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ELUCIDATING THE EFFECTS OF FORMALIN AS FOOD PRESERVATIVE ON GROSS AND HISTOARCHITECTURE OF VITAL ORGANS OF SWISS ALBINO MICE

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ABSTRACT

Formalin usage as food preservative in Bangladesh has dramatically increased recently, especially in fish, fruits, and many other food products, putting the vast majority of people in serious health risk. With this background, the study was aimed to detect the alterations of gross structure and histoarchitecture of vital organs in Swiss albino mice due to the consumption of formalin. Mice were divided into five groups at the age of four weeks. Normal drinking water was supplied to the control group of mice. Mice of group 1, 2, 3 and 4 were supplied normal drinking water mixed with formalin at 1%, 5%, 10% and 20% concentrations, respectively up to week 24. Mice treated with varying concentrations of formalin for 24 weeks was assessed for the weight change of their liver, heart, kidney and lung weights. Investigations were also conducted on histological alterations in the liver, heart, kidney and lung. After 24 weeks of treatment of mice with different concentrations of formalin, the weight of all four major organs (liver, heart, kidney and lung) was decreased in all formalin-treated mice in comparison to control mice. Petechial hemorrhages, congestion, necrosis, and parenchymatous cell degeneration have been found in liver. Kidney sections have shown decreased Bowman's space, hypertrophied glomerulus and tubular dilatation. Leucocyte infiltration, necrosis, and rupture of connective tissue have been seen in the heart sections. Lung sections have shown pulmonary alterations, including hemorrhages, thicker alveolar walls, and inflammatory cells. It was, concluded that formalin has adverse effects on vital organs of Swiss Albino mice.

Keywords: Formalin, food preservatives, toxicity, Swiss albino mice

Introduction

Formalin is a cadaverous chemical that is composed of 37–40% dissolved formaldehyde (FA) in water in an aqueous solution (Kawamata and Kodera 2004). It easily polymerizes colorless gas at room temperature and pressure, is flammable, and is strongly reactive with a wide range of chemicals (WHO 2006, Wilbur *et al.* 1999). It interacts with different biological components to quickly diffuse in any tissues, including the liver, when taken orally or intraperitoneally (Smith 1992). In addition to air exposure, which is the main way that FA enters the body, it can also enter through food and water consumption (NRC 1980). Although formalin was originally used as an embalming fluid and fixative, it is now used in many different facets of daily life. Since formalin is most commonly used as a food preservative, there is an increasing health risk of formalin poisoning (Restani *et al*. 1992, Tomkins *et al*. 1989). Long-term ingestion 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, liver and kidneys (Wahed *et al*. 2016, Mamun *et al*. 2014). Following ingestion, formalin is readily absorbed from the digestive tract (Research Triangle Institute and Disease Registry 1999). Recent studies have shown that animal exposure to formalin can cause hepatotoxicity (Rumchev *et al*. 2002). Formalin is carcinogenic and mutagenic, even at low concentrations, and it is detrimental to a wide variety of species (Nouh and Selim 2013).

The most crucial organs for survival are thought to be the vital organs. Maintaining the health of one's key organs is essential. Liver, lungs, brain, heart, and kidneys of mice are their essential organs. Any anomaly in these organs will have an impact on overall health, which results in significant financial losses for the production of animals. Any type of pathological state affecting the organs suggests that the organs and systems, together with all of the microorganisms that live there, are diseased (Kelly 1985).

A significant number of people in developing countries like Bangladesh are at considerable risk for serious health problems due to the unregulated use of formalin in various food products and drinking water (Smedje and Norbäck 2001, Franklin *et al*. 2000). Bangladesh is a tropical country. Its climate is hot and humid. As a result, perishable goods like vegetables are more likely to expire quickly. Formalin use in fish, fruits, and many other food items has dramatically escalated in Bangladesh in recent times, putting the vast majority of the people at serious danger for health problems (Mamun *et al*. 2014). However, very few research papers have been published to investigate the effect of formalin as a food preservative on histoarchitecture of vital organs of living individuals. There is a lack of information about the effects of formalin as a food preservative on the vital organs of mice. Therefore, this research work was performed to investigate the effects of formalin as a food preservative on gross and histoarchitecture of vital organs of Swiss albino mice that occur due to drinking of formalin.

Materials and Methods

Chemicals: The chemical compound known as formalin, which makes up between 37 and 50 percent of an aqueous solution of formaldehyde (Kawamata and Kodera 2004), is typically used in the production of textiles, plastics, papers, paint, and construction materials. It is also well known for its ability to preserve human remains. For this experiment, formalin (RCI Labscan Ltd.®, Bankok, Thailand) was purchased.

Animals: Three weeks old specific pathogen-free (SPF) Swiss albino male mice (*Mus musculus*) were purchased from the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and were housed in the laboratory animal containment facility, department of Pathobiology, Faculty of Veterinary Medicine and Animal Science (FVMAS), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Mice were housed for a week before being used in this study so they could become adjusted to their new surroundings. At the age of four weeks, mice were randomly selected into five groups and ear-coded. Standard mice pellets were used as normal feed.

The mice were housed in compartmentalized rectangular plastic cages wrapped with wire mesh. The cages were kept in a well-ventilated room at 26 ± 2°C and a relative humidity of 70-80% with natural day and light. The mice were cared in proper hygienic conditions.

Experimental design: Twenty (20) male mice at the age of four weeks were randomly divided into five groups and were ear-coded, each group comprising four mice. Groups were marked as control, group 1, group 2, group 3 and group 4.

Standard mice pellets collected from icddr,b were used as normal feed. Normal drinking water was supplied to the control group of mice. Mice of groups 1, 2, 3, and 4 were supplied normal drinking water mixed with formalin at 1%, 5%, 10% and 20% concentration, respectively orally up to study week 24.

Clinical examination: During the experimental period, the mice were under close observation and they were examined very carefully. Their feed consumption and water intake were carefully recorded.

Sample collection: After completing 24 study weeks of treatment of mice with different concentrations of formalin, mice were euthanized with ketamine hydrochloride (Gonoshasthaya Pharmaceuticals Ltd.®, Dhaka, Bangladesh) as recommended dose (100mg/kg body weight) during sacrifice. During necropsy, different important organs (liver, heart, lung and kidney) were collected from the mice of both control and formalin-treated groups to investigate the effect of formalin. Weight of collected organs of each mouse was measured with the help of a digital balance.

Sample preservation: Following necropsy, the vital organs were collected as soon as possible using a sterile scalpel and scissors to avoid any damage to the organs. These organs were cut into slices and fixed in a 10% formalin solution.

Histopathological study: Histopathological examination was performed by the following procedure. Fixed tissue sections were processed, paraffin-embedded, sectioned and routinely stained with Hematoxylin and Eosin (H & E) stain (Biognost®, Zagreb, Croatia) as per standard procedure (Luna 1968).

Gross and histological study: The gross and histological studies were performed in the Pathology Laboratory of BSMRAU, Gazipur. The gross study was performed by visual observation. The average length and width of all the collected organs were also measured. Detailed histological studies were completed using low and high-power light microscopy.

Photomicrography: Photomicrography was taken using photomicrographic camera (ZEISS AxioCam ERc5s) facilities facilitated by the Department of Gynecology, Obstetrics and Reproductive Health of BSMRAU.

Statistical analysis: Finally, at the end of a histopathological study, all the data were compiled, compared, and analyzed for constructive interpretation. Statistical analysis was performed using SPSS (IBM@ Version 21.0, USA). All results were represented as the Means ± S.D. For the comparison, a one-way analysis of variance (ANOVA) was carried. Differences were considered to be statistically significant when the *p*-value was less the 0.05.

Results and Discussion

Weights of different vital organs including liver of the formalin-treated mice have been evaluated, and the obtained results have shown a significant change in the relative weight of these organs in the formalin-treated group in comparison to the control group (Table 01). Weight of the liver was decreased in all formalin-treated group of mice except mice of group 1 in comparison to the control group after 24 study weeks. Significant reduction of weight of the liver of mice was observed in mice of group 2 (1.70 ± 0.095 gm), group 3 (1.34 ± 0.035 gm) and group 4 (1.21 ± 0.008 gm) compared to the control group (2.49 ± 0.01 gm) (Table 1). The highest reduction in the weight of the liver was observed in group 4 (Table 1). According to the study, mice exposed to formalin had decreased liver weight as a result of their decreased body weight. Similar results were reported in mice treated with formalin (Afrin and Islam 2021).

The liver serves as the main organ in animal's detoxifying system. The body's intermediate metabolism of carbohydrates, proteins, lipids, and amino acids is mostly controlled by the liver. It has a number of metabolizing enzymes that are crucial to the biotransformation of

Table 01. Weight of liver, heart, kidneys and lungs after completing study week 24 (Mean ± S.D.)

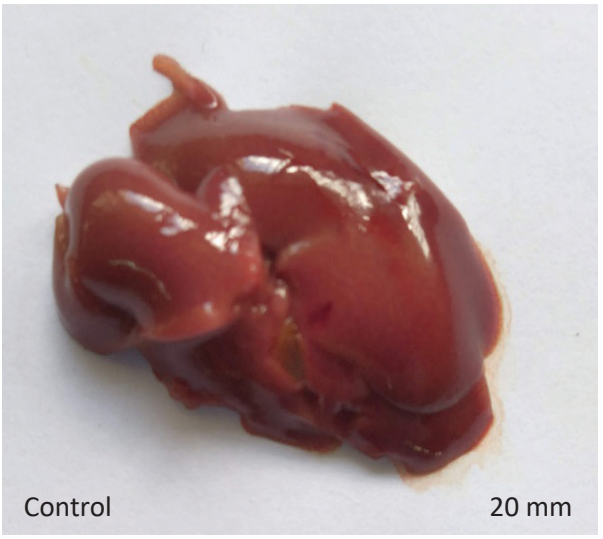
Organ at experimental week 24		Control	Group 1 (1%)	Group 2 (5%)	Group 3 (10%)	Group 4 (20%)
Liver	Weight (gm)	2.49±0.01	2.62±0.20	1.70±0.095	1.34±0.035	1.21±0.069
	Level of Significance		NS	*	*	*
Heart	Weight (gm)	0.31±0.01	0.33±0.021	0.28±0.024	0.26±0.037	0.27±0.008
	Level of Significance		NS	*	*	*
Kidneys (left + right)	Weight (gm)	0.49±0.02	0.54 ±0.11	0.45±0.02	0.47±0.01	0.48±0.024
	Level of Significance		NS	*	*	NS
Lungs	Weight (gm)	0.29±0.01	0.33±0.037	0.24±0.005	0.21±0.04	0.21±0.037
	Level of Significance		NS	*	*	*

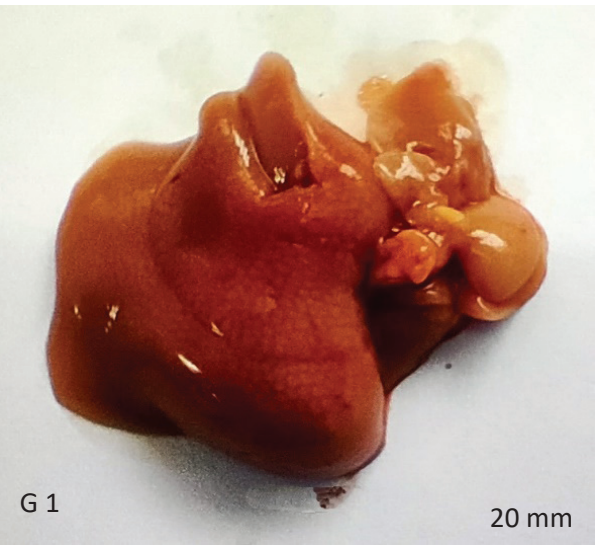
* Significant ($p<0.05$), NS = Non-significant ($p>0.05$).

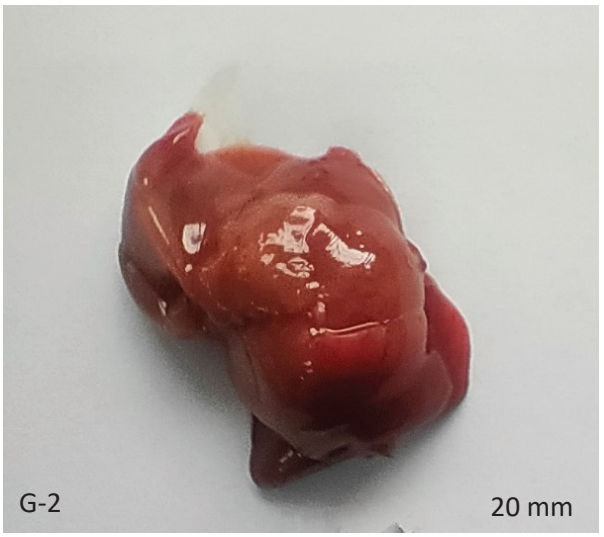
xenobiotics. Less or more harmful metabolites are produced as a result of this procedure (Sharif *et al.* 2016). Thus, oxidative stress in mouse tissue, which is likely caused by the toxic effects of formalin, may be responsible for the damage that has been detected in the liver and kidney. The gross morphological changes in the liver of formalin-exposed mice revealed a dark color with irregular surfaces (Figure 1) which was also found in other reports (Afrin and Islam 2021). Grossly formalin-treated groups, the existence of petechial hemorrhages, and liver congestion in mice point to an irritating impact of formalin (Figure 1). Mice and rats exposed to formalin orally developed similar liver abnormalities, according to reports (Afrin *et al*. 2016, Til *et al*. 1989, Tobe *et al*. 1989).

Weight of heart was decreased in all formalin-treated group of mice except mice of group 1 in comparison to the control group after study week. Significant reduction of weight of heart of mice was observed in mice of group 2 (0.28 ± 0.024 gm), group 3 (0.26 ± 0.037 gm) and group 4 (0.27 ± 0.008 gm) ($p < 0.05$) compared to control group (0.31 ± 0.01 gm) mice (Table 01).

Comparing the experimental group of mice to the control mice, size of the heart was significantly decreased in all experimental group of mice (Figure 2).







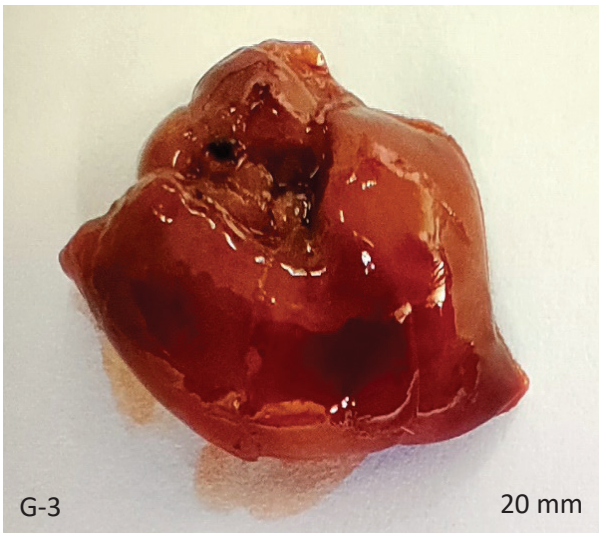
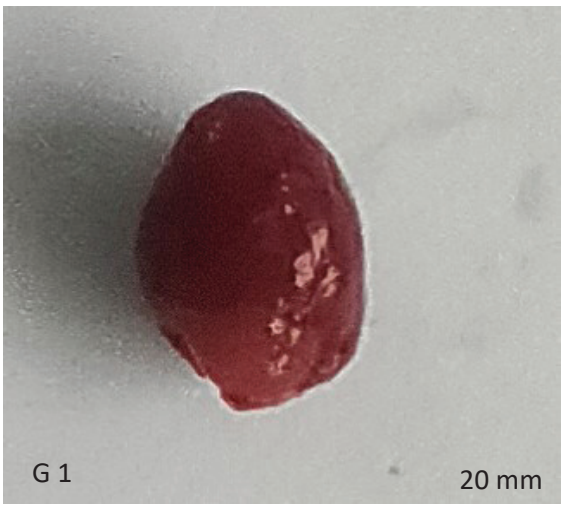
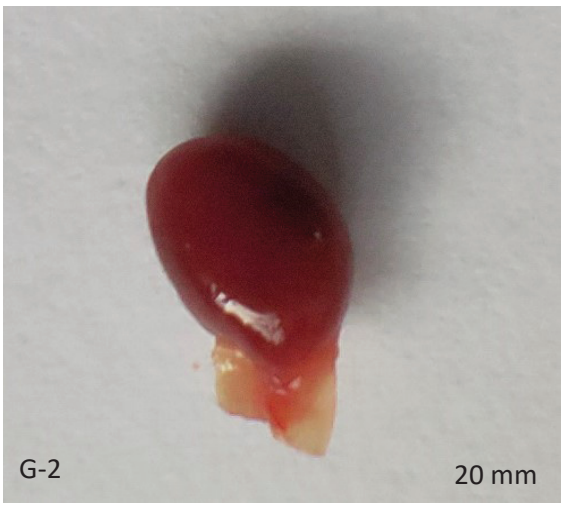


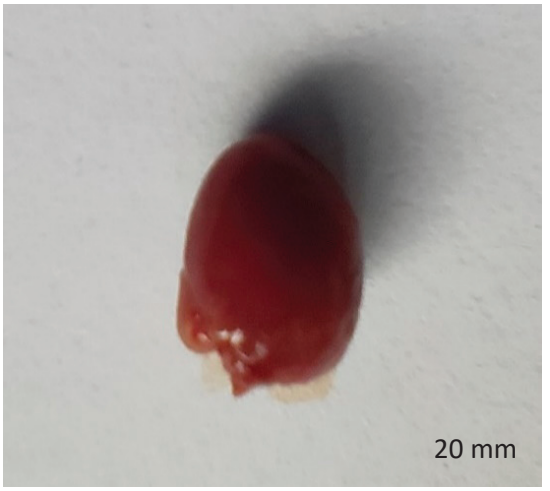


Figure 1. Liver of mice of different experimental group. Hemorrhagic & congested liver were found in the group 2, group 3 and group 4.









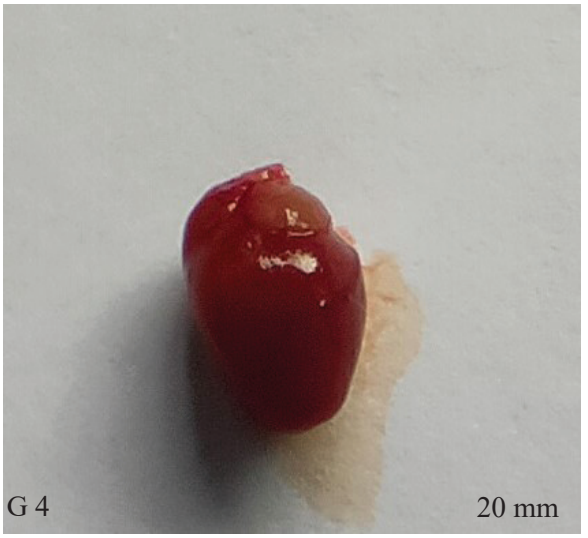


Figure 2. Heart of mice of different experimental group. Hemorrhage was observed on base of heart of group 2.

Weight of kidney was decreased in all formalin-treated mice except mice of group 1 in comparison to the control group after 24 study weeks. Significant reduction of weight of kidney of mice was observed in mice of group 2 (0.45 ± 0.02 gm) and 3 (0.47±0.01 gm) ($p < 0.05$) compared to control group (0.49±0.021 gm) mice (Table 01). Bowman's space in the kidney is reduced when formalin is taken orally (Figure 5). Similar results were reported in mice treated with formalin (Afrin and Islam 2021). This is a result of its strong dose-dependent metabolic reactivity. Previous experimental research has shown that oral administration of formalin to rats has harmful effects on their urinary systems. Rats exposed to formalin exhibited hematuria, anuria, metabolic acidosis, circulatory failure, and renal papillary necrosis (Til *et al*. 1989).

Weight of lungs was decreased in all formalin-treated mice except mice of group 1 in comparison to the control group after 24 study weeks. Significant reduction of weight of lungs of mice was observed in mice of group 2 (0.24 ± 0.005 gm), 3 (0.21 ± 0.04 gm) and 4 (0.21 ± 0.037 gm) ($p < 0.05$) compared to control group (0.29 ± 0.014 gm) mice (Table 01). The lung, which receives the full cardiac output, is a vital component of breathing. Additionally, the lung's wide surface of the pulmonary vascular bed contributes to host defense and the management of circulating amounts of biologically active chemicals (Fishman *et al*. 1998).

The mice in the control group exhibited normal brown coloration, consistency, and size of the liver lobes. Dorsal and ventral surfaces of the liver lobes were uniformly shaped and had smooth, consistent boundaries (Figure 1). Mice treated with a higher dose of formalin attained substantial morphological changes in their livers, including decreased weight, uneven surfaces, and black coloration (Figure 1). Increased congestion across the lobes and petechial hemorrhages on the ventral and dorsal surfaces indicated the liver's extensive vasculature (Figure 1). Mice belonging to group 4 have the most severe morphological changes in their liver, which include uneven surfaces, decreased weight, and a darker hue (Figure 1). The mice in group 4 exhibit the most severe morphological changes in their liver, which include a darker color, decreased weight, and uneven surfaces (Figure 1).It is believed that formalin produces cytotoxicity by directly interacting with tissue components (Kimbell *et al*. 2001). After intraperitoneal, oral, or inhaler administration, formalin rapidly diffuses into the liver, brain, and testis, among other organs (Chang *et al*. 1981). In the present investigation, oral formalin treatment causes hepatic parenchymatous cell degeneration, centrilobular necrosis, dilated sinusoidal gaps, and lymphocytic aggregations (Figure 5A). Another experiment also revealed lesions of this type (Afrin *et al*. 2016). Rats exposed to FA had mild edema, moderate hepatocyte degeneration, and hyperplasia of Kupffer cells

in their liver tissue (Uçmakli *et al*. 2013). Furthermore, the effects of formalin on hepatocytes and the intra- and extrahepatic bile ducts were evident (Dumont *et al*. 1996). Its metabolism-responsiveness is the reason for this. Formalin is found to be an essential metabolic bridge in all mammalian cells and to be involved in the synthesis of thymidine, purines, and certain amino acids (Neuberger 1981). Formalin dehydrogenase, a NAD-dependent enzyme, quickly transforms formalin into formic acid in the liver and blood through a glutathione (GSH)-dependent mechanism. Consequently, formic acid either partially enters the body's one-carbon pool or oxidizes further in the liver and erythrocytes to carbon dioxide and water. Primates exhibit this response more gradually than do dogs or rodents. The remaining non-metabolized formic acid and other minor metabolites are removed via urine, feces, or exhaled air (IARC 2006), and the proportionate amounts depend on the mode of administration (Galli *et al*. 1983, Upreti *et al*. 1987).

Grossly, hemorrhage was observed on base of heart of group 2 (Figure 2). Histologically, disruption of connective tissue has been observed as well as leucocyte infiltration and necrosis (Figure 5B). Intra myocardial spaces were increased in the sections of heart in formalin-treated group of mice, similar result was found previously (Wang *et al*. 2023). As an adaptive response to stress, the heart increases blood flow, strengthening the body's defenses against invasion (Ings *et al*. 2012). Thus, the heart is vital to the body's defense; but, several environmental pollutants may potentially disrupt or impair cardiac function. Exposure to formaldehyde has an impact on the development of hypertension (Wang *et al*. 2023). Leucocyte infiltration, necrosis, congestion, disruption, and destruction of connective tissue were all evident in the acquired results (Figure 5B). Fish heart tissues treated with hospital effluents, such as catfish, showed histological abnormalities such as necrosis and cellular infiltration (Afsa *et al*. 2021). Pharmacological effluent exposure has caused cellular degeneration and inflammatory cell infiltration in the endocardium of rodents (Sharif *et al*. 2016).

Grossly, size of the kidneys was decreased in all experimental group of mice in comparison to control group of mice (Figure 3). Histologically, in formalin treated groups the Glomeruli was found to be shrieked and ruptured (Figure 5C). Renal tubules have leucocytic infiltrations, degenerated tissue and congestion of renal glomerulus along with hemorrhage were also noticed (Figure 5C).

Following the systemic administration of formalin, abnormal glomerular patterns and expanded tubular and glomerular basal membranes were seen (Zararsiz *et al*. 2007). Moreover, it was demonstrated that systemic formalin treatment caused distal tubule dilatation, vacuolization, and intratubular vascular congestion (Zararsiz *et al*. 2007). They added that glomerular and tubular degeneration, as well as congestion and tubular dilatation, were detected following the injection of formalin. These severe nephrotoxic effects of formalin are demonstrated by these histological changes in the renal tissue. Grossly, compared to the control group of mice, the size of the lungs was reduced in all experimental groups of mice (Figure 4). There were no obvious lesions in the control group. Only the group treated with 20% formalin had hemorrhages visible in its lungs. However, no signs of necrosis or degeneration were found.

This study found similar pulmonary alterations, including hemorrhages, thicker alveolar walls, pulmonary blood vessel dilation, and inflammatory cells invasion (Figure 5D) that of the results of the lungs of rabbits after 40% formaldehyde (formalin) exposure (Bansal *et al*. 2011), also detected in rats (Sheela and Sreedevi 1991, Kamata *et al*. 1996, Turkoglu *et al*. 2008), mice (Guangyan *et al*. 2004) and in children (Casset *et al*. (2006). In case of all four organs, the histopathologic changes were severe in the highest concentrated formalin-treated mice. The widespread use of formalin in the preservation of fish, fruit, and other food products puts public health at danger. These days, food is preserved using this chemical, which is commonly used to stop dead bodies from decomposing. Formalin consumption has both immediate and long-term health hazards. Consuming formalin-contaminated food over an extended period of time can lead to several detrimental health consequences, such as cancer and problems with the heart, intestines, respiratory system, brain system, and nephrology (Wahed *et al*. 2016, Mamun *et al*. 2014).

Conclusion

The study was conducted to examine the toxic effects of formalin, and the gross and histological changes in the vital organs (liver, kidneys, lungs and heart) of Swiss albino mice. Results revealed that in comparison to the

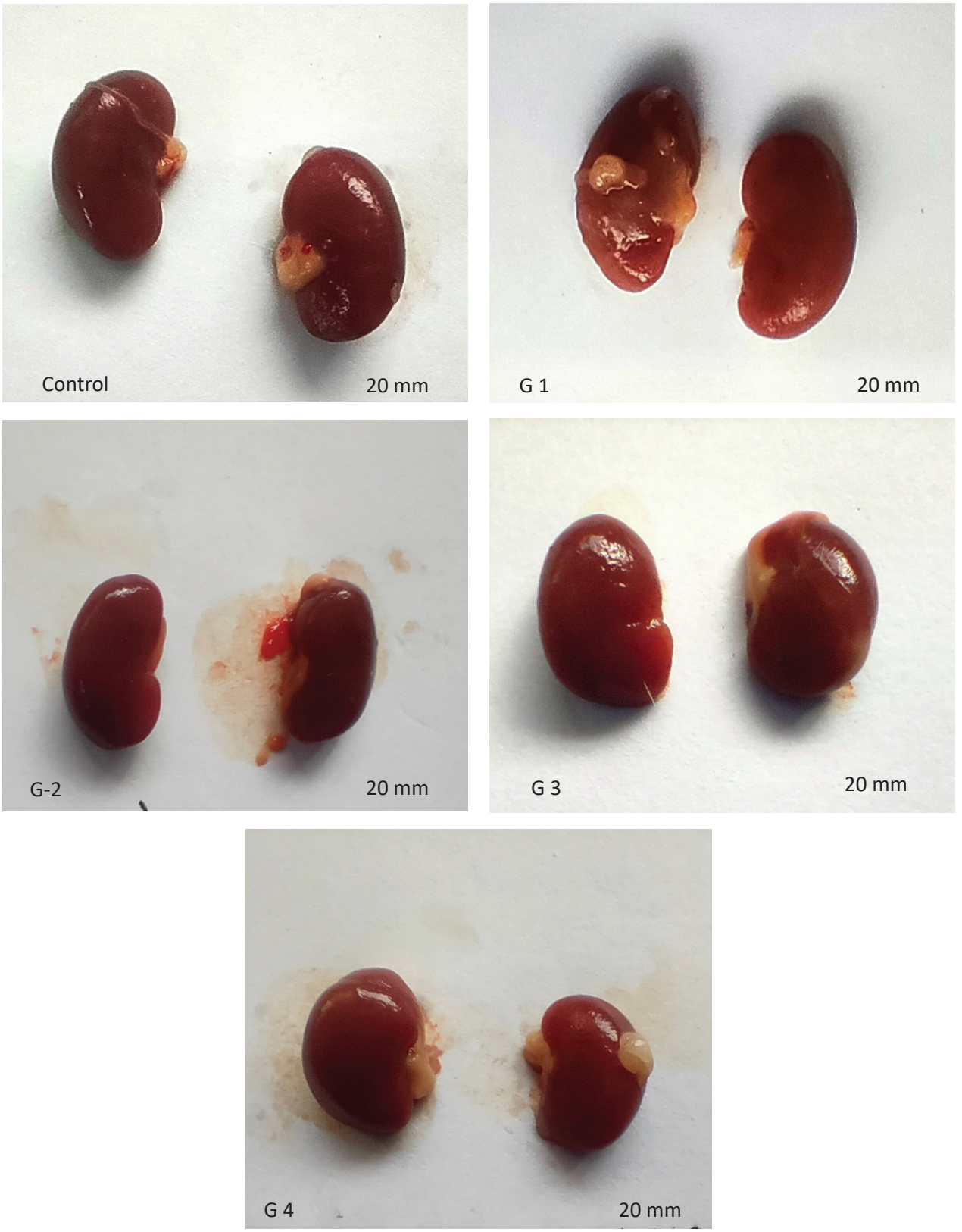


Figure 3. Kidneys of mice of different experimental group. Congested kidney was found in group 4.

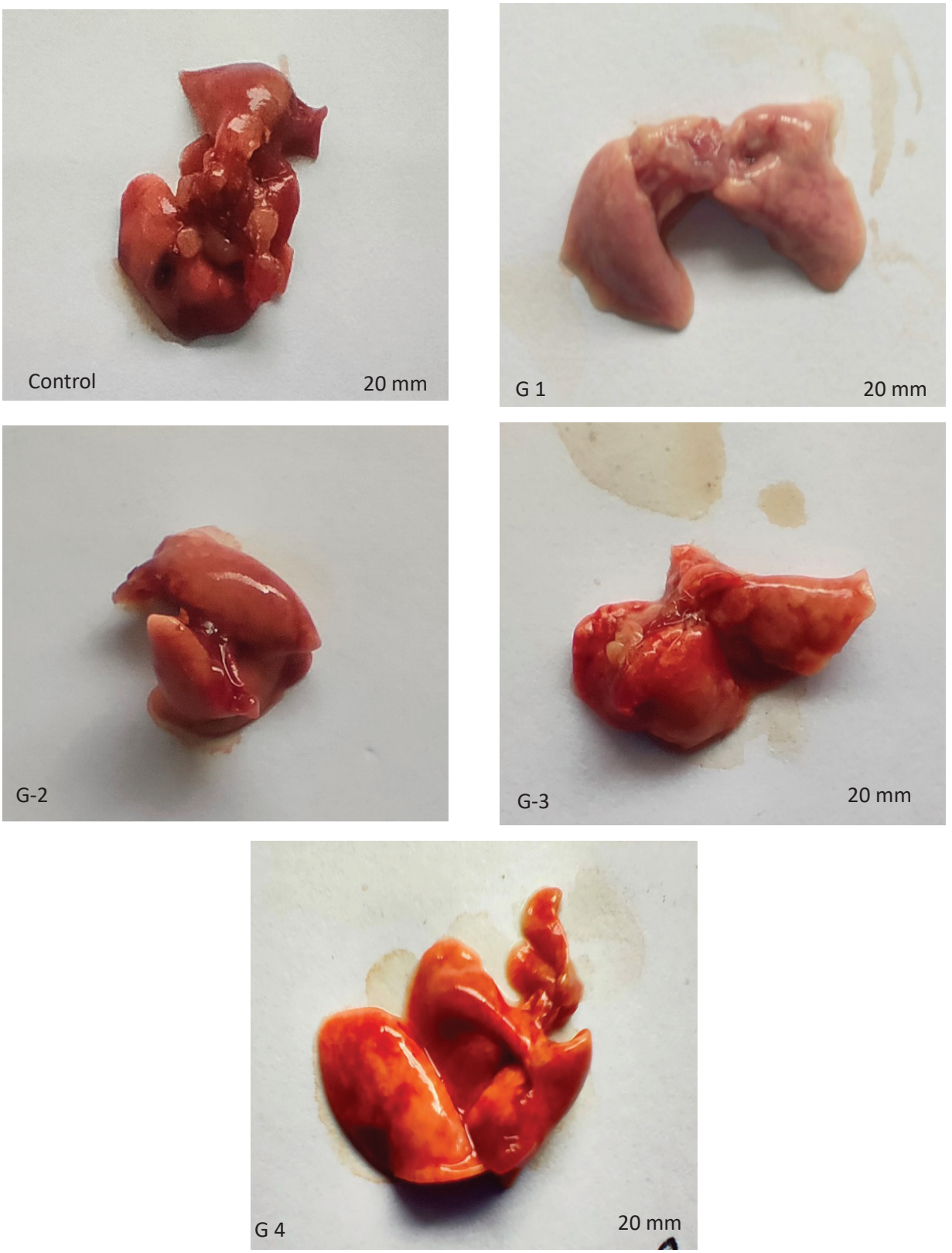


Figure 4. Lungs of mice of different experimental group. Congested lobe was found in group 2, group 3 and group 4.

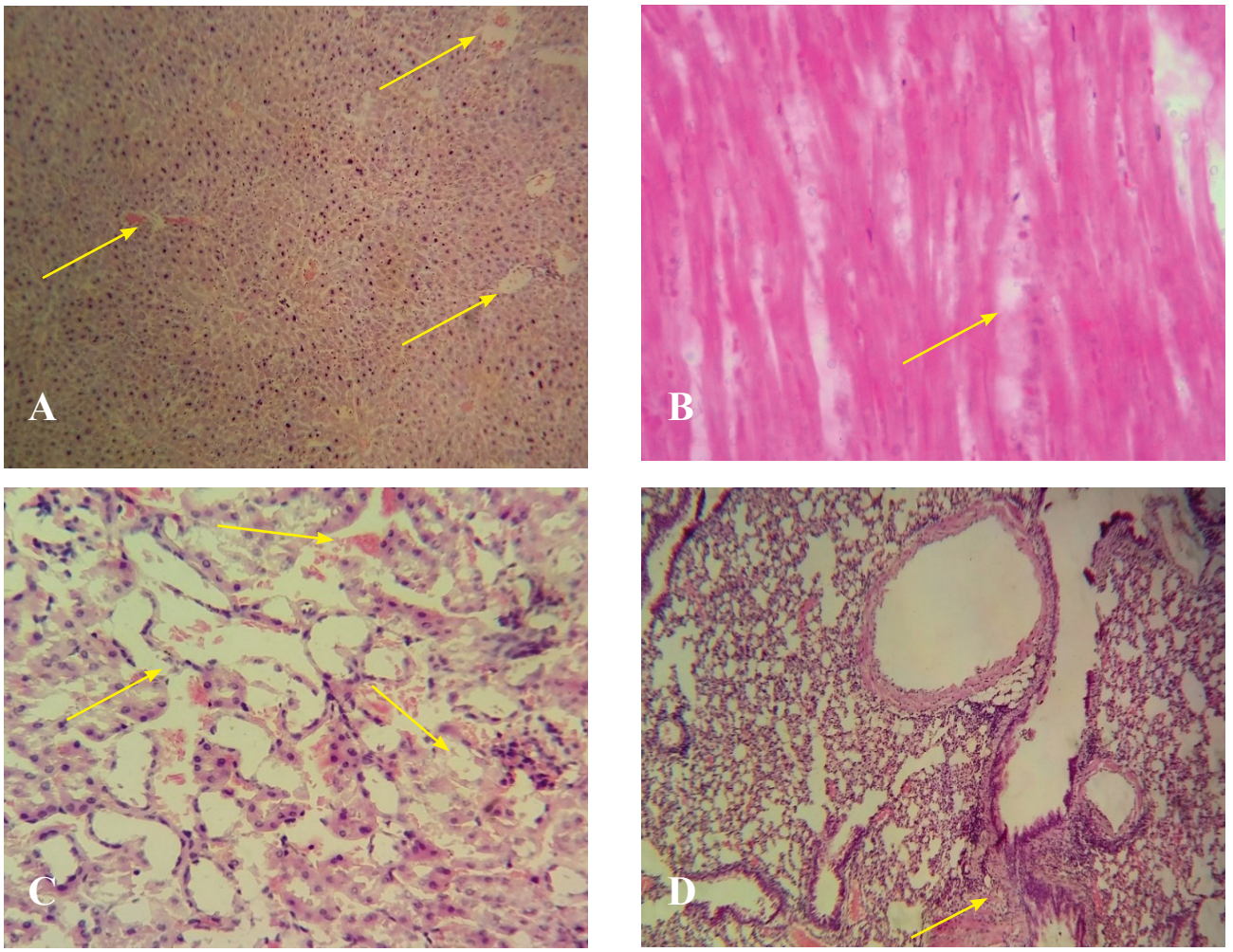


Figure 5. A. Histology of liver of mice. Congestion in the central vein, necrosis of hepatocyte, hepatic sinusoidal dilatation was observed (H & E, X 10); B. Histology of heart of mice. Increased intra myocardial spaces; C. Histology of kidney of mice. Hypertrophied glomerulus and tubular dilatation were observed; D. Histology of lungs of mice. Congestion in the lobes were observed; (H & E, X 40).

control group, the weight of the four main organs namely liver, lungs, heart and kidneys were significantly reduced in all formalin-treated mice. We also observed gross and histopathological changes in those organs, particularly liver and kidneys have shown more alterations. With an increase in concentration, the harmful effects of formalin become more obvious.

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Ethics approval

The study on mice experimentation was performed under the guidance of the Animal Research Ethics Committee, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (FVMAS/AREC/2023/15).

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