed that formaldehyde administration (200 mg/kg during 10 days) can induce testis degeneration and spermatogenesis arrest. The histological changes among seminiferous tubules due to spermatogenesis arrest in this study are similar to the findings of Zohu's et al. research [24].

Furthermore, the findings of our study about the decrease in spermatogenic cells were the same as those from Shah's et al. research [20]. The morphometric findings of our study were in accordance with the histopathological results. Another study, which surveyed the effect of welding fumes on the seminiferous epithelial cells in rats, showed a correlation between morphometric and histopathological changes [12].

With regard to the findings of our study in chronic exposure and in the normal conditions of the dissection laboratory, low concentration formaldehyde exposure over a long time can be seen to cause histopathological and morphometric alteration to the seminiferous tubules, and these changes are related to the duration of exposure.

The histopathological changes in the spermatogenesis process and the seminiferous tubules have been explained by the different mechanisms. According to the Ma et al. study [13], the histopathological changes in the spermatogenesis process and seminiferous tubules are related to the cytotoxic effect of formaldehyde. Another mechanism was explained by Feldman [5], who reported that intraperitoneal administration of formaldehyde caused the arrest of nucleic acid synthesis and proteins.

In addition, formaldehyde can increase the production of reactive oxygen species in many tissues [7, 18, 25]. Reactive oxygen species, including single oxygen, hydrogen peroxide, super oxide anions and hydroxyl radicals, are important mediators of cellular injury and play an important role in oxidative damage. Zhou et al. [25] also showed that formaldehyde inhalation decreased the effectiveness of the testicular antioxidant system but brought about a prominent increase in the testicular lipid peroxidation product malondialdehyde in the testis of rats exposed to formaldehyde. Malondialdehyde is one of the most important products of lipid peroxidation and interferes with protein biosynthesis by forming adducts with DNA, RAN and protein [4]. Similar phenomena are often observed after exposure to chemicals and gamma radiation that cause testicular damage [16, 25, 22]. This suggests that oxidative stress is an important mechanism of testicular damage [25].

Moreover, Ozen et al. [17] showed that subacute and subchronic formaldehyde vapour inhalation have caused growth retardation and altered levels of trace elements, including copper, zinc and iron in the testicular tissue of the rat. The other possible mechanism in oxidative stress may therefore be due to altered levels of trace elements in formaldehyde exposure.

Some research has also shown that an increase in the level of iron can cause indirect oxidative stress. This means that an increase in iron, either by making a complex with soluble cellular chelating agents such as ADP or directly by oxidative stress, can have an adverse effect on spermatogenesis [8, 11]. A decrease in zinc and copper levels may, moreover, cause oxidative stress in the testis tissue owing to formaldehyde exposure, as these two trace elements are co-factors of zinc-copper cytoplasmic superoxide dismutase (Zn, Cu-SOD) [8].

In conclusion, the results of this study showed that chronic formaldehyde exposure at the concentration and duration mentioned can cause histopathological and morphometric alterations in the seminiferous epithelium in rats. In addition, it seems that there is a relationship between the severity of exposure-induced changes and the duration of exposure.

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