ASIA PACIFIC JOURNAL of MEDICAL TOXICOLOGY
APJMT 12;4 http://apjmt.mums.ac.ir December 2023

ORIGINAL ARTICLE

Effects of Thirty-Day Exposure of Quails (Coturnix Coturnix) to Formalin Vapor on Biochemical, Immunological, and Histological Features of Liver and Kidney

EMAN F. ALBAGHDADY[1], EMAN IBRAHIM AL HACHEM[1], ABDULRAZZAQ B KADHIM[1*]

[1]Department of Anatomy and Histology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

Abstract

Background: Formalin is a highly toxic chemical that causes tissue damage. Formalin is metabolized by the liver and erythrocytes and excreted via the urinary and digestive tracts. This passage may propose toxicity in these systems. Thus, the current study was conducted to examine serological, and histological changes in the kidney and liver of formalin-vapor-exposed quails (FVEQs) (*Coturnix coturnix*) to promote awareness of formalin toxicity in the farms of Japanese quails.

Methods: The study included the recruitment of 16 adult quails divided into four equal groups; control group (no formalin exposure), FVEQ1 group (exposed to formalin for 10 days), FVEQ2 (exposed to formalin for 20 days), and FVEQ3 (exposed to formalin for 30 days). All FVEQs were exposed to formalin vapor twice daily (morning and evening, 2hrs/each). Each group was reared in a separate airtight chamber during the experiment time (10, 20, or 30 days). blood samples for renal and liver, IgG levels, and histopathological features were examined.

Results: The findings showed significant (p<0.05) increases in the levels of blood creatinine, urea, and uric acid in the FVEQ groups. Liver enzymes, ALP, ALT, and AST revealed significant (p<0.05) increases in the FVEQ groups. FVEQ1 group recorded significantly (p<0.05) the highest IgG level after 10 days of formalin exposure, which significantly (p<0.05) decreased after 10 days without formalin exposure, and immunity was slightly restored. The histopathological characteristics of the kidney were increases in the thickness of renal corpuscles, glomeruli, and the Henle loop of the medullary cone. For the liver, the findings showed increases in the parenchymal aggregation of lymphoid cells. There were amyloid depositions in hepatocyte spaces, which were squeezed and atrophied, creating intercellular gaps and reducing cell compactness.

Conclusion: The inhalation of formalin vapor may damage the kidney and liver tissues and eventually their functioning in formalin-exposed quails.

Keywords: Formalin, Toxicity, Quails, Liver, Kidney

How to cite this article: Albaghdady EF, Al Hachem EI, Kadhim AB.Effects of Thirty-Day Exposure of Quails (Coturnix Coturnix) to Formalin Vapor on Biochemical, Immunological, and Histological Features of Liver and Kidney. *Asia Pac J Med Toxicol 2023; 12(4):137-144.*

INTRODUCTION

Formalin exhibits the properties of being flammable, lacking odor, possessing toxicity, and displaying high solubility in water. Formaldehyde is commonly found in various sources such as cigarette smoke, emissions from automobiles, fuel oil, natural gas, exhaust from vehicles powered by fossil fuels, furniture, and the fumes emitted by chipboard paint. Disinfectants and sterilizers are commonly employed by educational institutions, higher education establishments, as well as biological, forensic, and pathological laboratories to safeguard tissues, surgical specimens, organs, and viscera [1]. Hence, formaldehyde is classified as a carcinogenic substance due to its propensity to induce cancer in animals, particularly in the nasal region, when exposed to elevated levels over an extended period. [2].

Occupational exposure to formaldehyde has been associated with multiple factors, such as the dosage and concentration of formalin, insufficient ventilation, suboptimal temperature, and inadequate relative humidity levels [3]. The inhalation of formalin has been observed to impact plasma proteins, leading to their conversion into formic acid by the liver and red blood cells. This process has been associated with the development of severe toxicity and increased mortality rates. The presence of formic acid in the bloodstream leads to the development of a significant metabolic acidosis, while also impairing liver function through the inhibition of cholinesterase, succinate oxidation, and anaerobic glycolysis [4].

Formic acid induces tissue hypoxia and elevates acid burden through its inhibitory effect on mitochondrial cytochrome c oxidase [5, 6]. The livestock industry employs formalin mists and fumigants as means of disinfecting, incubating eggs, and sterilizing equipment to mitigate the risk of disease and minimize the presence of bacterial and parasitic organisms [7,8,9,10]. In order to mitigate the presence of pathogens, the United States allows for a maximum formalin content of 2.5 kilograms per ton in

*Correspondence to: Abdulrazzaq Baqer kadhim, PhD, Department of Anatomy and Histology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.
Tel: 07812352090, Email address: abdulrazzaq.alrabei@qu.edu.iq

poultry feed [11]. It was reported that broilers at eight weeks old, revealed reductions in feed intake and body mass, occurrence of local necrosis, as well as crop and intestinal hemorrhage after the administration of formalin in feed at different concentrations [12, 13].

The primary aims of this study was to examine serological, and histological changes in the kidney and liver of formalin-vapor-exposed quails (FVEQs) to promote awareness of formalin toxicity in the farms of Japanese quails.

MATERIALS & METHODS

Experimental procedure

The study was carried out at the Department of Anatomy and Histology, Veterinary College, University of Al-Qadisiyah. The study was performed from September to November 2022, adhering to international standards for the care and use of animals. The quails were supplied in the Animal House facility located at the abovementioned institution. The current study was conducted to examine serological, and histological changes in the kidney and liver of FVEQs to promote awareness of formalin toxicity in the farms of Japanese quails. The study included the recruitment of 16 adult quails divided into four groups; control group (no formalin exposure), FVEQ1 group (exposed to formalin for 10 days), FVEQ2 (exposed to formalin for 20 days), and FVEQ3 (exposed to formalin for 30 days). All FVEQ groups were exposed to formalin vapor twice daily (morning and evening, 2hrs/each), as 37% formalin was dissolved in water. Each group was reared in a separate airtight chamber during the experiment time (10, 20, or 30 days). The subjects were kept under controlled laboratory conditions, specifically at a temperature range of 25-30°C, with unrestricted access to food and water. At the end of the exposure periods, the birds were euthanized, and blood from heart, kidney, and liver tissues were collected.

Liver enzyme profile

The test tubes containing the blood were positioned at an inclined angle and left at a temperature of 32°C for 30 minutes. The serum was isolated from the coagulated blood through a centrifugation process at a speed of 3000rpm for 20 minutes, followed by an additional centrifugation at the same speed for 10 minutes. The supernatant was subsequently collected using a micro-pipette, and transferred into an Eppendorf tube. The tube was then stored at -20°C. In this study, serum samples were employed to assess the hepatic and renal function, specifically measuring the levels of Aspartate aminotransferase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP). The analysis was conducted using the Beckman Coulter AU480 and Biorad D10 instruments, both of which are manufactured in California, United States. The concentrations of urea, creatinine, and uric acid were determined using UV kinetic methodology in commercially available kits (Human Gesellschaft for Biochemical und Diagnostics mbH, Germany). These kits effectively detected variations in levels of liver enzymes, as well as creatinine, urea, and uric acid. Furthermore, the serum samples from the four experimental groups were employed to assess the concentration of quail IgG using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Zeptomatrix, United States).

Histopathological examination

After the administration of deep chloroform anesthesia, the internal visceral organs, such as the liver and kidney, were spotted via the ventral mid-line thoracoabdominal incision. Subsequently, the liver and kidney specimens were excised and promptly immersed in a 10% neutral buffered formalin (NBF) solution for a duration of 48 hours, in accordance with established histological protocols. These specimens were then subjected to microscopic examination, employing the hematoxylin and eosin staining technique, to identify any potential histological alterations.

Statistical analysis

The raw data were analyzed using a one-way ANOVA (IBM SPSS Statistics 23.0) at a significance level $p \leq 0.05$. The results were expressed as mean ±SEM.

RESULTS

Clinical findings

During the exposure period to formalin, the treated quail group exhibited various clinical signs, including nervousness, depression, anxiety, persistent coughing, reduced appetite and water intake, dullness, unsteady gait, sitting with closed eyes, and decreased responsiveness to disturbances. The observed signs exhibited greater prominence during the morning and evening, immediately following exposure to FA, compared to the rest of the day. Consequently, the weight of the treated quail exhibited a gradual decrease in all experimental groups when compared to the control group. The average weight of the control group's normal birds was 200 ± 0.66gm, while the treated groups had average weights of 188 ± 1.86gm, 154 ± 0.004gm, and 130 ± 0.43 gm, respectively. Significant ($p< 0.05$) differences were observed in all groups at a significance level of $p< 0.05$.

Hematological analysis

In the control group, the mean WBC count, Lym %, Gran%, Mid%, RBC, HGB, and PLT were 81.45±51.33, 92.8 ±53.28, 1.2±0.69,3.17±1.81,17.9±9.23, and 9±4.61, respectively. In the FVEQ1, the mean count of WBC, Lym %, Gran%, Mid%, RBC, HGB, and PLT values were 125.27±57.75, 95.83 ±55.02, 0.73±0.25, 3.63±2.19, 3.91±2.07, 19.66±10.39, and 4.68±2.30, respectively. In the FVEQ2, the mean count of WBC, Lym%, Gran%, Mid%, RBC, HGB, and PLT values were 143.6±78.28, and 93.6±52.63, 0.76±0.5, 3.5±1.9, 3.28±1.84, 20.7±10.9, and 3.5±1.73, respectively. In the FVEQ3, the mean count of WBC, Lym%, Gran%, Mid%, RBC, HGB, and PLT values were 145.3±83.54, 93.68±55.31, 0.66±0.28, 4.63±2.30, 4.33±2.20, 22.23±11.54, and 2.66±1.73, respectively. The results showed a gradual rise in the WBCs, RBCs, HGB, Lym, and a gradual decrease in the PLTs (thrombocytopenia), and Gran (granulocytopenia). There were significant ($p<0.05$) differences in all groups in all parameters (Table 1).

Findings of serum analysis

The results of the renal and hepatic function parameters revealed, at the end of the third week, that all FVEQ groups showed significant ($p<0.05$) increases compared with the

control group. In the control group, the urea, creatine, uric acid, ALP, ALT, and AST values were 4.06 ±2.30, 0.2±0.50, 10.36±6.08, 275.66±166.3, 274±162.2, and 13.16±7.50, respectively. In the FVEQ1 group, the urea, creatine, uric acid, ALP, ALT, and AST were 5.03±2.48, 0.1±0.05, 8,76±5.42, 588±333.3, 328.66±173.20, and 10 ±5.56, respectively. In the FVEQ2 group, the urea, creatine, uric acid, ALP, ALT, and AST were 4.66±2.51, 0.1±0.05, 8.26±5.19, 622±346.3, 411±192.22, and 3.66±1.15, respectively. In the FVEQ3 group, the urea, creatine, uric acid, ALP, ALT, and AST were 6.23±3.46, 0.01±0.09, 10.5±5.77, 999.6±569.5, 785±545.03, and 5.33±2.88, respectively. The findings showed significant (p<0.05) increases in the levels of blood creatinine, urea, and uric acid in the FVEQ groups. Liver enzymes; ALP, ALT, and AST, revealed significant (p<0.05) increases in the FVEQ groups (Table 2).

FVEQ1 group recorded significantly (p<0.05) the highest IgG level after 10 days of formalin exposure, which significantly (p<0.05) decreased after ten days without formalin exposure, and immunity was slightly restored (Figure 1).

Histological features

The histological features of the kidneys in Japanese quail

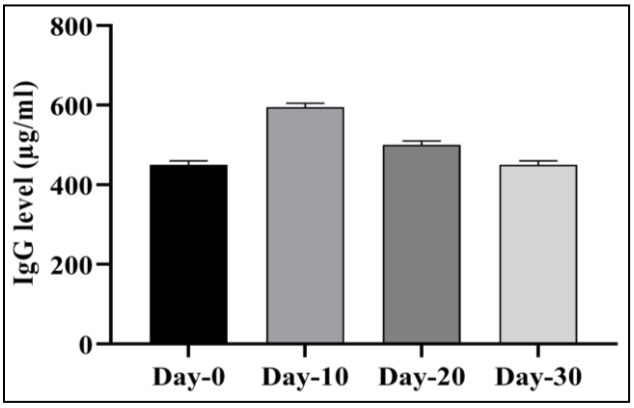

Figure 1. Effects of exposure to formalin vapor on IgG level in quails

Table 1. Complete blood parameters of quails after exposure to formalin vapor

Parameters	Control Group	FVEQ1 Group	FVEQ2 Group	FVEQ3 Group
	Mean± SEM	10 Days	20 Days	30 Days
WBC	81.45±51.32	125.27±57.73	143.6±78.28	145.3±83.53
	a	b	c	d
Lym%	92.82±53.28	93.83±55.02	95.83±56.02	97.56±58.88
	a	b	c	d
Gran%	1.2±0.69	0.73±0.28	0.70±0.50	0.66±0.28
	a	b	b	c
Mid%	5.8±3.05	3.36±2.19	3.5±1.90	3.3±1.08
	a	b	b	c
RBC	3.17±1.81	3.5±2.07	3.91±1.90	4.33±2.01
	a	b	c	d
HGB	17.96±9.23	19.66±10.23	20.7±10.96	22.23±11.54
	a	b	c	d
PLT	9±4.6	4.86±2.30	3.5±1.70	2.66±1.73
	a	b	c	d

Table 2. Blood kidney and liver function parameters of quails after exposure to formalin vapor

Parameters	Control Group	FVEQ1 Group	FVEQ2 Group	FVEQ3 Group
	Mean ± SEM	10 Days	20 Days	30 Days
Urea	4.13±0.23 a	4.66±0.3 b	5.08±0.80 c	6.23±0.68 d
Creatinine	0.2±0.1 a	0.1±0.0 b	0.1±0 b	0.1±0 b
Uric acid	10.36±1.50 a	8.76±0.70 b	8.26±0.6 c	8.20±0.4 c
ALP	275.77±17.21 a	586±55.7 b	622±69.5 c	999.6±87.6 d
ALT	274.44±8.23 a	328±60.3 b	411±84.4 c	785±265 d
AST	13.16±0.7 a	10±5.7 b	5.33±0.7 c	3.33±1.5 d

were found to be like those observed in poultry and other species, both in terms of general histological details and histochemical properties. The control group exhibited kidney tissue sections that displayed a consistent structure, with an enclosing capsule, and a well-distributed arrangement of glomerulus and tubules (Figures 2A and 2B). The illustration in Figure 2C demonstrates the anatomical structures of Bowman's capsule and Bowman's fissure, which enclose the glomerulus within the renal corpuscles. The morphology of the proximal convoluted tubule exhibited a longitudinal and rounded configuration. On the other hand, the distal convoluted tubule (Figure 2D) exhibits a convex shape. The collecting ducts serve as the boundary point between the dense and thin limbs of Henle, as depicted in Figure 2, E.

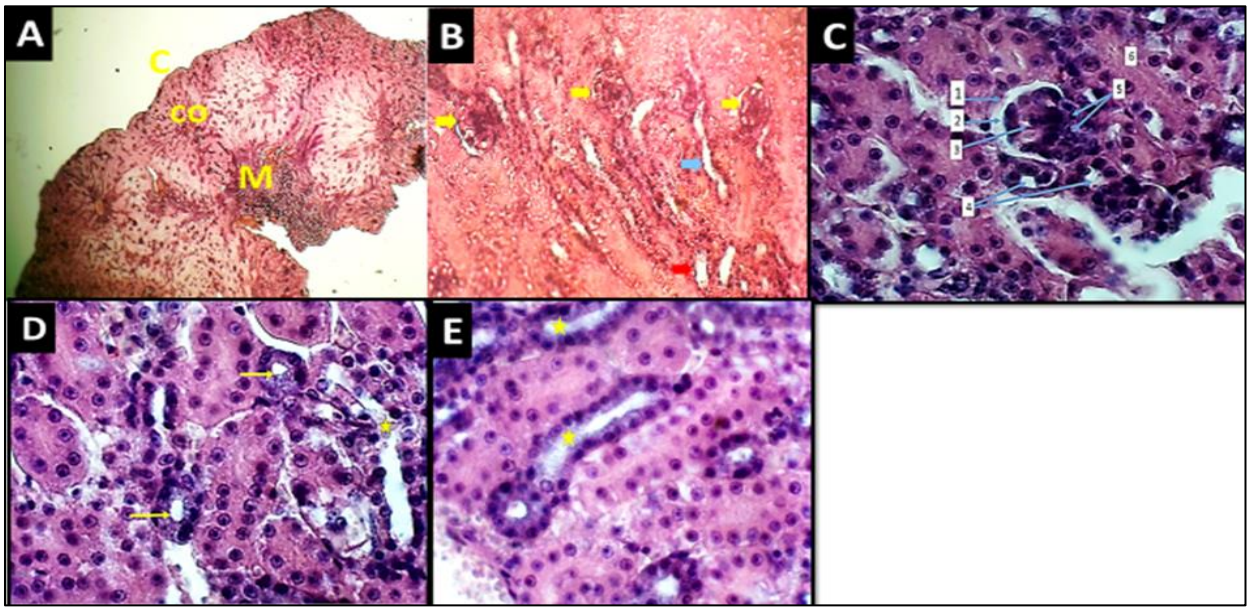

Figure 2. Microscopic section of healthy quail kidney (control group). A. It shows capsule, cortex, and medullary con. H&E stain 40X. B. Renal corpuscle (yellow arrow), proximal convoluted tube (red arrow), distal convoluted tube (blue arrow) H&E 100X. C. 1-renal corpsules, 2-space, 3-glomerula, 4-DCT, 5-mesengial cells, and 6-PCT H&E 200x. D. Proximal convoluted tubules (yellow arrow) and Henle loop (yellow star) H&E 200X. E. Collecting tubules (yellow star) H&E 400X

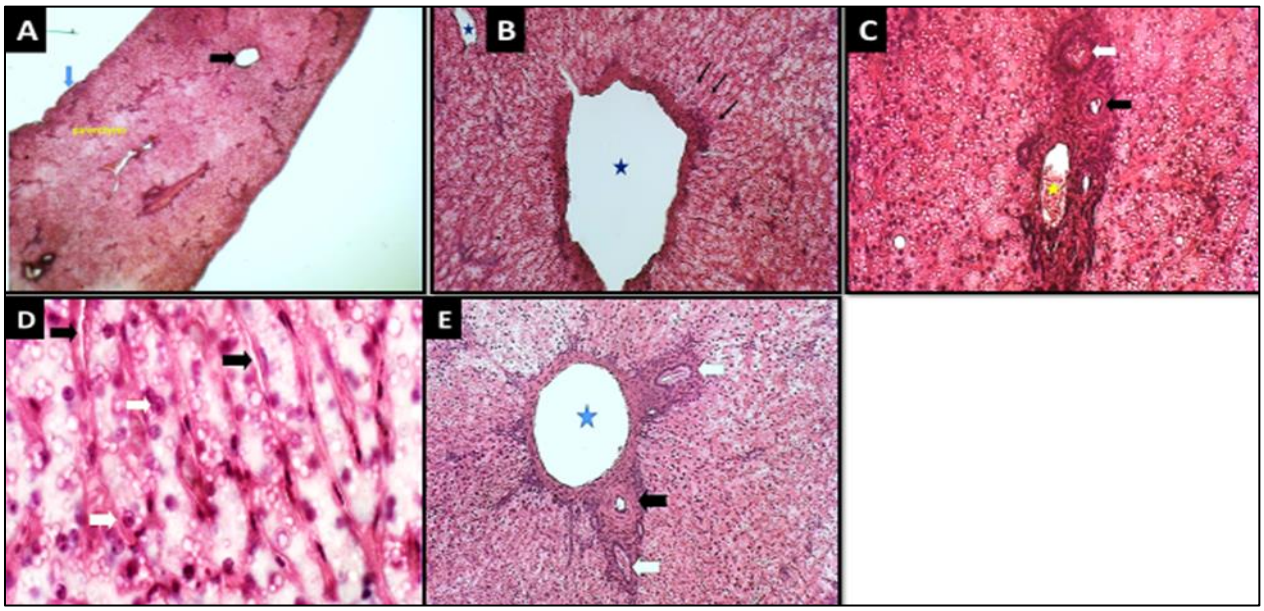

Figure 3. Microscopic section of healthy quail liver (control group). A. capsule (blue arrow), central vein (black arrow), and parenchyma. H&E 40X. B. Central vein (blue star) and hepatocytes (black arrow). H&E 200X. C. Central vein (yellow star), hepatic artery (black arrow), and intralobular duct (white arrow). H&E 200X. D. Nucleus of hepatocyte (white arrow), sinusoid (black arrow), and Kuffer cells (yellow arrow). H&E 400X. E. Portal area; central vein (blue arrow), hepatic artery (black arrow), and interlobular duct (black arrow). H&E 400X.

In the control group, the microscopic liver sections revealed normal hepatic tissue structure. There was no evidence of degenerative or necrotic changes. The parenchyma of the quail liver appeared to lack lobular structures. It was surrounded by a slender capsule of loose connective tissue and mesothelium (Figure 3A). In a longitudinal micrograph of the liver tissues, the hepatocytes appeared as hepatic lines and were arranged in pairs between the hepatic sinusoid and the central vein (Figure 3B). There are numerous sizes and shapes of hepatocytes, with polyhedral being the most common. Each hepatocyte contained one or two typically large, spherical, and eccentric nuclei and possessed a dark ovoid nucleolus. Figure 3C depicts sinusoids lined with flattened endothelial cells, including erythrocytes and macrophages (Kuffer cells). Four to six hepatocytes, bile canaliculi, and intralobular bile ducts appeared in the transverse orientation (Figure 3D). The portal region of the liver displayed interlobular arteries, veins, and the bile duct. Extracts of cuboidal tissue bordered the interlobular bile duct. The portal area branches were surrounded by smooth muscle fibers and lined with endothelial cells. There was abundant connective tissue that supported the portal tracts (Figure D and E).

Histopathologic features

In the FVEQ groups, the most frequently observed features in kidneys were shriveled and ruptured glomeruli with leukocyte infiltrations in renal tubules, degenerated tissue, and congestion of renal glomerulus with hemorrhage in the capsule (Figure 4A). In addition, there were epithelial hyperplasia, crowding of epithelial nuclei, hypereosinophilia, degeneration, and epithelial cell loss in the proximal and distal tubules (Figure 4B). Moreover, there was thickness in the renal corpuscles, glomeruli (Figure 4C), and the Henle loop of the medullary cone (Figure 4D).

In the FVEQ groups, the most frequently observed features in liver sections were increases in the parenchymal aggregation of lymphoid cells. There were lesions, such as congestion in the central vein, tissue degeneration, sinusoid enlargement, minor hemorrhages, and increases in the Kuffer cells (Figures 5A and B). There were amyloid depositions in hepatocyte spaces, which were squeezed and atrophied, creating intercellular gaps, and reducing cell compactness (Figure 5C).

DISCUSSION

The FVEQ groups exhibited clinical signs, including nervousness, depression, and persistent cough. These symptoms were attributed to the triggering effects of formalin on the nervous system, as well as its irritating effects on the respiratory system, as documented in previous studies [15, 16]. Furthermore, the findings indicate that the body weight of birds that were subjected to FA exposure exhibited decreases following the designated exposure period. The observed phenomenon can be attributed to a decrease in appetite, diminished intake of food and water throughout the exposure period, as well as intensified clinical manifestations, including anxiety, unsteady gait, and increased susceptibility of the esophagus to damage, resulting in impaired swallowing and subsequent decline in body mass. Furthermore, it is worth noting that the quail body may experience deficiencies in essential vitamins and proteins [17], resulting in clinical manifestations that are like

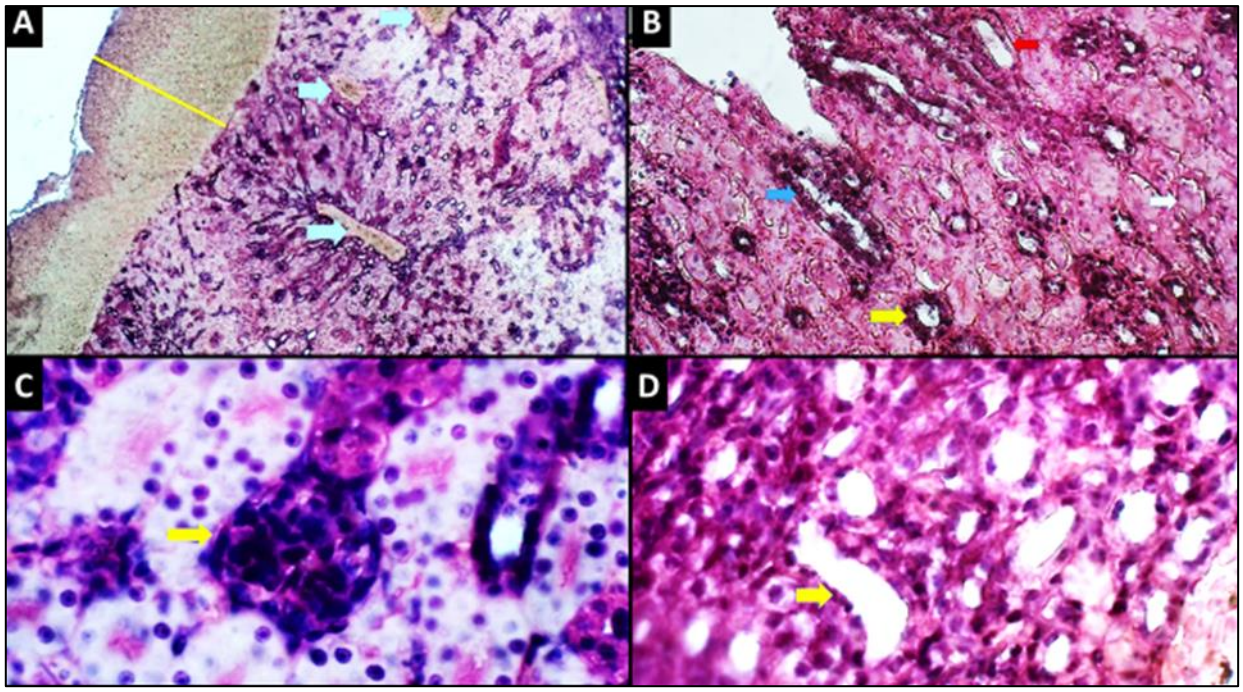

Figure 4. Histopathological section of kidneys in formalin-vapor-exposed quails. A. Congestion of capsule (yellow line) and parenchyma (blue arrow). H&E 100X. B. Hypertrophy and irregular epithelial cells of proximal and distal convoluted tubules. H&E 200X. C. Thickness of renal corpuscles and glomeruli (yellow arrow). H&E 400X. D. Thickness of the Henle tubules (yellow arrow). H&E 400X

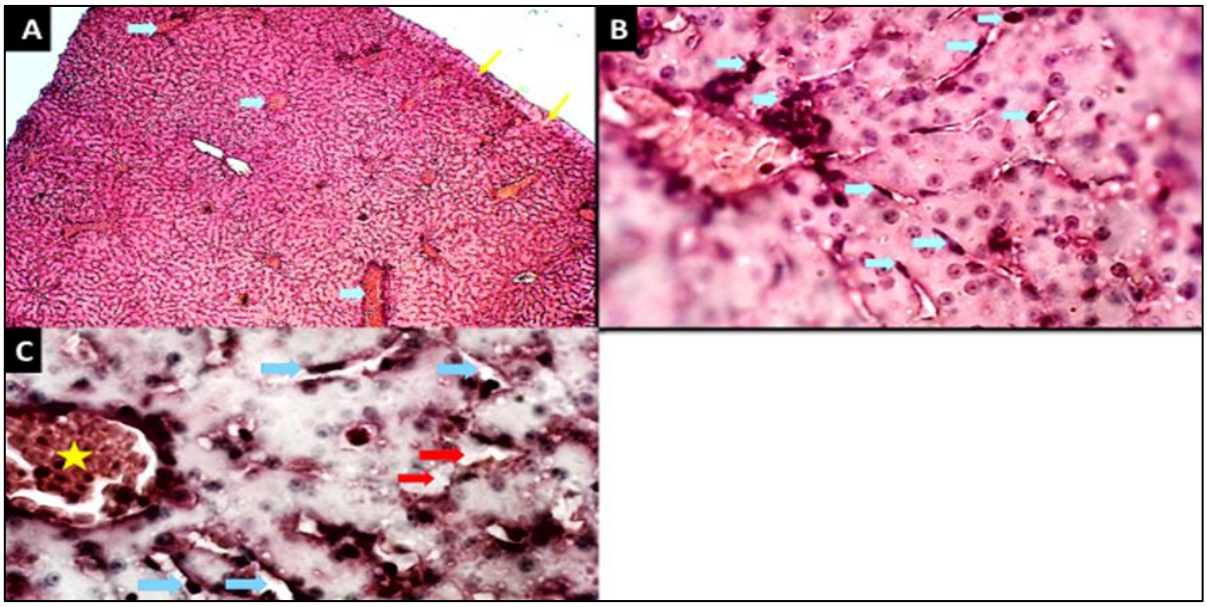

Figure 5. Histopathological section of liver in formalin-vapor-exposed quails. A. Hypertrophy and congestion of capsule (yellow arrow) and hepatocytes and central vein (blue arrow). H&E 40X. B. Increases in Kuffer cells (blue arrow). H&E 400X. C. Dilated central vein (yellow star) and sinusoid (blue arrow), deposition of amyloid (red arrow), and spaces between hepatocytes is compressed and atrophied. H&E 1000X.

those described in our study [18]. Like our investigation, in a study in broiler chickens [19,20], the study findings showed increases in the proportion of white blood cells and lymphocytes commonly observed as a physiological response to the inhalation of formalin. Consequently, the condition gives rise to infections or damage to the hepatic and renal tissues, thereby impacting the functionality of the immune system. Nevertheless, the present discovery contradicts the results reported by [21], wherein it was observed that exposure to formalin leads to a decline in immune response.

The presence of formalin, a tissue irritant, and its ability to induce tissue hypoxia, resulting in bone marrow stimulation and increased production of RBCs, suggest that an elevated level of RBCs and hemoglobin may serve as an indicator of a healthy condition in both the kidney and the liver. There are two reasons that support this argument. Initially, it is important to consider the tissue-irritating properties of formalin. This contrasts with the results reported by [22], which indicate that formalin exposure in hens led to a decrease in the count of RBCs and hemoglobin, which agrees with the present study that also observed a decline in the number of RBCs and hemoglobin [23]. Based on previous studies [24,25], it has been demonstrated that formalin exposure induces immune system dysfunction, resulting in a reduction in the proportion of platelets, granulocytes, and mid cells within the bloodstream. This finding aligns with the results reported in the study conducted by [26].

The liver primarily engages in the intermediary metabolism of carbohydrates, proteins, lipids, and amino acids, and detoxification of some chemicals. Based on the findings referenced [27], the consumption of fatty acids (FA) at different levels and through various modes of intake leads to alterations in the hepatic metabolic pathway. The evidence indicated that FA caused the destruction of hepatocytes, intrahepatic and extrahepatic bile ducts as a result of its metabolic reactivity [28]. This result aligns with the findings of the present study conducted on quails. Based on the findings of previous studies [29], it has been observed that cytotoxicity appears when a substance interacts directly with the constituents of biological tissues. FA rapidly permeates various organs after intraperitoneal, oral, or inhalation administration [30]. Several tissues, such as the liver, brain, and testis, are included in this category. The researchers in this study utilized enzyme activity measurements in hepatocytes, including ALT, AST, and ALP, to assess the degree of liver tissue damage and evaluate liver functionality. The findings presented in this study are consistent with the findings reported by [31], which characterize these enzymes as highly responsive indicators of liver injury and reflective of the detrimental impact of formalin on the functioning of hepatocytes. There was a notable rise in the concentrations of all these enzymes, except for AST. In contrast, a study conducted by researchers [32] and [33] reported a reduction in the serum activity of enzymes, namely ALP, AST, and ALT, in rats that were administered higher doses of formalin through their drinking water. These doses included 1.8, 21, and 109 mg/kg of body weight. The obtained results are inconsistent with the findings of the present study.

The initial cohort (FVEQ1), spanning a duration of ten days, observed a notable elevation in IgG levels consequent to an immune response triggered by FA exposure. Nevertheless, there was a gradual decline in the proportion within the FVEQ2 group (20 days) and the FVEQ3 group (30 days) due to infection with immune dysfunction that persisted in various body tissues, including the liver and kidneys. This resulted in the development of an immune disorder that led to a decline in IgG levels, which is in line with previous findings. The avian subjects that were administered the FA exhibited elevated levels of blood urea, creatinine, and uric acid in comparison to the group that was not subjected

to FA. The reabsorption of urea by the renal tubules is influenced by pathological changes in the glomeruli, which subsequently impact the rates of filtration. Symptoms such as dehydration, fever, and chemotaxis appear because of a malfunction in the filtration mechanism. These symptoms promote the process of tubular reabsorption of salt, leading to an elevation in the urea-to-creatinine ratio. Furthermore, there is an increased presence of uric acid [34, 35].

Based on the research conducted by [36, 37], the introduction of formalin into bodily tissues was found to induce toxicity, resulting in the development of ulcers and severe tissue infections. In cases where formalin is highly concentrated or remains in contact with the tissue for an extended duration, these adverse effects may escalate to the extent of tissue perforation. This substance is also causally linked to the development of cirrhosis of the liver, and it is also implicated in instances of failure.

The inadvertent addition of FA to food for the purpose of preservation results in detrimental effects on renal function and the development of hepatic tumors. Formaldehyde, even at minimal concentrations, possesses a considerable capacity to induce harmful health consequences in individuals exposed to it. Upon exposure, it induces negative effects on the skin and elicits an allergic response in ocular tissues. Consequently, it is categorized as a Group 1 carcinogen, denoting its classification as having the highest level of certainty in terms of its carcinogenicity. When inhaled directly, it generates irritation in the nasal mucous membranes, induces inflammation in the trachea, and impairs respiratory function. This elucidates the various pathological abnormalities that have been observed in the liver and kidneys of quail birds, which aligns with the conclusion reached in previous studies [38, 39, 40].

CONCLUSION

The findings of this study indicate that prolonged exposure to formalin vapor may result in adverse effects on the histological composition and functional performance of the kidney and liver in the quail subjected to formalin vapor exposure Hence, it is advisable that all individuals who handle formalin adhere to time-limited exposure guidelines and work in adequately ventilated environments.

ACKNOWLEDGMENT

The authors would like to acknowledge the Department of Anatomy, Histology, and Embryology, College of Veterinary Medicine, University of Al-Qadisiyah for supporting this research.

Conflict of Interest: None to be declared.
Funding: None.

REFERENCES

1. Agarwal AR, Garg RL.: Formalin toxicity in hydatid liver disease. Anesthesia;1983;38(7): 662-665 https://doi.org/10.1191/096032700678815954
2. Babar AM, Khan MZ, Shabbir A, Khan A, Bachaya HA, Anwar MI. Toxico-pathological effects of formalin (37% formaldehyde) feeding in broiler chicks. Pakistan Vet J. 2001; 21(1): 13-16. https://doi.org/10.1191/0960327105ht543oa
3. Bernstein RS, Styner LT, Elliot LJ, Kimbrough R, Falk H, Blade L. Inhalation exposure to formaldehyde: An overview of its toxicity, epidemiology, monitoring, and control. Am Ind Hyg Assoc J.1984;45(11): 778-85. https://doi.org/10.1080/15298668491400601
4. Bosetti C, McLaughlin JK, Tarone RE, Pira E , La Vecchia C. Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006. Ann Oncol. 2008; 19(1):29-43. https://doi:10.1093/annonc/mdm202
5. Brown R. (1996): FDA approved using formaldehyde in poultry feed. Feed Stuff; 15: 40. | https://doi.org/10.3389/fvets.2019.00188
6. Cheney JE, Collins CH. Formaldehyde disinfection in laboratories: Limitations and hazards. Br J Biomed Sci. 1995; 52(3):195-201. PMID: 8527997
7. Davies RC. Effect of regular formalin foot baths on foot lameness in dairy cattle. Vet Rec. 1982; 111(17):394. doi: 10.1136/vr.111.17.394.
8. Dietz P, Bohm R, Strauch D. Effectiveness and safety of formaldehyde gas and aerosols of peracetic acid and hydrogen peroxide. Zentralbl Veterinarmed B 1980; 278: 268-79. DOI: 10.1139/m92-104
9. Egwurugwu JN, Oham aeme MC, Izunwanne D, Ugwuezumba PC, Ngwu EE, Elendu MU, Nwamkpa P, Ekweogu CN. Assessment of the Inhalational Effects of Formalin on the lipid profile of Wistar Albino Rats. Res J Health Sci. 2018; 3(1).
10. Fair WR. Formalin in the treatment of massive bladder hemorrhage. Techniques, results and complications. Urology.1974; 3(5):573-6. doi: 10.1016/s0090-4295(74)80250-5.
11. Flyvholm MA, Menne T. Allergic contact dermatitis from formaldehyde: A rare study focusing on sources of formaldehyde exposure. Contact Dermatitis.1992; 27(1):27-36. doi: 10.1111/j.1600-0536.1992
12. Giannakopoulos X, Grammeniatis E, Chambilomatis P, Baltogiannis D. Massive haemorrhage of inoperable bladder carcinomas: treatment by intravesical formalin solution. Int Urol NephroI.1997; 29(1):33-8. doi: 10.1007/BF02551414.
13. Hansen J, Olsen JH. Formaldehyde and cancer morbidity among male employees in Denmark. Cancer Causes Control 1995; 6(4):354-60. doi: 10.1007/BF00051411
14. Kilburn KH. Neurobehavioral impairment and seizures from formaldehyde. Arch Environ Health. 1994;49(1):37-44. DOI: 10.1080/00039896.1994.9934412
15. Kita T, Fujimura M, Myou S, Ishiura Y, Abo M, Katayama N, Nishitsuji M, Yoshimi Y, Nomura S, Oribe Y. Potentiation of allergic bronchoconstriction by repeated exposure to formaldehyde in guinea-pigs in vivo. Clin Exp Allergy. 2003;33: 1747-1753. https://doi.org/10.1016/S0761-8425(06)73414-3
16. Kochhar R, Nanda V, Nagi B, Mehta SK. Formaldehyde induced corrosive gastric cicatrization: a case report. Hum Toxicol. 1986; 5 (6): 381-382. https://doi.org/10.1177/096032718600500609
17. Koppel C, Baudisch H, Schneider V, Ibe K. Suicidal ingestion of formalin with fatal complications. Intensive Care Med.1990;16: 212-214. https://link.springer.com/article/10.1007/BF01724806
18. Mathai V, Seow-Choen F. Endoluminal formalin therapy for hemorrhagic radiation proctitis. Br J Surg. 1995; 82 (2): 190. https://doi.org/10.1002/bjs.1800820216
19. Peckham MS. Poisons and toxins in poultry. Dis Poult.1980; 8: 790-91.
20. Restani P, Galli CL. Oral toxicity of formaldehyde and its derivatives. Crit Rev Toxicol.1991;21(5):315-328.

21. Roche B, Chautems R, Marti MC. Application of formaldehyde for treatment of hemorrhagic radiation-induced proctitis. World J Surg.1996; 20(8):1092-4. doi: 10.1007/s002689900166.
22. Saclarides TJ, King DG, Franklin JL, Doolas A. Formalin instillation for refractory radiation-induced hemorrhagic proctitis. Report of 16 patients. Dis Colon Rectum.1996; 39 (2): 196-199. . https://doi.org/10.1007/BF02068075
23. Sarnak MJ, Long J, King AJ. Intravesicular formaldehyde instillation and renal complications. Clin Nephrol.1999; 51(2):122-5. DOI: 10.5152/tud.2013.010
24. Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann NYAcad Sci.2002; 982(1): 87-105. DOI:10.1111/j.1749-6632.2002.tb04926.x
25. Usmanmaz SE, Akarsu ES, Vural N. Neurotoxic effects of acute and sub-acute formaldehyde exposures in mice. Environ Toxicol Pharmacol.2002; 11(2):93-100. doi: 10.1016/s1382-6689(01)00109-0.
26. Williams JE. Formalin destruction of Salmonella in poultry litter. Poult Sci.1980, 59(3): 2717-24. DOI: 10.1590/1806-9061-2022-1631
27. Witek TJ, Tosum T. Formaldehyde concentration in the blood of humans and Fisher 344 rats exposed to formaldehyde under controlled conditions. Am Ind Hyg Assoc J.1985; 46 (1): 1-3. DOI: 10.1080/15298668591394275
28. Kimbell J, Overton J, Subramaniam R. Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment. Toxicol Sci. 2001; 64(1):111-21. doi: 10.1093/toxsci/64.1.111.
29. Chowdhury AR, Gautam AK, Patel KG, Trivedi HS. Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. Indian J Physiol Pharmacol. 1992; 36(3):162-8.
30. Anwar M I, Khan MZ, Muhammad G, Bachaya A, Babar AM. Effects of dietary formalin on male Japanese quails' health and testicular pathology (Coturnix coturnix Japonica). Vet. Hum. Toxicol.2001; 43(6), 330-333. DOI: 10.29244/jintp.v18i1.30993
31. Holladay SD, Smith BJ, Gogal RM. Exposure to formaldehyde at therapeutic levels decreases peripheral blood lymphocytes and hematopoietic progenitors in the pronephros of tilapia Oreochromis niloticus. Aquat Biol. 2010; 10(3), 241-247. DOI: https://doi.org/10.3354/ab00282
32. Zhang Y, Liu X, McHale C, Li R, Zhang L, Wu Y, Ding S. Bone marrow injury induced via oxidative stress in mice by inhalation exposure to formaldehyde. PloSone.2013;8(9)e74974. DOI: 10.1371/journal.pone.0074974
33. 33.Dwyer J, Murphy R, González-Vázquez, A, Kovarova L, Pravda M, Velebny V, Heise A, Duffy GP, Cryan SA. Translational Studies on the Potential of a VEGF Nanoparticle-Loaded Hyaluronic Acid Hydrogel. Pharmaceutics.2021; 13(6), p.779. DOI: 10.3390/pharmaceutics13060779
34. Khan A, Hussain SM, Khan MZ. Effects of formalin feeding or administering into the crops of white leghorn cockerels on hematological and biochemical parameters. Poult Sci. 2006; 85(9):1513-9. doi: 10.1093/ps/85.9.1513.
35. Leisegang K, Henkel R. The in vitro modulation of steroidogenesis by inflammatory cytokines and insulin in TM3 Leydig cells. Reprod Biol Endocrinol. 2018; 16(1):26. doi: 10.1186/s12958-018-0341-2.
36. Tang X, Bai Y, Duong A, Smith MT, Li L. Formaldehyde in China: production, consumption, exposure levels, and health effects. Environ Int. 2009; 35(8),1210-1224.doi:10.3390/pharmaceutics 130607
37. Baugh EH, Ke H, Levine AJ, Bonneau RA, Chan CS. Why are there hotspot mutations in the TP53 gene in human cancers? Cell Death Differ. 2018; 25(1), 154-160. DOI: 10.1038/cdd.2017.180
38. Kareem DA, Jassem ES, Daaj SA, Al-Khalad WJ. Morphological and histological study of the testes in adult duck. Plant Arch,2022; 20(Supl.2), 751-755. https://doi.org/10.3390/vetsci9090485
39. Shahbaa Kh. Al-Taee; Muna H. Al-Zubaidy; Khayrea A. Mustafa; Hana Kh. The role of Saccharomyces cerevisiae to alleviate copper sulfate toxicity in Cyprinus carpio, Iraqi J Vet Sci 2023;37(3) 573-580 DOI: 10.33899/ijvs.2023.135148.2449
40. Jaber MT, Al-Jumaa ZM, Al-Taee SK, Nahi HH, Al-Hamdany MO, Al-Salh MA, Al-Mayahi B. Bioaccumulation of heavy metals and histopathological changes in muscles of common carp (Cyprinus carpio L.) in the Iraqi rivers', Iraqi J Vet Sci. 2020;35(2):245-249. DOI: 10.33899/ijvs.2020.126748.1368