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EXPERIMENTAL INVESTIGATION OF HEPATOCELLULAR CHANGES IN *WISTAR RATS* EXPOSED TO CABALT-ENRICHED BEER: HEPATOCELLULAR EFFECT OF CABALT-ENRICHED BEER IN *WISTAR RATS*

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ABSTRACT

Alcohol consumption and exposure to metallic contaminants have been associated with progressive liver injury and metabolic dysfunction. This study investigated hepatocellular changes in *Wistar* rats exposed to cobalt-enriched beer. Thirty male Wistar rats weighing 168-193 g were randomly assigned into six groups (A-F) containing five rats each (n=5). Groups A-D received graded concentrations of cobalt chloride (0.01-0.04 g/100 mL $CoCl_2$) in Budweiser® lager beer, Group E received beer only, while Group F served as the control. Treatments were administered via oral gavage for 45 days while, body weight changes, gross pathological findings, and histopathological alterations of liver tissues were evaluated using standard Hematoxylin and Eosin staining techniques after experimentation. Results showed significant dose-dependent reductions in body weight gain among cobalt-treated groups compared with the control group ($p < 0.05$). Gross examination of liver tissues revealed enlargement, paleness, vascular congestion, and pinpoint hemorrhages in cobalt-treated rats. Histopathological evaluation demonstrated progressive hepatocellular degeneration characterized by vascular congestion, inflammatory cell infiltration, fatty changes, sinusoidal dilatation, and hepatocyte necrosis, particularly in Groups C and D exposed to higher cobalt concentrations. The beer-only group showed only mild vascular congestion and slight hepatocellular degeneration, while the control group maintained normal hepatic architecture. The findings indicate that cobalt enrichment potentiates alcohol-induced hepatotoxicity through mechanisms likely involving oxidative stress, inflammatory activation, and mitochondrial dysfunction. This study highlights the potential public health risks associated with metallic contaminants in alcoholic beverages and emphasizes the need for strict quality control and monitoring of cobalt exposure in beverage production systems.

Keywords: Alcohol-induced liver injury; Cobalt chloride toxicity; Hepatotoxicity; Hepatocellular effects; Wistar rats

Introduction

Alcohol consumption remains a major global public health concern because of its strong association with metabolic disorders, cardiovascular diseases, neurological impairment, and progressive liver injury (Gilpin & Molina, 2026; Wagner et al., 2026). Chronic alcohol use disorder has been recognized as a multifactorial chronic disease characterized by long-term physiological and metabolic dysfunction capable of affecting multiple organ systems, particularly the liver (Gilpin & Molina, 2026). The liver serves as the principal organ responsible for alcohol metabolism, detoxification, and biotransformation of xenobiotics; consequently, it is highly susceptible to alcohol-induced oxidative stress and hepatocellular injury (Berasain et al., 2023). Persistent alcohol exposure has been implicated in the pathogenesis of fatty liver disease, alcoholic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma through mechanisms involving oxidative stress, inflammation, mitochondrial dysfunction, and progressive loss of hepatic functional identity (Berasain et al., 2023; Gan et al., 2025).

Recent toxicological evidence suggests that the hepatotoxic effects of alcohol may be intensified by metallic additives, contaminants, and process-induced toxicants introduced during industrial processing and brewing (Alizadeh et al., 2025). Process-induced toxicants generated during food and beverage production have increasingly attracted scientific attention because of their potential adverse health implications, including oxidative injury, inflammatory responses, and cellular degeneration (Alizadeh et al., 2025). Among these toxic substances, cobalt compounds have emerged as important toxicological agents due to their documented deleterious effects on biological tissues and organ systems (Chen & Lee, 2026). Cobalt is an essential trace element required in minute quantities for normal physiological activities, particularly as a constituent of cobalamin (vitamin B12). However, excessive exposure to cobalt compounds has been associated with severe pathological alterations involving the cardiovascular, neurological, renal, and hepatic systems (Chen & Lee, 2026). Cobalt toxicity may result from occupational exposure, environmental contamination, dietary intake, or industrial applications involving cobalt-containing compounds (Teschke, 2022). Among these compounds, cobalt chloride ($CoCl_2$) has been extensively studied because of its potent ability to induce oxidative stress, lipid peroxidation, mitochondrial dysfunction, inflammatory responses, and apoptosis within hepatic tissues (Karagözoğlu et al., 2026).

Experimental investigations have demonstrated that cobalt chloride exposure causes significant alterations in liver enzymes, hepatocellular degeneration, vascular congestion, sinusoidal dilatation, inflammatory infiltration, and tissue necrosis in laboratory animals (Iji et al., 2023). The hepatotoxic effects of cobalt are believed to occur primarily through excessive generation of reactive oxygen species (ROS), depletion of endogenous antioxidant defenses, disruption of cellular respiration, and activation of inflammatory signaling pathways (Hamad et al., 2025; Teschke, 2022). Oria et al. (2022) further reported that cobalt exposure induced oxidative stress and histomorphological alterations in experimental animal tissues, emphasizing the systemic toxic potential of cobalt compounds. Historically, cobalt salts were incorporated into beer production to stabilize foam and improve the visual appearance of lager beers. However, the industrial application of cobalt additives in brewing became controversial following reports associating cobalt-enriched beer consumption with severe cardiotoxic and hepatotoxic effects in humans (Chen & Lee, 2026). Although the use of cobalt additives in brewing has considerably declined, concerns regarding cobalt-associated toxicity remain relevant because environmental and dietary exposure to cobalt compounds persists (Karagözoğlu et al., 2026). Simultaneous exposure to alcohol and cobalt may further potentiate hepatic injury because both substances independently induce oxidative stress, inflammation, and hepatocellular degeneration (Iji et al., 2023).

The liver performs several essential physiological functions including detoxification, bile production, glycogen storage, protein synthesis, metabolism of xenobiotics, and regulation of biochemical homeostasis (Berasain et al., 2023). Hepatocytes, which constitute the major functional cells of the liver, are continuously exposed to toxic metabolites and circulating xenobiotics during metabolic processing and are therefore highly vulnerable to toxic injury (Gan et al., 2025). Exposure to hepatotoxic agents such as cobalt chloride and alcohol may consequently result in cellular degeneration, inflammatory reactions, fatty infiltration, vascular congestion, fibrosis, and necrosis, ultimately impairing hepatic function and compromising systemic homeostasis (Berasain et al., 2023). Histopathological evaluation remains one of the most reliable methods for assessing hepatic injury induced by toxic substances because it enables direct visualization of structural and cellular alterations within liver tissues. Experimental animal models, particularly Wistar rats, are widely utilized in toxicological investigations due to their physiological and metabolic similarities to humans (Karagözoğlu et al., 2026). Previous experimental studies involving albino rats have demonstrated that

cobalt chloride exposure produces varying degrees of hepatic degeneration depending on dose, duration, and route of exposure (Akinrinde et al., 202; Iji et al., 2023; Kadry & Ali, 2023).

Despite increasing scientific interest in heavy metal toxicity and alcohol-related liver injury, there remains limited contemporary literature specifically investigating hepatocellular alterations associated with exposure to cobalt-enriched alcoholic beverages. Furthermore, few recent studies have examined the histomorphological changes induced by cobalt-containing beer formulations in experimental animal models (Oria et al., 2022; Teschke, 2022). Understanding these hepatic alterations is important because consumers may unknowingly be exposed to trace metallic additives and process-induced toxicants capable of potentiating alcohol-induced liver injury (Alizadeh et al., 2025). Therefore, this study was designed to experimentally investigate hepatocellular changes in Wistar rats exposed to cobalt-enriched beer. The study specifically evaluates the histopathological alterations associated with prolonged exposure to cobalt-containing beer in order to contribute contemporary toxicological evidence regarding the hepatic safety implications of cobalt-enriched alcoholic beverages.

Materials and Methods

Experimental Design

This experimental study was designed to investigate hepatocellular changes in Wistar rats following exposure to cobalt-enriched beer. A completely randomized experimental design was adopted using graded concentrations of cobalt chloride ($CoCl_2$) administered alongside beer exposure to evaluate dose-dependent hepatic alterations.

Experimental Animals

Thirty (30) healthy male *Wistar* rats aged between 2 and 4 weeks and weighing between 168 g and 193 g were used for the study. The animals were obtained from the Animal House Unit of the School of Basic Medical Sciences, Igbinedion University, Okada, Edo State, Nigeria. The rats were acclimatized for a period of two weeks before commencement of the experiment under standard laboratory conditions. The animals were housed in stainless steel wire-mesh cages at an ambient temperature of 28-32°C under normal light-dark cycles. Standard commercial rat feed (Top Feed Growers Mash, Nigeria) and clean water were provided *ad libitum* throughout the experimental period. The cages were cleaned regularly to maintain hygienic conditions and minimize environmental contamination.

Preparation of Cobalt-Enriched Beer Solutions

Cobalt chloride ($CoCl_2$) was used to prepare graded concentrations of cobalt-enriched beer solutions. Commercially available Budweiser® lager beer was utilized as the alcoholic vehicle for cobalt administration. Based on preliminary compositional information regarding cobalt additives in beer formulations, different concentrations of cobalt chloride were prepared and administered to the experimental animals.

Statement on Dose Selection

The experimental concentrations of cobalt chloride used in this study were adapted based on previously published toxicological studies demonstrating sub-lethal hepatotoxic effects of cobalt exposure in experimental animals (Iji et al., 2023; Kadry & Ali, 2023). Since the present study was designed to evaluate chronic hepatocellular alterations rather than acute toxicity, LD_{50} determination was not performed. The selected concentrations were therefore chosen to represent graded sublethal exposures capable of inducing progressive hepatic injury without causing mortality during the experimental period.

Grouping of Experimental Animals

Groups A-D received graded concentrations of cobalt chloride preparations, while Group E received only Budweiser® lager beer without additional cobalt supplementation. Group F served as the control group and received neither cobalt chloride nor beer exposure. The thirty Wistar rats were randomly divided into six groups consisting of five rats per group as follows:

Table 1. Grouping of Experimental Animals

Group	Treatment
Group A	0.01 g/100 mL $CoCl_2$ solution
Group B	0.02 g/100 mL $CoCl_2$ solution
Group C	0.03 g/100 mL $CoCl_2$ solution
Group D	0.04 g/100 mL $CoCl_2$ solution
Group E	Budweiser® lager beer only
Group F	Control (standard feed and water only)

The above table 1 showed the grouping of experimental rats and its corresponding treatments

Administration of Experimental Treatments

The experimental treatments were administered orally for a duration of forty-five (45) consecutive days. Animals were monitored daily throughout the study period for behavioural changes, feeding pattern, physical appearance, and signs of toxicity. Body weights of the animals were measured weekly using a digital weighing balance and recorded to assess weight changes during the experimental period.

Sample Collection

On the forty-sixth day, the experimental animals were sacrificed following light chloroform anesthesia. Liver tissues were carefully excised through abdominal dissection and examined grossly for observable pathological changes including discoloration, enlargement, congestion, and hemorrhage. The harvested liver samples were rinsed in normal saline to remove blood stains and immediately fixed for histopathological processing.

Histological Processing

Liver tissues were fixed in 10% buffered formalin for 24 hours. Following fixation, tissue samples were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in molten paraffin wax using standard histological procedures. Paraffin-embedded tissues were sectioned at approximately 5 µm thickness using a rotary microtome. The tissue sections were mounted on clean glass slides and stained routinely using Hematoxylin and Eosin (H&E) staining technique for microscopic examination.

Hematoxylin and Eosin Staining Procedure

The mounted tissue sections were deparaffinized in xylene and rehydrated through descending grades of ethanol to water. Sections were stained with Ehrlich's hematoxylin, differentiated in acid alcohol, blued in Scott's tap water substitute, and counterstained with eosin. Thereafter, sections were dehydrated in ascending grades of alcohol, cleared in xylene, and mounted with DPX mounting medium.

Histopathological Examination

Prepared liver sections were examined under a binocular light microscope using ×10 and ×40 objective lenses for assessment of hepatocellular alterations. Histopathological features evaluated included hepatocellular degeneration, inflammatory cell infiltration, vascular congestion, sinusoidal dilatation, fatty changes, necrosis, and distortion of hepatic architecture. Photomicrographs of representative histological findings were obtained using an Olympus photomicroscope fitted with a digital imaging system.

Ethical Consideration

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. Animal handling, treatment administration, and sacrifice were performed with minimal stress and discomfort to the experimental animals. All protocols followed the guidelines of the Igbinedion University Teaching Hospital Ethical Review Committee (Approval no. IUTH/R.25/VOL 2/23).

Statistical Analysis

Body weight data obtained before and after treatment were expressed as mean values. Statistical comparisons between experimental groups and control were performed using Student's t-test, with values of $p < 0.05$ considered statistically significant.

Results

Clinical Observations and Body Weight Changes

All rats remained active throughout the study period, and no mortality was recorded in any of the experimental groups. However, body weight gain was observed to decrease progressively in cobalt chloride-treated groups (Groups A-D) compared with the control and beer-only groups. Group F (control) recorded the highest mean weight gain throughout the experimental period.

Table 2 presents the mean body weights of experimental rats before and after 45 days of treatment. Rats exposed to increasing concentrations of cobalt chloride demonstrated dose-dependent reductions in body weight gain. Group D, which received the highest concentration of cobalt chloride (0.04 g/100 mL $CoCl_2$), exhibited the least weight gain compared with the control group. Statistical analysis showed that the reductions in body weight observed in cobalt-treated groups were significant at $p < 0.05$.

Table 2: Effect of Different Concentrations of Alcohol on the Body Weights of *Wistar* Rats

Cobalt Salt Conc. (g/100 mL)	Group	Pre-mean weight (g)	Post-mean weight (g)	d	d^2	P-value
0 (Control)	F	180.03	223.00	140.95	198773.95	–
0.01	A	182.18	218.67	-136.49	18630.69	P < 0.05
0.02	B	181.73	170.00	-97.28	9462.42	P < 0.05
0.03	C	182.38	138.00	-55.63	3094.14	P < 0.05
0.04	D	181.93	135.00	-53.46	2857.44	P < 0.05
Beer Only	E	182.38	138.00	-55.63	3094.14	P < 0.05

Key

- d = Difference between the pre mean weight and the post mean weight values.
- d^2 = Squared value of the difference between the pre mean weight and the post mean weight values.
- Conc. = concentration

Gross Pathological Findings

Gross examination of liver tissues revealed varying degrees of enlargement, paleness, vascular congestion, and pinpoint hemorrhages in Groups A-D compared with the control group. The livers from the control animals appeared reddish-brown with normal size and texture. Mild congestion was observed in the beer-only group (Group E), whereas the cobalt-treated groups demonstrated progressively severe gross pathological changes with increasing cobalt concentration.

Histological Findings

Histopathological examination of liver sections stained with Hematoxylin and Eosin (H&E) revealed remarkable differences between the control and experimental groups. The control group (Group F) demonstrated normal hepatic architecture characterized by intact central veins, well-arranged hepatocytes, and preserved sinusoids. Rats exposed to cobalt-enriched beer (Groups A-D) exhibited progressive dose-dependent hepatocellular alterations. Group A showed mild vascular congestion and hepatocellular degeneration. Group B demonstrated moderate hepatocellular degeneration accompanied by inflammatory cell infiltration and vascular congestion. More severe pathological lesions were observed in Groups C and D, including fatty changes (vacuolation), hepatocyte necrosis, inflammatory infiltration, sinusoidal dilatation, and severe vascular congestion. The beer-only group (Group E) displayed mild vascular congestion and slight hepatocellular degeneration compared with the more severe lesions observed in cobalt-treated groups. Overall, the histopathological findings indicate that exposure to cobalt-enriched beer induced progressive hepatic injury characterized by inflammation, degeneration, fatty change, and necrosis in Wistar rats.

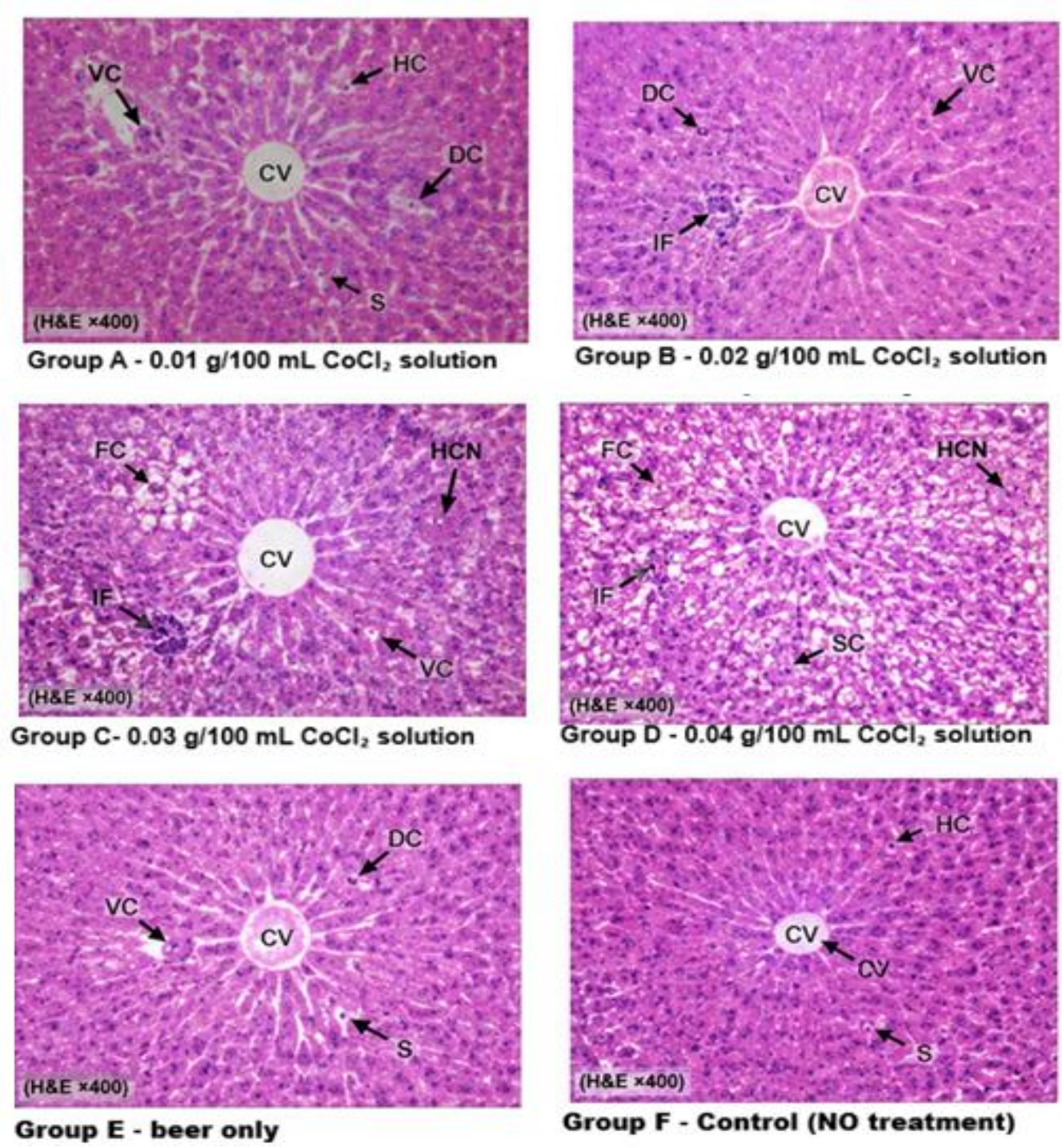

Plate 1. Photomicrographs of liver sections from experimental rats (H&E ×400).

- Central vein; HC - Hepatocytes; S - Sinusoids; VC - Vascular congestion;
DC - Degeneration of hepatocytes; IF - Inflammatory cell infiltrate;
FC - Fatty change (vacuolation); HCN - Hepatocyte necrosis;
SC - Sinusoidal dilatation.

Discussion

The present study investigated the hepatocellular effects of cobalt-enriched beer in Wistar rats and demonstrated that prolonged exposure to cobalt chloride produced significant dose-dependent hepatic injury characterized by reduced body weight gain, gross pathological abnormalities, and severe histopathological alterations in liver tissues. The findings obtained from this investigation strongly indicate that cobalt supplementation in alcoholic beverages potentiates alcohol-induced hepatotoxicity through mechanisms involving oxidative stress, inflammatory responses, vascular alterations, and hepatocellular degeneration (D'Arcangelo et al., 2026; Lee et al., 2025).

One of the important observations in this study was that all experimental animals remained alive throughout the experimental period, and no mortality was recorded despite the presence of significant hepatic lesions. This finding suggests that the administered cobalt concentrations produced chronic sublethal hepatotoxicity rather than acute lethal toxicity. Chronic exposure to toxic substances often results in progressive tissue injury before the manifestation of overt mortality, particularly in hepatic tissues that possess substantial regenerative capacity (Berasain et al., 2023). The absence of mortality observed in this study therefore does not exclude severe toxicological effects but rather indicates progressive chronic hepatic injury induced by prolonged cobalt and alcohol exposure.

The results presented in Table 2 demonstrated significant reductions in body weight gain among cobalt-treated groups compared with the control group. The reductions were statistically significant at $p < 0.05$, indicating that cobalt exposure had measurable biological effects on the metabolic status and physiological growth of the animals. Group D, which received the highest concentration of cobalt chloride (0.04 g/100 mL $CoCl_2$), exhibited the least body weight gain, thereby confirming a dose-dependent toxicological response. The progressive reduction in body weight gain observed across Groups A-D may be attributed to impaired nutrient utilization, metabolic dysfunction, oxidative stress, reduced appetite, and systemic toxicity induced by cobalt exposure (Lee et al., 2025).

Similar findings have been reported by Akinrinde et al. (2023), who observed that cobalt exposure disrupted physiological homeostasis and produced systemic toxic effects in experimental rats. Wagner et al. (2026) further reported that alcohol-related metabolic dysfunction is associated with adverse systemic health outcomes including weight abnormalities and organ dysfunction. The significant decline in body weight gain observed in this study therefore supports the hypothesis that combined alcohol and cobalt exposure interferes with normal metabolic and physiological activities.

The control group recorded the highest body weight gain and maintained normal physical activity throughout the experimental period, further validating that the observed toxicological effects in cobalt-treated groups were treatment-induced rather than environmentally mediated. Preservation of normal physiological growth in the control animals strengthens the reliability of the experimental model and confirms the hepatotoxic influence of cobalt-enriched beer exposure (Li et al., 2026).

Gross pathological examination of liver tissues revealed varying degrees of enlargement, paleness, vascular congestion, and pinpoint hemorrhages in Groups A-D compared with the control group. These findings indicate progressive hepatic damage and circulatory disturbances associated with cobalt toxicity. Enlargement and congestion of the liver may result from inflammatory edema, impaired hepatic circulation, and vascular disturbances induced by oxidative stress. The gross pathological changes observed in the cobalt-treated groups correlated strongly with the microscopic lesions identified in Plate 1, particularly vascular congestion, sinusoidal dilatation, and inflammatory infiltration. Similar hepatic congestion and hemorrhagic alterations have been reported in cobalt-induced hepatotoxicity studies involving experimental animals (Kadry & Ali, 2023; Hamad et al., 2025). The mild congestion observed in the beer-only group suggests that alcohol alone possesses moderate hepatotoxic potential, although the lesions were substantially less severe than those observed in cobalt-treated groups.

Histopathological examination provided the most significant evidence of cobalt-induced hepatotoxicity in this study. Plate 1A demonstrated normal hepatic architecture in the control group, characterized by intact hepatocytes, preserved sinusoids, and well-defined central veins. The preservation of normal liver architecture in the control group confirms that the histopathological lesions observed in the treatment groups resulted directly from cobalt and alcohol exposure. This finding is consistent with previous reports indicating that healthy hepatic tissues typically maintain organized hepatocyte arrangement and intact sinusoidal structures in the absence of toxic injury (Berasain et al., 2023).

Plate 1B, representing Group A (0.01 g/100 mL $CoCl_2$), revealed mild vascular congestion and early hepatocellular degeneration. These mild lesions suggest that low-dose cobalt exposure initiates hepatic injury primarily through early vascular and oxidative

mechanisms. Vascular congestion may impair hepatic blood flow and oxygen delivery, thereby predisposing hepatocytes to metabolic stress and degeneration. Chen and Lee (2026) explained that cobalt toxicity commonly involves oxidative injury and vascular dysfunction capable of initiating cellular degeneration even at relatively low exposure levels.

Moderate hepatocellular degeneration accompanied by inflammatory cell infiltration and congestion was observed in Plate 1C corresponding to Group B (0.02 g/100 mL $CoCl_2$). The progression from mild degeneration in Group A to inflammatory infiltration in Group B suggests activation of hepatic inflammatory pathways following sustained cobalt exposure. Inflammatory infiltration observed microscopically may represent recruitment of immune cells in response to oxidative stress-mediated hepatocellular injury. Similar inflammatory responses have been documented in cobalt chloride-induced hepatic injury where reactive oxygen species (ROS) trigger cytokine release and inflammatory activation (Hamad et al., 2025; Iji et al., 2023).

More severe pathological alterations were observed in Groups C and D as shown in Plates 1D and 1E. These groups demonstrated marked fatty changes, severe vascular congestion, sinusoidal dilatation, inflammatory infiltration, and hepatocyte necrosis. The fatty vacuolation observed in these groups indicates impaired lipid metabolism and accumulation of intracellular fat droplets within hepatocytes. Alcohol metabolism itself disrupts mitochondrial β-oxidation and promotes triglyceride accumulation in hepatocytes, while cobalt-induced oxidative stress further impairs lipid metabolism and membrane integrity (Gilpin & Molina, 2026; Berasain et al., 2023). The combined effect of alcohol and cobalt exposure may therefore have synergistically enhanced hepatic steatosis and cellular degeneration observed in the present study.

The hepatocyte necrosis observed prominently in Plate 1E suggests advanced hepatocellular injury associated with high-dose cobalt exposure. Necrosis may result from severe oxidative stress, ATP depletion, mitochondrial dysfunction, and irreversible membrane damage induced by excessive reactive oxygen species generation (Marcheggiani et al., 2026). Teschke (2022) explained that cobalt and other heavy metals induce molecular pathways involving lipid peroxidation, oxidative stress, inflammatory activation, and mitochondrial injury, ultimately resulting in hepatocyte death. Karagözoğlu et al. (2026) similarly reported severe oxidative stress-mediated hepatocellular degeneration and necrosis in rats exposed to cobalt chloride. The present findings therefore corroborate existing evidence that cobalt toxicity produces progressive dose-dependent hepatic necrosis (Hullon et al., 2026; Zhang et al., 2025).

Sinusoidal dilatation observed in Groups C and D further indicates disruption of hepatic microcirculation and vascular architecture. Severe sinusoidal expansion may impair nutrient and oxygen exchange within hepatic tissues, thereby aggravating hepatocellular degeneration and inflammatory injury. The correlation between sinusoidal dilatation observed microscopically in Plate 1 and gross vascular congestion observed macroscopically strongly supports the occurrence of progressive vascular pathology in cobalt-induced hepatotoxicity.

Interestingly, the beer-only group (Plate 1F) demonstrated only mild vascular congestion and slight hepatocellular degeneration compared with the more severe lesions observed in cobalt-treated groups. This observation suggests that although alcohol alone possesses hepatotoxic properties, cobalt enrichment substantially amplifies hepatic injury. Alcohol use disorder has been recognized as a chronic disease associated with inflammatory and metabolic disturbances capable of compromising liver function (Gilpin & Molina, 2026). Gan et al. (2025) further emphasized that chronic alcohol exposure remains one of the leading causes of progressive liver disease globally. However, the comparatively mild lesions observed in the beer-only group in this study suggest that cobalt chloride acts synergistically with alcohol to intensify oxidative and inflammatory hepatic damage.

The pathological findings observed in this study may largely be explained by oxidative stress-mediated mechanisms. Cobalt chloride has been shown to induce excessive generation of reactive oxygen species, depletion of antioxidant enzymes, mitochondrial dysfunction, and inflammatory signaling activation within hepatic tissues (Karagözoğlu et al., 2026; Hamad et al., 2025). Simultaneously, alcohol metabolism generates acetaldehyde and free radicals capable of further enhancing oxidative injury and membrane lipid peroxidation. The synergistic interaction between cobalt-induced oxidative stress and alcohol-induced hepatotoxicity may therefore explain the progressive severity of lesions observed across Groups A-D as shown in Table 2 and Plate 1.

The findings of the present study also support increasing concerns regarding process-induced toxicants and metallic contaminants in foods and beverages. Alizadeh et al. (2025) emphasized that contaminants introduced during industrial food processing may exert significant adverse health effects including oxidative injury, inflammation, and organ toxicity. The present findings therefore raise important public health concerns regarding exposure to metallic additives and

contaminants in alcoholic beverages and their potential contribution to chronic liver disease development (Stem et al., 2025; Zhang et al., 2026).

Overall, the present study demonstrated that prolonged exposure to cobalt-enriched beer induced significant dose-dependent hepatocellular injury in Wistar rats. The observed pathological alterations including vascular congestion, inflammatory infiltration, fatty degeneration, sinusoidal dilatation, hepatocellular degeneration, and necrosis strongly indicate that cobalt chloride potentiates alcohol-induced liver toxicity through oxidative and inflammatory mechanisms. These findings contribute important contemporary evidence regarding the hepatotoxic potential of cobalt-containing alcoholic beverages and highlight the need for strict regulation and monitoring of metallic contaminants and additives in beverage production systems.

Limitation of the Study

Despite the significant findings obtained in this study, certain limitations should be acknowledged. First, the study utilized a relatively small sample size of thirty Wistar rats, which may limit the generalizability of the findings. Although the observed hepatocellular alterations were significant, larger experimental populations may provide more robust statistical power and broader toxicological interpretation.

Secondly, the study focused primarily on histopathological assessment of hepatic tissues without evaluating biochemical markers of liver function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, or oxidative stress biomarkers. Inclusion of these biochemical parameters would have provided additional insight into the functional and molecular mechanisms underlying cobalt-induced hepatotoxicity.

Another limitation is that only male Wistar rats were used in the experiment. Sex-related physiological and hormonal differences may influence toxicological responses; therefore, inclusion of female animals could have provided a more comprehensive understanding of cobalt-induced hepatic injury.

The study was also limited to a single duration of exposure (45 days), which may not fully represent long-term chronic exposure conditions commonly associated with alcohol consumption in humans. Extended exposure studies may provide deeper insight into progressive hepatic fibrosis, cirrhosis, or carcinogenic changes associated with prolonged cobalt exposure.

Furthermore, only Hematoxylin and Eosin (H&E) staining technique was employed for histological evaluation. Advanced histochemical, immunohistochemical, and molecular techniques could have provided more detailed information regarding inflammatory mediators, apoptotic pathways, oxidative stress markers, and fibrotic changes within hepatic tissues.

Finally, although the study demonstrated significant hepatocellular alterations associated with cobalt-enriched beer exposure, the exact molecular mechanisms responsible for the observed hepatic injury were not directly investigated. Further studies involving molecular toxicology, oxidative stress assays, and gene expression analyses are therefore recommended to elucidate the precise pathways involved in cobalt-induced hepatotoxicity.

Conclusion

This study demonstrated that prolonged exposure to cobalt-enriched beer caused significant dose-dependent hepatocellular injury in Wistar rats. The observed alterations included reduced body weight gain, vascular congestion, inflammatory infiltration, hepatocellular degeneration, fatty changes, sinusoidal dilatation, and hepatocyte necrosis. Histopathological lesions were more severe in cobalt-treated groups compared with the beer-only and control groups, indicating that cobalt potentiates alcohol-induced liver toxicity. The findings suggest that cobalt-containing alcoholic beverages may pose serious hepatotoxic risks and highlight the need for strict regulation and monitoring of metallic additives and contaminants in beverage production. Regulatory agencies should enforce stringent quality control measures to minimize metallic contamination in alcoholic beverages.

Conflict of Interest - Nil

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