



## Elucidating the Effects of Formalin as Food Preservative on Hematological Profile and Fertility of Swiss Albino Mice

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### Abstract

In Bangladesh, the use of formalin as a food preservative has recently expanded significantly. With this backdrop, the study was attempted to discover changes in the fertility, gross structure, and histoarchitecture of the male reproductive system and hematology in Swiss albino mice as a result of formalin use. Up until experimental week 24, four groups of four mice each were given normal drinking water mixed with formalin at doses of 1%, 5%, 10%, and 20%, respectively, orally. A control group of mice (n=4) were given normal drinking water only. After 24 weeks of treatment, the testicular weight, sperm concentration, morphology, gonadosomatic index (GSI), and hematological profile were evaluated. Testicular histological changes were also evaluated. Formalin-treated mice had significantly lower testes weight, GSI, and sperm count ( $P<0.05$ ) than the control group. In formalin-treated mice, hematocrit value (HCT) and hemoglobin (Hb) concentration were dramatically reduced; percentage of morphologically healthy epididymal sperm also reduced significantly. In the formalin-treated animals, histopathology indicated degenerative alterations in seminiferous tubules, a lower number of spermatogenic cells, the buildup of spermatozoa with exudates in the tubular lumen, and the displacement of the seminiferous tubule from the basement membrane. According to the findings, formalin has a negative impact on hematological profile and reproductive health of male mice.

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### Introduction

Formalin is a colorless, pungent chemical that is commonly used in the construction, paint, textile, plastic, and paper industries. It is also well recognized for its ability to preserve human corpses. Formalin is made up of 37–40% formaldehyde gas dissolved in

water (Kawamata and Kodera, 2004). Escalating global competition in the food industry often prompts the preference for the least expensive preservation technique, driving many to opt for food additives as a practical solution. Within this array of choices, formaldehyde (FA) emerges as a widely employed

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preservative (Guzman *et al.*, 2018). However, long-term use of food tainted with formalin can result in a number of harmful health conditions, including cancer and difficulties with the heart, digestive tract, respiratory system, nervous system, and kidneys (Mamun *et al.*, 2014). It has long been believed that FA, a chemical carcinogen and common environmental pollutant, has detrimental effects on reproduction and development of humans and animals (Duong *et al.*, 2011). Several studies indicated that inhalation or administration of FA affects Leydig cells, and significantly lowers blood testosterone levels and sperm motility (Zhou *et al.*, 2006a). Following FA treatment, there is evidence that a diminished testicular antioxidant system is associated with testicular tubule atrophy, degeneration of seminiferous epithelial cells, and changes in Leydig cell count, all of which lead to reduction in sperm quality (Tang *et al.*, 2003; Zhou *et al.*, 2006b).

Long-term formalin consumption with meals can have an impact on hematocrit (HCT) and hemoglobin (Hb) levels, among other hematological changes (Farshad *et al.*, 2023). Formalin may diminish the mean corpuscular hemoglobin concentration (MCHC), Hb, HCT, and red blood cell (RBC) concentrations. (Abd-Elhakim *et al.*, 2016).

Nonetheless, relatively few research has been published looking into the impact of formalin on the macroscopic and histoarchitecture of various organs in living organisms. Therefore, the purpose of this study was to look into how the food preservative formalin affects male Swiss albino mice's hematological profile, reproductive health, and testicular histoarchitecture.

## Materials and Methods

### Chemicals

The chemical compound known as formalin (RCI Labscan Ltd.<sup>®</sup>, Bangkok, Thailand), which makes up between 37 and 50 percent of an aqueous solution of formaldehyde, was purchased.

### Animals

Three-week-old male Swiss albino mice (*Mus musculus*) that are specific pathogen free (SPF) were obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The mice were maintained in the laboratory animal

containment facility of the Department of Pathobiology at the Faculty of Veterinary Medicine and Animal Science (FVMAS), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Prior to being employed in this study, the mice were housed for a week to allow them to adjust to their new surroundings. They were fed regular mouse pellets obtained from the icddr,b.

The mice were housed in wire mesh-covered rectangular plastic cages separated into divisions. The cages were housed in a room with natural light, good ventilation, a temperature of 26±2°C, and 70-80% relative humidity. The mice received proper hygienic treatment.

### Experimental Design

Twenty (20) male mice aged four weeks were randomly separated into five groups and ear tagged, with each group consisting of four mice. The groups were identified as control, group 1, group 2, group 3, and group 4.

Mice from the control group were given normal drinking water without formalin. Whereas, mice in groups 1, 2, 3, and 4 were given drinking water mixed with formalin at concentrations of 1%, 5%, 10%, and 20%, respectively, until study week 24.

During the experiment, the mice were closely monitored and studied. Their feed and water intake were also closely monitored.

A digital balance was used to determine initial body weight of each mouse. Body weight was measured on the first day of the experiment and then every 15 days after that until the end of the experiments.

### Hematological Analyses

After completing 24 weeks of treatment, the mice were anaesthetized with ketamine hydrochloride as the recommended dose (100mg/kg body weight) before sacrifice, and a thoracotomy was done at the BSMRAU Pathology Laboratory.

Heart bleeds were carried out into heparinized capillary tubes for the determination of various hematological parameters. Total leukocyte count (TLC), differential leukocyte count (DLC), total erythrocyte count (TEC), HCT value, Hb

concentration, packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) were determined by calculating mean value $\pm$ SD.

#### *Effects of Formalin on Testicular Weight and Gonadosomatic Index (GSI)*

At the end of the research week 24, the testes of experimental and control male mice were extracted and weighed at the time of sacrifice. The GSI was determined by dividing the testis weight by the body weight of each mouse and represented as a percentage (Anbara *et al.*, 2021).

#### *Effects of Formalin on Epididymal Sperm Count and Sperm Morphology*

Following terminal sacrifice, the left cauda epididymis was excised, weighed and minced with sterile and fine scissors in 10 mL Dulbecco modified Eagle medium (DMEM) containing 10% fetal calf serum and incubated for 6 hours at 37°C. The total number of sperms per 10 mg cauda epididymis were counted under a light microscope using trypan blue dye exclusion technique (Khan *et al.*, 2008). Sperm morphology was evaluated using a differential interference contrast (DIC) microscopy of wet-mount semen 'fixed' in isotonic formol saline solution at approximately 20x magnification. All types of sperm morphological abnormalities were recorded, classified, and documented in record sheet. The morphological defects in the head (normal, amorphous, pin-head, and banana shaped), hook (knobbed, bent, short, no hook), and tail pieces (tail folded overhead, two tails, and coiled tails) were investigated in a total of 100 spermatozoa/mouse.

#### *Gross and Histopathological Study*

During necropsy, testes were extracted from mice in both the control and formalin-treated groups to investigate the effect of formalin. The testes were weighed, sliced, and preserved in a 10% formalin solution. Hematoxylin and Eosin (H&E) stain was routinely added to fixed tissue sections after they had been processed, paraffin embedded, and sectioned according to standard procedure (Luna, 1960).

The gross and histological examinations were carried out in the Pathology Laboratory, Department of Pathobiology, BSMRAU, Gazipur. The gross investigation was carried out through eye observation. The average length and width of all collected organs were also determined. Low and high-power light microscopy were used to conduct detailed histological examinations.

#### *Photomicrography*

The Department of Gynecology, Obstetrics, and Reproductive Health at BSMRAU permitted the use of a photomicrographic camera (ZEISS AxioCam ERc5s) for photographing various histology samples.

#### *Data Interpretation*

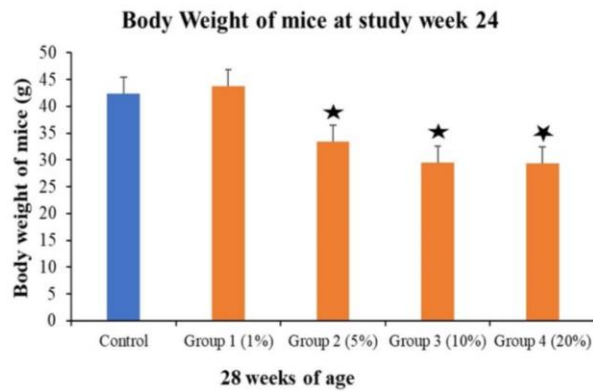
At the end of the study, all data were compiled, compared, and assessed for useful interpretation. The statistical analysis was carried out using SPSS (version 27; SPSS Inc., Chicago, IL, USA). Data were reported as Mean $\pm$ SD. A one-way analysis of variance (ANOVA) was used for comparison. When the *P*-value was less than 0.05, the difference was considered statistically significant.

## **Results and Discussion**

#### *Effects of Formalin on Body Weight of Mice*

At the start of the trial, the average body weight of mice was 27.32 $\pm$ 1.71 gram (g). Group 3 had the highest body weight (29.74 $\pm$ 0.64 g) compared to the control group (24.85 $\pm$ 1.22 g). There was no significant (*P*>0.05) difference in mice's body weight at the start of the trial.

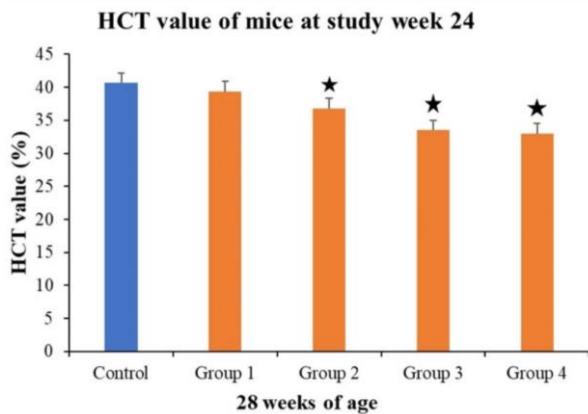
However, after research week 24, body weight in formalin-treated mice dropped as compared to the control group. Mice in groups 2 (33.42 $\pm$ 1.99 g), 3 (29.44 $\pm$ 2.18 g), and 4 (29.33 $\pm$ 2.98 g) lost significant (*P*<0.05) body weight than the control group (42.35 $\pm$ 1.0 g) (Figure 1). The mice in group 4 showed the greatest loss in body weight. Similar findings were observed in rats treated with 20% formalin (Til *et al.*, 1989; Kum *et al.*, 2007).



**Figure 1.** Effects of formalin on body weight of mice. Values are expressed as mean±SD. Significant ( $P<0.05$ ) differences are indicated with one asterisk.

#### Hematological Findings

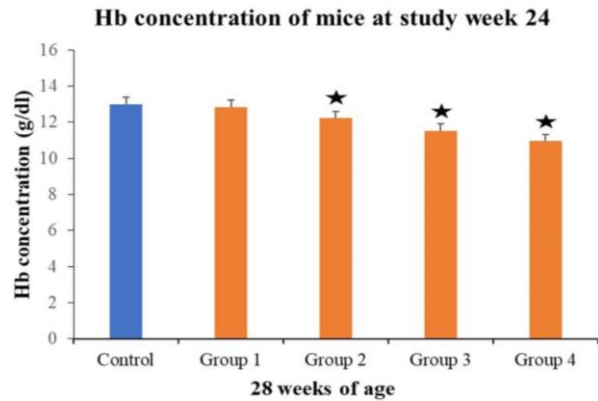
After research week 24, the main hematological changes were identified in HCT value and Hb concentration. Mice in Groups 2 ( $36.8\pm 1.34\%$ ,  $12.23\pm 0.17$  g/dL), 3 ( $33.5\pm 0.70\%$ ,  $11.53\pm 0.34$  g/dL), and 4 ( $32.98\pm 0.22\%$ ,  $10.95\pm 0.24$  g/dL) had significantly ( $P<0.05$ ) lower mean HCT values (Figure 2) and Hb concentrations (Figure 3) compared to the control group ( $40.65\pm 1.81\%$ ,  $12.985\pm 0.39$  g/dL). There was no substantial difference between TEC, TLC, and DLC.



**Figure 2.** Effects of formalin on HCT value in mice. Values are expressed as mean±SD. Significant ( $P<0.05$ ) differences are indicated with one asterisk.

According to a prior study, mice treated with formalin had considerably decreased amounts of RBC, HGB, MCHC, and HCT (Farshad *et al.*, 2023). Furthermore, formalin treatment has been shown to significantly reduce RBCs, Hb, MCHC, total WBC, lymphocyte, and basophile levels, as well as WBC phagocytosis

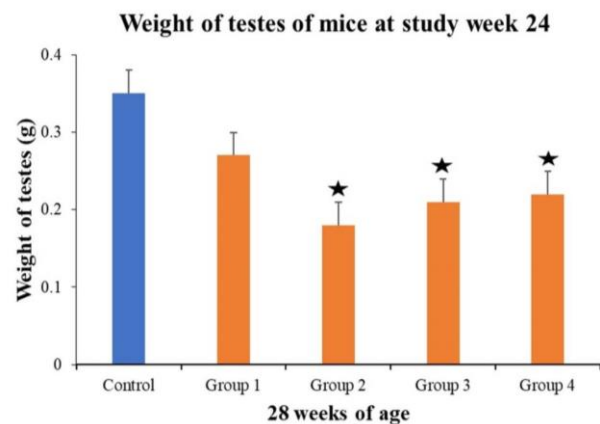
and lysozyme activity. In mice, however, MCV, PCV%, and reticulocyte levels increased dramatically (Abd-Elhakim *et al.*, 2016).



**Figure 3.** Effects of formalin on Hb concentration in mice. Values are expressed as mean±SD. Significant ( $P<0.05$ ) differences are indicated with one asterisk.

#### Effects on Weight of Testes

Compared to the control group ( $0.35\pm 0.005$  g), the weight of testes decreased significantly ( $P<0.05$ ) in Groups 2 ( $0.18\pm 0.025$  g), 3 ( $0.21\pm 0.02$  g), and 4 ( $0.22\pm 0.021$  g) (Figure 4). A significant ( $P<0.05$ ) drop in testes weight was found in mice from groups 2, 3, and 4 (Figure 4), which is comparable with findings reported by other authors for male rats (Tang *et al.*, 2003; Hegazy *et al.*, 2017).



**Figure 4.** Results of effects of formalin on weight of testes of male mice.

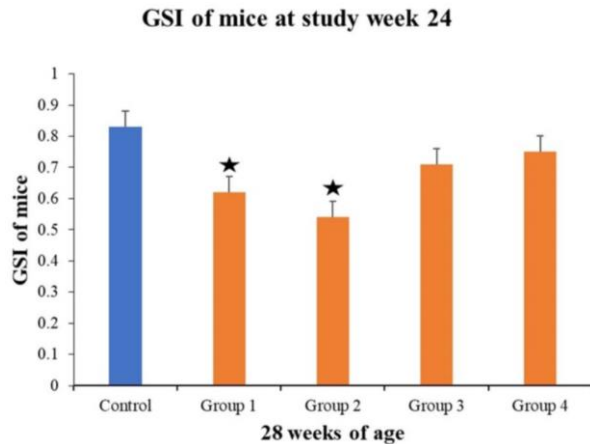
Values are expressed as Mean±SD. Significant ( $P<0.05$ ) differences are indicated with one asterisk.

#### Effects on Gonadosomati Index

After research week 24, all formalin-treated mice had



lower GSI than the control group (Figure 5). Groups 1 ( $0.62\pm 0.01$ ) and 2 ( $0.54\pm 0.01$ ) had significantly ( $P<0.05$ ) lower GSI levels. Groups 3 ( $0.71\pm 0.02$ ) and 4 ( $0.75\pm 0.03$ ) showed a nonsignificant reduction in GSI compared to the control group ( $0.83\pm 0.07$ ) (Figure 5).



**Figure 5.** Results of effects of formalin on GSI of male mice.

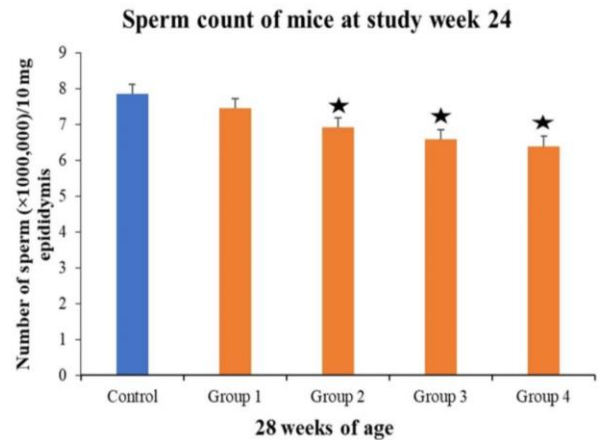
Values are expressed as Mean±SD. Significant ( $P<0.05$ ) differences are indicated with one asterisk.

#### Effects on Total Sperm Count

In week 24, all formalin-treated groups had lower epididymal sperm counts ( $P<0.05$ ) than the control group (Figure 6). In formalin-treated mice, sperm from the left cauda epididymis (10 mg) had significantly ( $P<0.05$ ) lower total sperm counts in Groups 2 ( $6.92\pm 0.11\times 10^6$ ), 3 ( $6.58\pm 0.13\times 10^6$ ), and 4 ( $6.39\pm 0.24\times 10^6$ ) than in the control group ( $7.85\pm 0.19\times 10^6$ ) (Figure 6).

Among the mice, those exposed to the highest concentration of formalin (20%) showed greatest drop in sperm count. Furthermore, formalin-treated mice had fewer morphologically healthy epididymal sperm than the control group. Several studies have reported that formalin has a severe noxious effect on the reproductive system of male rats, reducing semen quality, sperm count, motility, viability, and integrity (Majumder and Kumar 1995), as well as increasing the number of morphological abnormalities in sperm such as a decrease in the number of Leydig cells, suppression of spermatogenesis, and degeneration and calcification of testicular tissue (Sharma *et al.*, 1970). It has also been found that formalin significantly reduces sperm cell count, vitality, and

progressive motility while increasing the percentage of defective sperm (Vosoughi *et al.*, 2013). Other dietary additives have the negative impact of being reproductive toxicants, which causes reproductive toxicity.



**Figure 6.** Results of effects of formalin on sperm count of male mice.

Values are expressed as Mean±SD. Significant ( $P<0.05$ ) differences are indicated with one asterisk.

#### Effects on Sperm Morphology

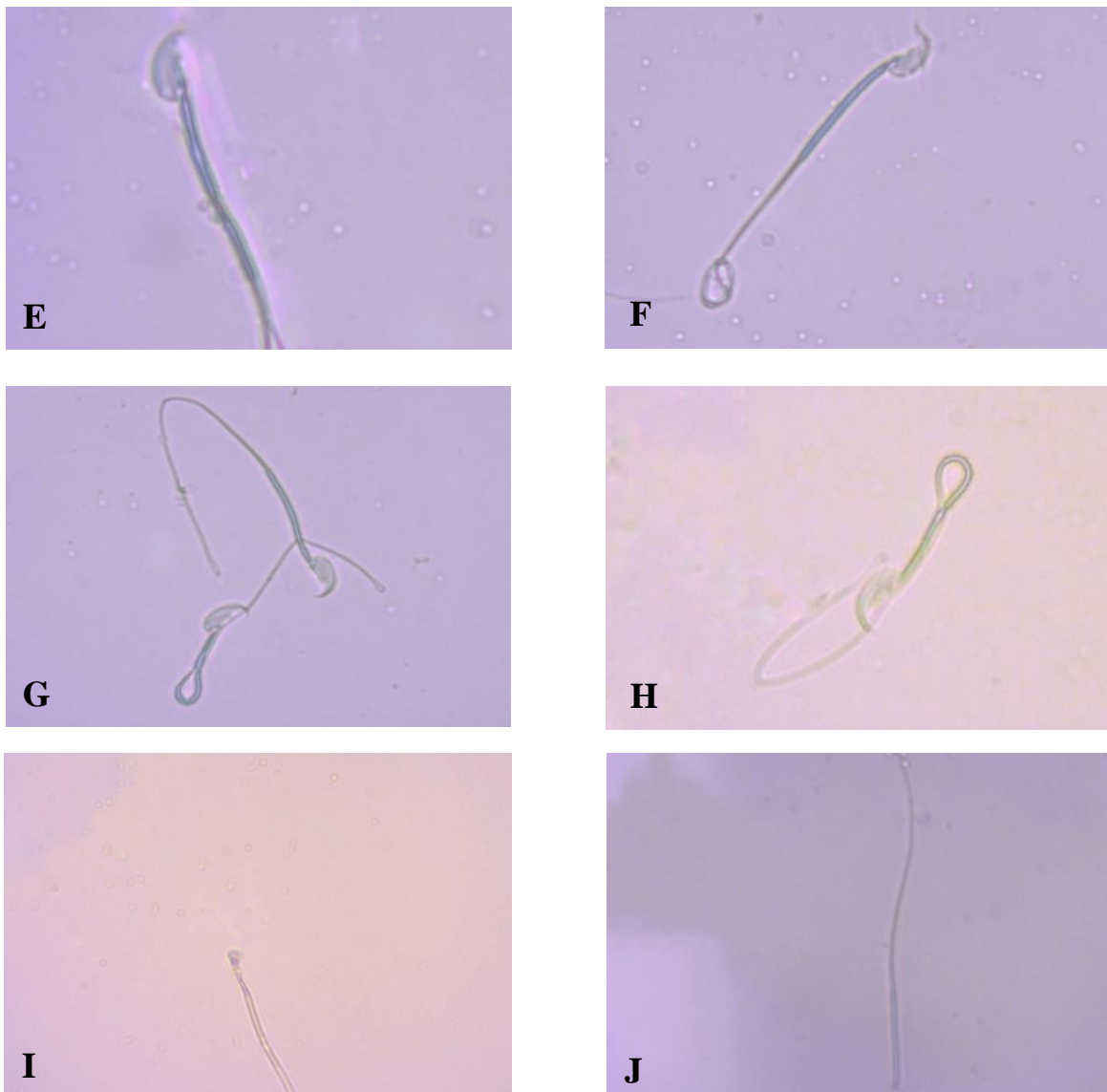
The percentage of morphologically healthy epididymal sperm was expressively reduced compared to the control group (Table 1) following 24 weeks of watering of mice with formalin (Table 1). It is also seen from the same table that mice in Groups 3 ( $59.0\pm 3.74\%$ ) and 4 ( $58.25\pm 2.22\%$ ) showed the greatest decrease in healthy sperm count compared to the control group ( $72.0\pm 3.5\%$ ). The increased count of defective sperm in the epididymis of formalin-treated mice included distal cytoplasmic droplet (Figure 7B), translocated cytoplasmic droplet (Figure 7C), amorphous head (Figure 7D), proximal cytoplasmic droplet (Figure 8E), coiled tail (Figure 7F), bent tail (Figure 7G), super coiled mid-piece (Figure 7H), microcephaly of the head (Figure 7I), and headless (Figure 7J). Figure 7(A) depicted a healthy sperm. The majority of morphologic abnormalities detected in the sperm of formalin-treated mice were distal cytoplasmic droplet, proximal cytoplasmic droplet, filamentous tail, and coiled tail (Table 1).

In Group 4, faulty sperm count was mainly made up of filamentous tails ( $4.75\pm 0.5\%$ ), coiled tails ( $4.5\pm 1.15\%$ ), and distal cytoplasmic droplets ( $4.25\pm 0.96\%$ ).

**Table 1.** Effects of formalin on the morphology of sperm in formalin-treated and control mice

Characteristics	Group 1	Group 2	Group 3	Group 4	Control
Healthy sperm (%)	69.75±1.83	64.75±2.75	59.00±3.74	58.25±2.22	72.00±3.50
Distal cytoplasmic droplet (%)	3.75±0.96	3.75±1.26	3.75±0.96	4.25±0.96	3.25±1.26
Translocated cytoplasmic droplet (%)	2.00±0.82	2.25±0.50	3.75±0.50	4.75±1.26	1.75±0.50
Filamentous tail (%)	3.25±0.82	4.75±0.10	4.75±1.71	4.75±0.50	3.75±1.26
Proximal cytoplasmic droplet (%)	3.50±0.96	3.50±0.96	4.75±0.96	4.25±1.00	2.75±0.10
Coiled tail (%)	3.75±0.96	3.00±1.15	4.00±1.71	4.50±1.15	3.75±0.10
Microcephaly (%)	2.25±0.82	2.75±1.26	3.25±0.96	2.50±1.26	1.75±0.58
Pyriform head (%)	2.00±0.50	3.00±0.82	4.25±0.50	4.75±1.26	2.25±0.50
Supercoiled midpiece (%)	1.75±1.26	2.75±1.29	3.75±0.82	3.50±1.26	1.50±0.29
Bent tail (%)	3.00±1.15	3.50±1.50	3.75±1.26	3.25±1.29	2.75±1.50
Others (%)	4.25±0.50	4.25±0.58	4.50±0.58	4.25±1.50	4.50±0.58





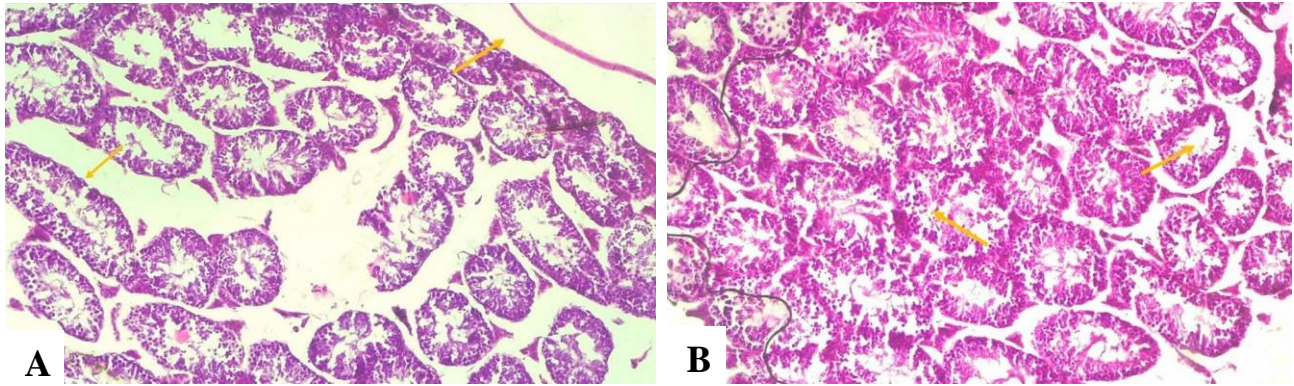
**Figure 7 (A–J).** Quantification of the level of morphologically defective sperm present in the cauda epididymis of formalin-treated male mice following 24 experimental weeks (20x). The increased count of defective sperm in the epididymis of formalin-treated mice were distal cytoplasmic droplet (B), translocated cytoplasmic droplet (C), amorphous head (D), proximal cytoplasmic droplet (E), coiled tail (F), bent tail (G), super coiled mid-piece (H), microcephaly of head (I), and head less (J). Figure A showed a healthy sperm.

#### *Gross and Histopathological Changes of Testes*

Grossly, there were no abnormalities in the testes, with the exception of a reduction in testes size in the formalin-treated group over the control group. The mice in Groups 3 and 4 exhibited the biggest loss in testes size when compared to the other groups; these two groups were given the highest dose of formalin.

There were no notable histopathological alterations

found in the control group's testicular sections. However, differences in testicular histopathology were seen in mice from Groups 2, 3, and 4. There were degenerative alterations in the seminiferous tubule. Furthermore, the formalin-treated group of mice had less spermatogenic cells, more spermatozoa with exudates in the tubular lumen, and seminiferous tubules were displaced from the basement membrane (Figure 8).



**Figure 8 (A–B).** Effects of formalin on histoarchitecture of male gonad (testes) of Swiss albino mice. The figures showed elongated seminiferous tubules and displacement of seminiferous tubule from basement membrane (A), and accumulation of spermatozoa with exudates in tubular lumen and degeneration of seminiferous tubules (B) (H&E, x10).

Previous studies reported that the rat and mouse testes have functional glutamate transporters and receptors (Gill *et al.*, 2000; Takarada *et al.*, 2004; and Hu *et al.*, 2004). Mono-Sodium glutamate (MSG) is a dietary supplement that is believed to target the testes. Another study found that MSG directly altered seminiferous tubule epithelial cells via glutamate receptors and transporters (AL-Sharkawy *et al.*, 2017). Furthermore, it was reported that the seminiferous tubules shrank in size and had a narrow lumen; additionally, the germinal epithelium lining the tubules showed no signs of spermatogenesis or necrosis; and the production of massive sperm cells in the male testes was caused by a food preservative called Sodium MetaBisulfite (AL-Sharkawy *et al.*, 2017).

### Conclusions

This study examined the effects of formalin on Swiss albino mice. The results showed that the mean HCT value and Hb concentration were significantly lower in all formalin-treated animals. In terms of male mouse sperm parameters, the formalin-treated group had considerably lower testes weight, GSI, and sperm count than the control group. The largest reduction was observed at increasing formalin concentrations. Grossly, no alterations were found in the gonads of male mice, with the exception of a reduction in size in the formalin-treated group when compared to the control group.

In the formalin-treated group of mice, histopathology

indicated degenerative alterations in seminiferous tubules, a reduction in the number of spermatogenic cells, the buildup of spermatozoa with exudates in the tubular lumen, and the displacement of seminiferous tubules from the basement membrane. The findings of this study show that formalin has a negative impact on hematological profile and testicular histoarchitecture, which might be used to improve both human and animal health by raising public knowledge of the repercussions.

### Acknowledgements

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### Declaration

There are no declared conflicts of interest involving the authors. The content of the paper has been read and approved by each co-author, and there are no financial conflicts of interest to report. We certify that the material is wholly original with no current considerations from other publishers.

### Authors' Contributions

MTI conceived the idea, designed the experiments, reviewed and edited the manuscript; MTI, FK and ANMAR supervised the study; MTH performed the sample collection, laboratory experiment and drafted the first version of the manuscript; SA and DR



performed the sample collection and laboratory experiment; AKT participated in the idea development, analyzed data and edited the manuscript; UH, FK, MNH, ANMAR and MGH reviewed and edited the manuscript. The completed manuscript has been read and approved by all authors.

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