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EFFECTS OF CONSUMING FORMALIN ADULTERATED FOODS ON THE TESTICULAR TISSUES IN RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

The experiment was conducted to analyze effects of administering various concentrations of formalin mixed feed on the testicular tissue in rats. A total of 24 healthy adult male rats (*Rattus norvegicus*, Long Evans strain) were randomly divided into 4 groups of which one remained as negative control and other groups with fed with 0.85 ppm, 3.4 ppm and 7.0 ppm concentrations of formaldehyde mixed feed. After a 30 days experimental period, all rats were killed both for Haematoxylin and Eosin (HE) staining of testis and sperm collection. We analyzed testes weights, the seminiferous tubule diameters, sperms in the cross-section of seminiferous tubules and qualitative evaluation of concentration and the motility of spermatozoa. After one month experimental period, although there was little variation in body weight gain of rats across different groups, but didn't differ significantly ($p < 0.05$). The average paired testes weight of the control and formaldehyde treated groups (0.85 ppm, 3.4 ppm and 7.0 ppm) were measured 6.5 ± 0.45 g, 6.38 ± 1.70 g, 6.45 ± 0.49 g and 6.40 ± 1.10 g, respectively. Paired testes weight did not differ significantly ($p < 0.05$) between control and formaldehyde treated groups. Cross sections of seminiferous tubules in the control and formaldehyde treated groups (0.85 ppm, 3.4 ppm and 7.0 ppm) were measured 488.4 ± 39.8 μ m, 505.4 ± 53.2 μ m, 486.4 ± 66.0 μ m and 484.2 ± 41.3 μ m, respectively. Diameters of seminiferous tubules in rats given high concentration of formaldehyde decreased slightly but did not differ significantly ($p < 0.05$). Those rats given 7.0 ppm formaldehyde mixed feed; severe effects were found. There were no spermatozoa in the lumen of seminiferous tubules with loosely disorganized interstitial tissues. Sperm motility and concentration of normal feeding testis was normal and good in the study. On the other hand, sperms motility and concentration seemed gradually decreased with effects of higher concentrations of formaldehyde in feed. In 0.85 ppm and 3.40 ppm concentrations of formaldehyde mixed feed, sperm motility was moderate. However, spermatozoa showed the lowest motility and the lowest density in rats supplied 7.0 ppm formaldehyde mixed feed compared with other groups in the present study. In conclusion, formaldehyde in the form of formalin directly affects the testicular tissues and decreases sperm number and their quality even at low dose.

Keywords: Formalin, Formaldehyde, Effects, Rats, Testis

INTRODUCTION

Formalin which is an aqueous solution of 37-40 percent formaldehyde is highly toxic and hazardous. Formalin is hazardous not only for human but also for other animals and birds (Logue *et al.*, 2011). According to reports of newspaper (Anonymous, 2013), formalin was detected by Fisheries Department and BSTI experts in fishes, meat, fruits and vegetables in various supermarkets and open markets. Formaldehyde was detected at the levels of a minimum 3.3 ppm to a maximum 7.46 ppm in fishes (Nomanuzzaman *et al.*, 2012). However, formaldehyde present in biological fluids or tissues and environment as a result of natural processes or from man-made sources and can be emitted slowly into the air (Norliana *et al.*, 2009).

Its detrimental effects may harm all tissues and organs of the body. Infertility and sterility of man and woman is increasing day by day specially in urban area in Bangladesh. It is assumed that consumption of formalinized food products might damage human gonad as well as decreases reproductive performance. According to Ozen *et al.*, (2005), inhaling formaldehyde (FA) gas may damage spermatogenic cells. But

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so far we know no research work has been done to evaluate the effect of consuming formalinized food in human body especially in testicular tissues. So, the piece of work was conducted to analyze effects of administering various concentrations of formalin mixed food products on the testicular tissue in rats with a view to evaluating weight of testes, the seminiferous tubule diameters, sperms in the cross-section of seminiferous tubules and qualitative evaluations of concentrations and the motility of spermatozoa.

MATERIALS AND METHODS

The experiment was conducted in the Laboratories of Departments of Anatomy and Histology and in the Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet, Bangladesh from January 3, 2014 to February 4, 2014 for a period of 30 days. As 1 to 3 spermatogenic cycles of rats occur at every 30 days (Clermont and Perey, 1957), the interventional period was set for 30 days in order to get effects. A total of healthy male 24 rats (*Rattus norvegicus*, Long Evans strain) (n=24) were purchased from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). Sexually matured (120 days old) male rats are randomly divided into 4 groups-A, B, C and D (n=6/group) and were placed in four wire boxes (each measuring 40 cm long ×40 cm breadth ×30 cm height). Group A was treated as control whereas group B were fed with 0.85 ppm formaldehyde mixed feed, group C fed with 3.40 ppm formaldehyde mixed feed and group D fed with 7.00 ppm formaldehyde mixed feed. Concentrations of formaldehyde were measured in water using formaldehyde detection kits ‘Formaldehyde Meter’ (Environment Sensor Co. USA). Specific concentration of formaldehyde mixed water such as 0.85 ppm, 3.40 ppm and 7.0 ppm was mixed by praying on feed and was immediately kept in tightly closed polythene bags separately. They were given *ad libitum* feed and water twice (in morning and in evening) in a day. Light and dark regime was 16:8 hour.

Table 1: Showing body weight gain of rats before starting (120 days old) and after the experimental period (150 days old)

Groups	Body weight (mean ± SD) (g) gain of rats Before starting of experiment	After experimental period	Level of Significance at 5%
Control group	364.67±21.54	398.67±19.20	NS*
0.85 ppm formaldehyde treated group	362.5±15.37	397.33±36.86	NS
3.4 ppm formaldehyde treated group	360.83±14.99	391.17±19.44	NS
7.0 ppm formaldehyde treated group	370.17±9.45	395.83±12.30	NS

*NS=Not Significant at 5% level of significance

Body weight of rats was taken before starting the experiment and during samples collection in the 30-days interventional period. Out of 6 rats from each group, four rats were killed with an overdose (1ml/rat) of ketamine hydrochloride (G-Ketamine®, Gonosastho Pharmaceuticals, Bangladesh). The remaining two rats from each group were subjected to sperm collection from the tails of epididymis. After laparotomy, the testes were carefully removed and the mass of the paired testes weighed. The tissue blocks of testes were then immersed in Bouin’s fluid and post-fixed for 24 hours, dehydrated in a graded alcohol series over 48 h, cleared in xylene, embedded in paraffin, and cut at 6-µm-thick by a rotatory microtome (Microm, GmbH, Germany). The randomly selected good sections were mounted on the glass-slides. For each rat, three histological glass slides with four tissue sections on each were prepared for Haematoxylin and Eosin (HE) staining. Sperms were collected from the tail of each epididymis and immediately diluted

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10 fold in normal saline. Then, pieces were placed in ten-fold volume of normal saline (w/v) in eppendroph tubes and were immediately gently sacked. One drop of solution was taken on glass slide and was covered with a coverslip. Then, sperm motility and sperm concentration were examined and recorded by using a compound light microscope (Olympus BX51, Japan). Some videos were captured by a video camera (Canon, Japan) from the focus of the microscope at same magnification and subsequently images were converted by video converter.

Histopathological changes of the stained sections were observed under a light microscope (Olympus BX51, Japan). Spermatogenic cells were identified by their cytoplasmic and nuclear morphology according to Breucker (1982). The seminiferous epithelia and number of spermatogenic cells were examined in each testis. Histomorphometric data were obtained from cross-sections of each testis using a photomicroscope (Olympus Digital Camera, DP12). The diameters of the seminiferous tubules were measured using ImageJ Software (National Institutes of Health, Bethesda, MD). The diameters were calculated from 10 randomly selected cross-sections of the seminiferous tubules in each rat and subsequently averaged. The seminiferous epithelia and number of spermatogenic cells were examined in each testis.

Morphometric data were collected on body weight of rats, paired testes weight and diameters of seminiferous tubules. Data were expressed as mean values \pm SD and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups by Tukey's Test.

RESULTS AND DISCUSSION

Our study was designed to evaluate the histopathology and morphometrics of the rat testis when they were exposed to various concentrations of formaldehyde in feed for 30 days. This was the first experimental report on the effects of consuming formalin adulterated food in Bangladesh. In our present study, before starting of experimental, the minimum body weight of rats in group B was 362.5 ± 15.37 g and the maximum body weight of rats in group A was 398.67 ± 19.20 g during sample collection (Table 1) across all the groups. But the average body weight didn't differ significantly.

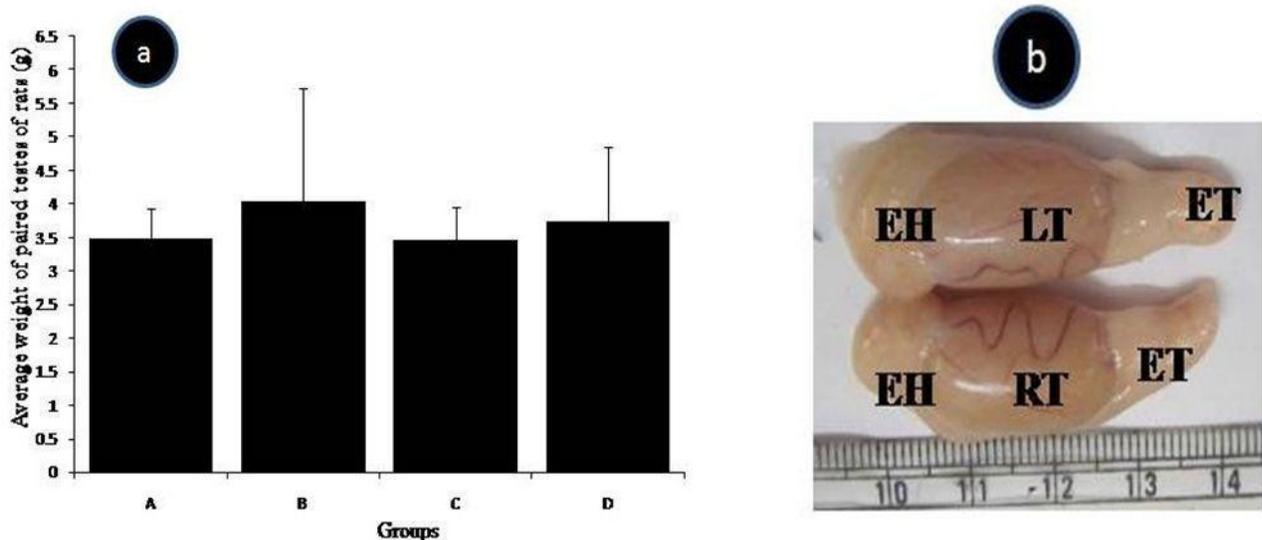


Figure 1: Showing paired testes weights at 150 days old rats (a) in the left. In the right (b) showing paired testes of a rat of control group. EH= head of epididymis, ET= tail of epididymis, LT=Left testis and RT= right testis

This finding is similar to the report in rats (Johannsen *et al.*, 1986) where they stated that body weight did not differ significantly. However, according to Zhou *et al.*, (2006), compared with control, the weight of testis of rats in the high-dose formaldehyde exposure group significantly decreased ($P < 0.05$) in the medium- and high-dose formaldehyde exposure groups. On the contrary, a reverse result

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showed that formalinized feed was given rats (Long Evans strain) whose average body weight much higher than those of normal adult rats of the Wistar strain, weighing 280 to 300 g (Lee and Fritz, 1972). This might be strain variations of rats. However, it may be plausible that chronic administration of formaldehyde even at low level may cause body weight loss and consequently death.

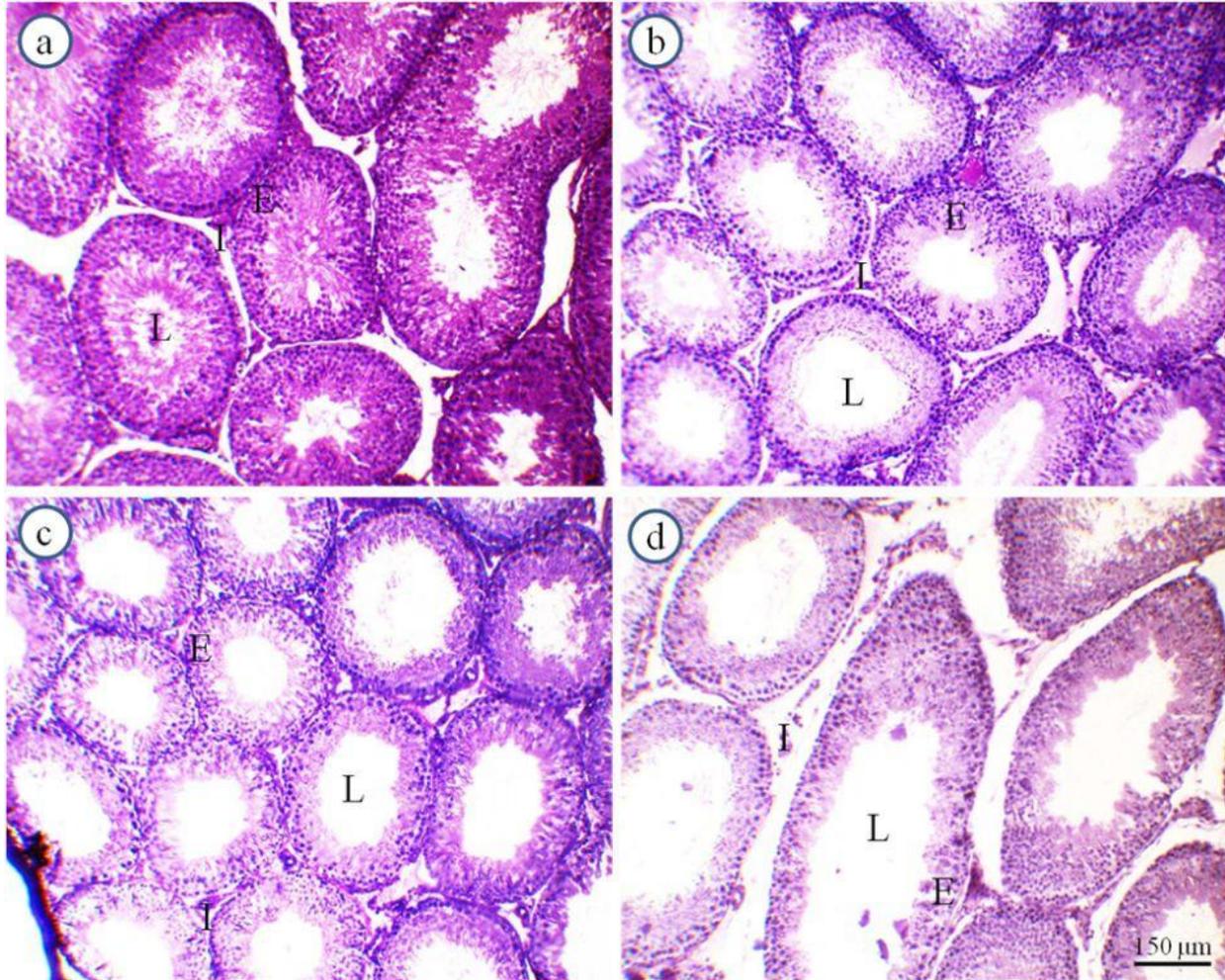


Figure 2: Showing testicular sections of rats. Note that control group (a); 0.85 ppm (b); 3.40 ppm (c) and 7.0 ppm (d) formaldehyde mixed feed groups. L, lumen and E, epithelium of seminiferous tubules. HE staining. Scale bar=150 µm

Average paired testes weight (Figures 1a, b) in the control group and formaldehyde treated groups were 6.5 ± 0.45 g, 6.34 ± 1.7 g, 6.45 ± 0.49 g, 6.40 ± 1.1 g, respectively. But average paired testes weight did not differ significantly between the control group and the formaldehyde treated groups after a 30-days experimental period. Although, it is reported that formaldehyde affects all tissue in body, effects of formaldehyde in testicular weight is not found in few experimental studies so far. But it is assumed that testes weight may decrease due to long term exposure of FA unlike our short term study.

In the present study, after intervention, cross sections of seminiferous tubules in the control group and other ascending concentrations of FA treatment groups were measured 488.4 ± 39.8 µm, 505.4 ± 53.2 µm, 486.4 ± 66.0 µm and 484.2 ± 41.3 µm, respectively (Figure 4). Diameters of seminiferous tubules in rats given high concentration of formaldehyde decreased slightly but did not differ significantly. However, another study showed that the convoluted tubules became atrophied, the layers of seminiferous epithelium decreased, the arrangement of seminiferous epithelium was in disorder in the medium- and high-dose

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formaldehyde exposure groups (Zhou *et al.*, 2006). In our present study, although diameters did not differ significantly, lumen contained few or no sperm in rats fed with formaldehyde mixed feed. Moreover, the epithelial height looked broken and loss of secondary and round spermatids. However, in some parts of testis, the seminiferous epithelium lost some types of spermatogenic cells in all treatment groups (Figures 2 a, b, c, d).

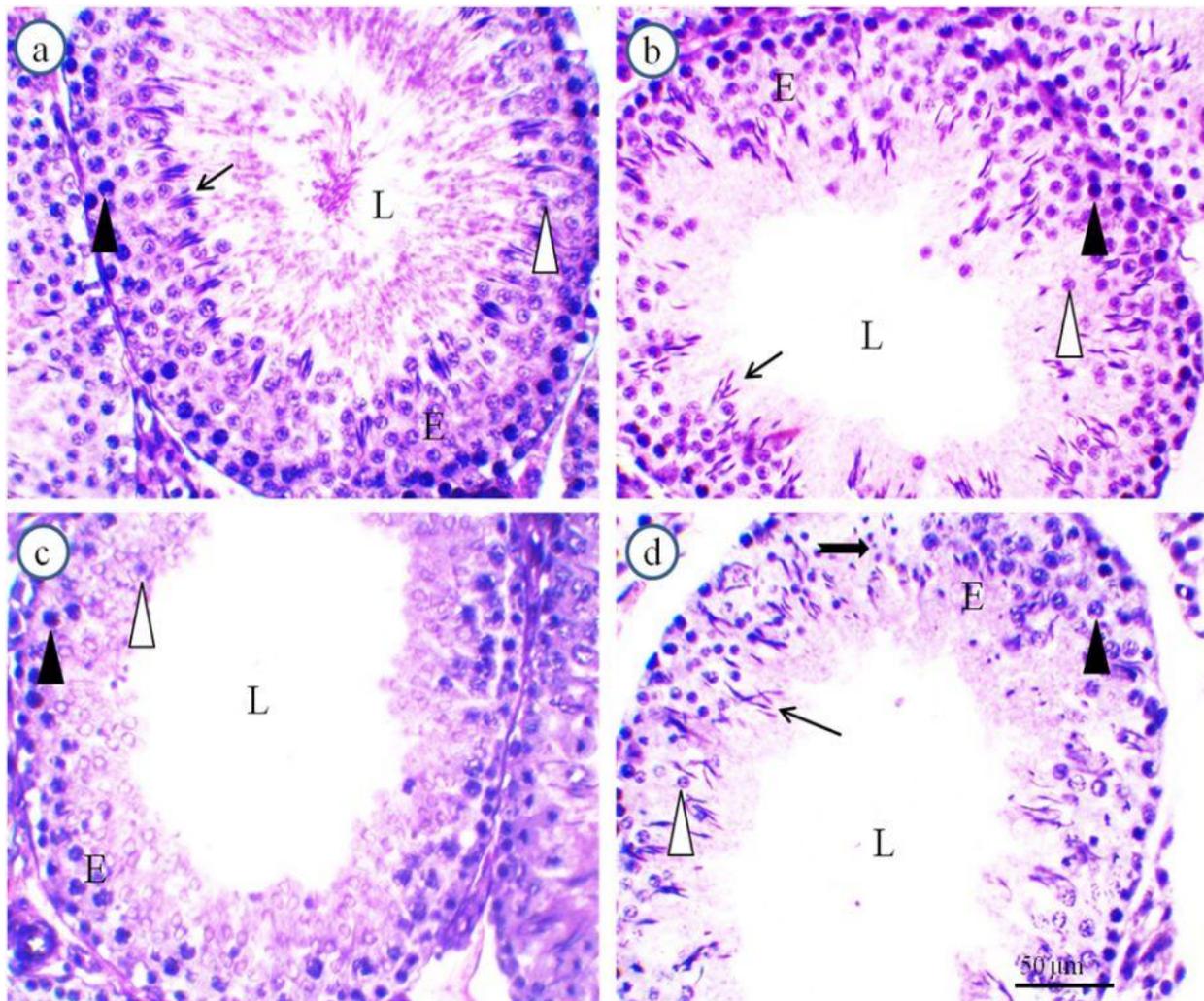


Figure 3: Showing the higher magnification of the sections of testis of rats. Note that control group (a); 0.85 ppm (b); 3.40 ppm (c) and 7.0 ppm (d) formaldehyde mixed feed groups. L, lumen and E, epithelium of seminiferous tubules; arrow=elongated spermatid, blank arrow head=round spermatid and solid arrow head= primary spermatocytes; arrow with tail divided=pycnotic nucleus. HE staining. Scale bar=50 µm

In present study, the rats fed with 0.85 ppm and 3.40 ppm formaldehyde mixed feed, most of the seminiferous tubules of the whole section contained few or no sperms. In some areas of the testicular section, all types of spermatogenic cells including spermatozoa were still found, but most part of the seminiferous epithelia started to have decreased its height (Figures 3 a, b, c, d) Although spermatogonia and primary spermatocytes were present; lumen of the seminiferous tubules was empty in most of the cases. Long term exposure of moderate dose of formaldehyde (2.46 mg/m³) showed decreased number of spermatogenic cells and the lumina were oligozoospermic in testes of Sprague-Dawley male rats (Zhou *et*

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al., 2011). A severe decrease in germ cells associated with spermatogenesis arrest and loss in germ cells and a thickening of the basal membrane of the seminiferous tubules were the detrimental effects of formaldehyde. Displacement of Sertoli and germinal cells were also evident (Golalipour *et al.*, 2007).

Severe effects were found in rats given high dose (7.0 ppm) of formaldehyde mixed feed in our present study. There were no spermatozoa in the lumen of seminiferous tubules with loosely interstitial tissues. The seminiferous epithelia looked disorganized with loss of secondary spermatocytes and round spermatids. In the seminiferous epithelium, some pycnotic nuclei of spermatogenic were found (Figures 3a, b, c, d). These findings might indicate apoptosis of germ cells. Our findings conforms the reports of Ozen *et al.*, (2008) in which they stated that long exposure of formalin may lead to apoptosis of spermatogenic cells and to collapse of seminiferous tubules. They also added that formaldehyde caused apoptosis of spermatogenetic and Leydig cells of testicular tissues. Formaldehyde exerts reproductive toxicity to adult male rats, and apoptosis may be one of the functional mechanisms involved in the reproductive toxicity of formaldehyde.

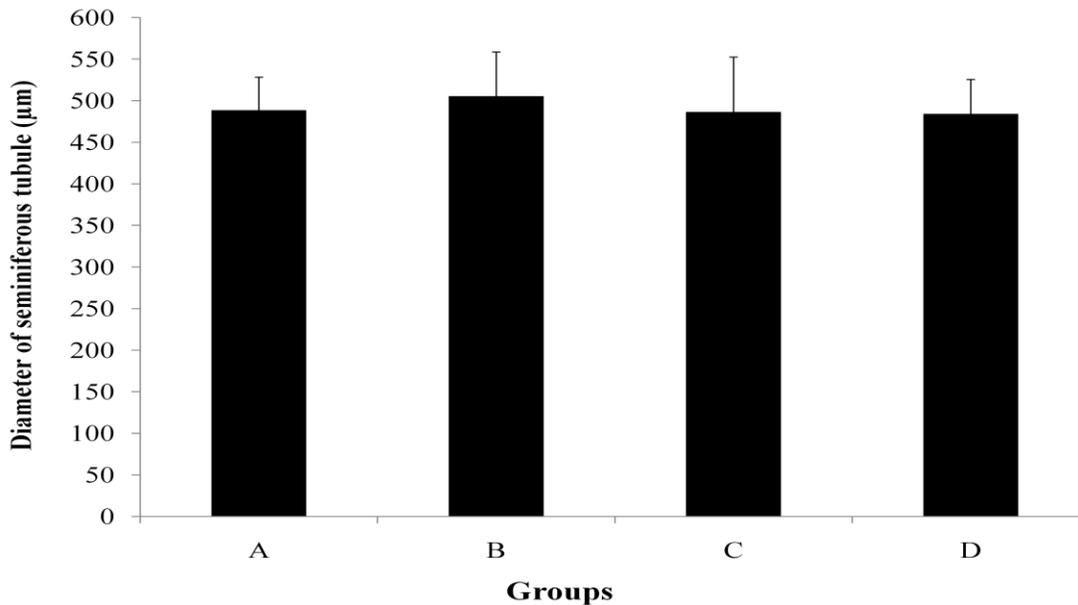


Figure 4: Showing diameters of the seminiferous tubules of rats in different groups where Group A was negative control; B 0.85 ppm, C 3.40 ppm and D 7.0 ppm formaldehyde mixed feed given to rats

Sperm motility and concentration of normal feeding testis was normal and good in the study. On the other hand, sperms motility and concentration seemed gradually decreased with effects of higher doses of formaldehyde in feed. In addition, spermatozoa of rats supplied 7.0 ppm formaldehyde mixed feed showed lowest motility and density compared with other groups in the present study (Figure 5). According to the report of Toru *et al.*, (1996) and Tsujii (1984), the sperm counts averaged 9.42×10^7 / ml³ collected from the tail of rat epididymis.

According to the report of Kempinas and Carvalho (1988) sperm concentration in albino rats ranged from 152.5 to 230.0 X 10⁷ spermatozoa / ml, with a mean of $187.7 \pm 5.6 \times 10^7$ spermatozoa / ml. In addition, the sperm count and sperm motility significantly decreased (P<0.05), the percent of abnormal sperm significantly increased (P<0.05) in the medium-and high-dose formaldehyde exposure rats (Zhou *et al.*, 2006). Environment Canada/Health Canada and WHO stated formaldehyde as weakly genotoxic, with effects most likely to be observed in vivo in cells from tissues or organs after the initial contact. The extent of histopathological changes are proportionate the formaldehyde concentration (Takahashi *et al.*, 1986).

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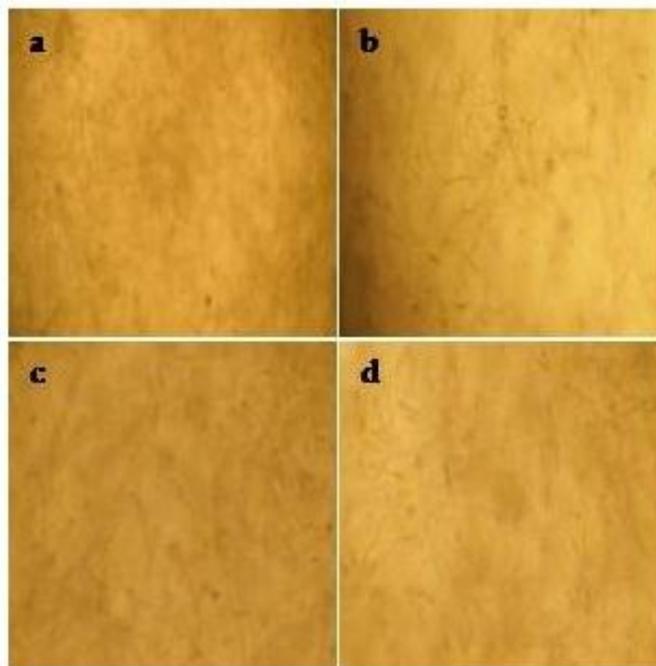


Figure 5: Showing density of sperms collected from tail of epididymis of rats. Figure a was negative control; b 0.85 ppm, c 3.40 ppm and d 7.0 ppm formaldehyde mixed feed given to rats. All figures were produced from the videos of the same magnifications

The widespread use of formalin in various foods is considered to be gravely dangerous for public health in Bangladesh. Despite different reasons for the unsafe treatment and adulteration of foodstuffs in Bangladesh, this study will increase public awareness among food manufacturers and consumers about harmful effects on sperm destruction by consuming formalin adulterated food. In addition, the obtained findings will help to take appropriate measures to combat the current food safety problems persisting in Bangladesh. In conclusion, formalin directly affects the testicular tissues and decreases sperm number and their quality even at low dose. Further study in this regard is necessary for long term exposure of formaldehyde including occupational personnel.

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