Formaldehyde-contaminated feed induces histopathological changes in the testes of adult pigeons (Columbia livia)

Mohammad Rabiul Karim1*, Alamgir Kobir1, Imam Hasan1, Munmun Pervin2 and Ahmed I. Abo-Ahmed3

1Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
2Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
3Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh 13736, Egypt.

*Corresponding author: Dr. Mohammad Rabiul Karim, Professor and Head, Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, E-mail: mraibiulkarim@bau.edu.bd

ABSTRACT: Formaldehyde (FA), a ubiquitous environmental pollutant, has long been suspected to possess reproductive toxicity. Here, we investigated the histopathological alteration of male gonads following exposure to FA-contaminated feed (40% aqueous solution of FA; 2.5 ml formalin/kg feed) in pigeons for 7 days. The mean body weights were not changed significantly in FA-contaminated feed exposed pigeons compared with control pigeons. The hemoglobin concentration was significantly decreased and serum enzyme aspartate transaminase (AST) was significantly increased in FA-exposed pigeons in comparison with control pigeons. Histologically, the structural components of the testes are the seminiferous tubules and interstitial tissues, which are surrounded by a connective tissue capsule. In control pigeons, the size and shape of seminiferous tubules were normal with a regular arrangement of spermatogenic cells. In FA-exposed pigeons, the testicular capsule was thickened and degeneration of spermatogenic cells in the seminiferous tubules was observed. The number of spermatogenic cells was significantly decreased in the seminiferous tubules of FA-exposed pigeons in comparison with control pigeons, indicating that the low exposure of FA affects the spermatogenic cells’ populations in male birds. The present results suggested that FA might cause infertility in birds.

KEYWORDS: Food contaminant, Formaldehyde, Histopathology, Testes, Birds.

INTRODUCTION

Contamination of foods with toxic chemicals possesses a serious threat to public health in Bangladesh due to poor health literacy and low level of awareness [1]. Bangladesh is a country of the tropical region with hot and humid weather. As a result, perishable food items tend to decay quickly [2]. Toxic food contaminants are now very alarming health hazards for human and animals. Food contaminants such as formaldehyde, xylene, ethane dimethane sulfonate, cleaner, toluene and methanol have a detrimental effect on testicular tissue function and structures [3, 4]. Formaldehyde (FA), the recently classified carcinogen and ubiquitous environmental contaminant, is widely used in the construction, textile, furniture, resin, medical, chemical and pharmaceutical industries and FA heavily impacts the everyday consumer [5]. Exposure to ubiquitous exogenous sources of FA at work, in residences, in food and medicine, possess a significant health risk [6]. Formalin (37% aqueous solution of FA) is highly germicidal and used as a disinfectant in poultry and livestock industries. Food and Drug Agency in USA has approved the addition of formalin in poultry feed for keeping it salmonella free [7]. FA treatment of feeds has been reported to have bactericidal effects without apparent loss of palatability or growth reduction in poultry and other food animals [8-11]. However, FA has a detrimental effect on the biological systems including the reproductive system of mammals [12, 13].

FA toxicity in multiple tissues of the exposed rats and mice, in liver, lymphocytes, heart, brain, lung and gonads was reported [13-15]. In rats, FA caused testicular atrophy and reduction in the testicular weight,
serum testosterone level, diameter of seminiferous tubules and seminiferous epithelial height [16-19]. However, comprehensive study on the histomorphological alterations of testes in FA-exposed birds has not been yet undertaken. Therefore, it is very important to know the toxic effects of FA contaminated feed on testicular tissues of pigeons.

**MATERIALS AND METHODS**

**Statement of the experiment**

The research work was conducted in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202 and Village-Roholy, Saturia, Manikganj-1800, Bangladesh during the period from July, 2018 to May, 2019 to assess the effects of FA-contaminated feed exposure on testicular tissue of pigeons.

**Ethical approval**

The present study and all experimental protocols were approved and performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh, Bangladesh [AWEEC/BAU/2019-40].

**Animals and experimental procedures**

Ten adult healthy male pigeons (*Columba livia*) (more than twelve month-old age, 106-146 gm; purchased from local markets at Saturia, Manikganj-1800) were housed in pigeon cages, and fed a standard diet (mustard, rice, sesame, wheat) twice daily and filtered tube-well water *ad libitum*. After one-week acclimatization, five pigeons were fed a FA-contaminated feed daily morning and fed a standard diet daily evening for 7 days. Previous studies reported that the 2.5 ml formalin/kg feed in broiler chicken and Japanese quails has no adverse effect on body weight and feed intake and suggestive as a beneficial dose [20, 21]. This dose was used in the experiment. Formalin (40% aqueous solution of stock FA powder) was mixed properly with pigeon feeds (2.5 ml formalin or 1000 mg/kg feed). The other five pigeons were used as control, fed a standard diet twice daily (morning and evening) for 7 days. After 7 days of FA-exposure (in morning of the 8th day), all pigeons were euthanized under deep anesthesia using 5 ml chloroform-soaked cotton in vacuum glass chamber for 3-4 minutes and then post-mortem examination was performed.

**Determination of hematobiochemical parameters**

After 7 days of FA-exposure, blood samples were collected from the wing vein of both control and FA-exposed pigeons for hematobiochemical parameters. The principles and procedures of hematobiochemical examinations were as follows:

**Total erythrocyte count (TEC)**

Total erythrocyte count was done following the method described by [10]. Well-mixed blood sample was drawn with red blood cell diluting pipette and immediately filled with the red cell diluting fluid. The total number of RBCs was calculated as number of cells counted x 10,000 and the result was expressed in million/cubic meter of blood.

**Hemoglobin concentration (Hb)**

Well-homogenized blood sample was drawn into the Sahli pipette and then the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. Water was added drop by drop to the tube containing acid hematin mixture. The solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in day light by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in g%.

**Serum enzyme aspartate transaminase (AST) analysis**

At necropsy, blood was collected from the heart. Test tube containing blood was placed in slanting condition at room temperature for 30 minutes. Sera were separated from clotted blood by centrifugation at 3000 rpm for 20 minutes and again for 10 minutes. Then the supernatant was collected in eppendorf tube by micro-pipette and stored in refrigerator at -20°C until use and analyzed for aspartate transaminase (AST).

**Postmortem examination and collection of testes for histopathology**

After 7 days of FA-exposure, both control and FA-exposed pigeons were weighed and then all pigeons were euthanized under deep chloroform anesthesia. The internal visceral organs including testes were visualized through ventral mid-line thoraco-abdominal opening and the gross anatomy was observed very carefully. Then,
the testes were removed and their colour and gross texture were observed. The weight of testes was measured and recorded. Subsequently, testicular tissues from the left and right testes were collected and immediately fixed in 10% neutral buffered formalin (NBF) by standard method. Briefly, NBF-fixed tissues were dehydrated with ascending graded alcohols and embedded in paraffin and sectioned at 6 µm in thickness using a sliding microtome (EuromaxR, Japan). The deparaffinized sections were stained with hematoxylin and eosin (HE) for histopathological examination. FA-induced changes were analyzed morphologically using light microscope (LABOMED, Labo America Inc., CA 94538). The spermatogenic cells were counted in at least 5 seminiferous tubules (clear cross sections which were sharply rounded) of each birds. The oblique or longitudinal section of seminiferous tubules were not considered for spermatogenic cell count.

Statistical analysis
All the achieved data were analyzed using student t-test and statistically evaluated with SPSS, version 18.0 software (IBM). The p-values of ≤0.05 were considered to indicate statistically significant, while p-values of <0.01 indicate highly significant results. All the results were expressed as mean ±SD.

RESULTS

Effects of FA on body weight and gross morphology of testes of pigeons
The mean body weights of adult pigeons were not changed significantly in FA-exposed pigeons in comparison with control pigeons (Figure 1A). The color, size and shape of both testes (right and left) were normal in control pigeons (Figure 2A). However, in FA-exposed pigeon, the testes showed uneven shape with pinpoint hemorrhage on their surface (Figure 2B). The weight of the testes was not change significantly in FA-exposed and control pigeons (data not shown).

Effects of FA on hematological parameters of pigeons
Total erythrocyte count showed no significant change in FA-exposed pigeons in comparison with control pigeons (Figure 1B). The hemoglobin concentration was significantly decreased in FA-exposed pigeons in comparison with control pigeons (Figure 1C). The values of AST were significantly increased in FA-exposed pigeons in comparison with control pigeons (Figure 1D), an indicative of liver injury and disturbance of body homeostasis.

Effects of FA on histoarchitecture of testes of pigeons
In HE-stained sections, regular histological structure without abnormalities was seen in testes of control pigeons (Figures 3A, 4A-B). The testis was surrounded by a capsule, which was composed mainly of dense collagenous fibrous connective tissue (Figure 3A). The structural components of the testis were the seminiferous tubules and interstitial tissues (Figure 4A-B, F). Spermiferous tubules are the structural and functional unit of testes. The sertoli cells and the spermatogenic cells lined the seminiferous tubules. The different stages of spermatogenic cells were found in several layers,

![Figure 1. Body weight and blood parameters analysis of FA-exposed adult pigeons. A. No significant changes of body weight were seen in control and FA-exposed pigeons. B. Total erythrocyte count (TEC) showed no significant changes but showing decreasing tendency in FA-exposed pigeons in comparison with control. C. Hemoglobin content (Hb, g%) was significantly decreased in FA-exposed pigeons compared with control pigeons. D. Serum enzyme, aspartate aminotransferase (AST) level was significantly increased in FA-exposed pigeon compared with control. Student t-test, *The p-values of ≤0.05 were considered to be statistically significant, **p-values of <0.01 indicate highly significant results. FA- Formaldehyde.](image)

![Figure 2. Gross anatomy of testes of adult pigeons. A. Showing normal color, size and shape of the right and left testes of control pigeons. B. Normal color and size, but uneven shape and pinpoint hemorrhage was seen in the both right and left testes of FA-exposed pigeons. FA- Formaldehyde. Bar = 1 cm.](image)

![Figure 3. Normal histological structure of testes of control pigeons. A and B. The seminiferous tubules and interstitial tissue. The seminiferous tubules are lined by spermatogenic cells. The interstitial tissue is composed of fat cells and blood vessels. F. Spermatogenic cells.](image)

![Figure 4. Structural components of the testis. A and B. The seminiferous tubules and interstitial tissue. The seminiferous tubules are lined by spermatogenic cells. The interstitial tissue is composed of fat cells and blood vessels. F. Spermatogenic cells.](image)
namely, the spermatogonia, spermatocytes, spermatids and finally mature spermatozoa (Figure 4A-B). The spermatogonia were rounded cells with rounded nuclei; and sertoli cells were tall cells with oval nuclei (Figure 4B). The interstitial tissues were narrow and showed clusters of Leydig cells (Figure 4A-B, F).

In FA-exposed pigeons, the FA-induced testicular changes were characterized by thickened capsule and degeneration of spermatogenic cells in the seminiferous tubules (Figure 3B). Irregular arrangement of spermatogenic cells in the seminiferous tubules of FA-exposed pigeons were seen (Figure 4D). Lumen of the seminiferous tubules became large and number of spermatogenic cells particularly; secondary spermatocytes and spermatid were reduced (Figure 4C-D). Primary spermatocytes were found separated from spermatogonia. The number of spermatogenic cells was significantly decreased in seminiferous tubules of FA-exposed pigeons in comparison with control pigeons (Figure 4E). The interstitial cells or Leydig cells were localized as cord or cluster between or among the seminiferous tubules (Figure 4 A-D, F-G). No changes were seen in the Leydig cells of both control and FA-exposed pigeons (Figure 4 F-G).

**DISCUSSION**

In the present study, the value of serum AST level was significantly increased, and the hemoglobin concentration was significantly decreased in FA-exposed pigeons in comparison with control pigeons. The total erythrocyte count showed decreasing tendency in FA-exposed pigeons. These results suggested liver injury and imbalance of body homoeostasis caused by FA exposure. No reports are available in birds, however serum enzyme AST, ALT was significantly increased in FA-exposed mice (oral, 5mg/kg body weight) [22]. FA treatment (oral, 10mg/kg body weight) resulted in
significant decreases in RBCs, hemoglobin, total WBC and lymphocytes in mice [23].

FA exposure through feed induced a significant reduction of spermatogenic cells and widening of the lumen of the seminiferous tubules in the testes of pigeons. There was no available literature on FA effect on testicular tissues of avian species. However, the present results were consistent with that of other formaldehyde exposure research on male rats and mice although the dosage, duration and route of administrations of FA were different [13, 21, 24-27]. Low levels of formalin (10 ml/kg feed), when fed to Japanese quails (Coturnix coturnix japonica), decreased testicular weight and diameter of seminiferous tubules [21]. Intraperitoneal administration of FA with dosages of 0.2, 2 and 20 mg/kg can cause degeneration and necrosis of the secondary spermatocytes, spermatogenic cells and spermatozooids [15]. FA vapor (10 mg/m³) for two weeks’ exposure, caused atrophy of the seminiferous tubules, a decrease in the number of spermatogenic cells and disorganization of the seminiferous epithelial cells of male rats [26]. FA-induced change in rat testes are characterized by thickened capsule, spermatogenesis arrest, a decrease in the number of spermatogenic cells and atrophy of seminiferous tubules in rats [28,29], which were reproduced in the present study following FA contaminated feed exposure in testes of adult pigeons.

CONCLUSIONS

The present findings revealed that the toxic feed contaminant, FA, has detrimental effects on the testes of adult pigeons. FA was found to cause degeneration of spermatogenic cells, separation of primary spermatocyte from spermatogonia and irregular arrangement of spermatogenic cells in the seminiferous tubules, these histological changes indicated that it might affect the reproduction of birds. Therefore, it is very alarming if the human being exposed to this toxic food contaminant, it may cause reproductive failure.

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AUTHOR CONTRIBUTIONS

The experiment was designed by MRK and MP. MRK, AK and IH undertook the experiment; MRK and IH interpreted the results putting efforts on statistical analysis with AK; MRK and IH wrote up the draft. MRK, MP and AIA checked the manuscript critically. All authors read and agreed on the final version of the manuscript.

CONFLICTS OF INTEREST

Authors declared that they have no conflict of interest.

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