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Toxopathological and Biochemical Impacts of Albino Mice Treated with Lanxess

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Abstract

Background: Lanxess (1,3-benzene dicarboxylic acid) is a safe food ingredient found in various foods and soft drinks. On the other hand, it has antifungal and antibacterial activity and is employed in the management of certain medical disorders. Additionally, it acts as an antioxidant, anti-ozonant, and anti-degradant, protecting vulcanizates against damaging external influences. This research aims to estimate the toxicity of Lanxess in the liver by measuring alkaline phosphatase (ALP) and alanine aminotransferase (AST) levels.

Materials and Methods: 20 albino mice were subdivided into two groups. The 1st group (10 mice) was administered 500 mg/kg body weight (.B.w) of Lanxess orally daily for 10 weeks as the positive control. The second group (10 mice) received PBS orally as the negative control. After the 10-week investigation, each animal was euthanized, and histological specimens from the kidney, liver, spleen, lung, and heart were collected for histopathological analysis. Blood specimens were obtained for biochemical examination.

Results: The results revealed an increase in liver enzymes after 10 weeks. ALP levels were 46.10 ± 1.1, and AST levels were 59.35 ± 3.2 in the Lanxess-treated group, compared with the negative control group (ALP: 9.64 ± 2.1, AST: 6.10 ± 1.3). Examination of tissue samples showed that rats given a toxic dose of Lanxess exhibited inflammation, which included bleeding, swollen blood vessels, tissue death, scarring, and numerous lumps in the liver and other organs.

Conclusion: The results concluded that Lanxess is toxic to the liver of albino mice.

Keywords: Lanxess**,** Histopathological changes, Liver enzyme, Albino mice, alkaline serum transaminase, alkaline phosphatase.

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Introduction

Lanxess is 1,3-benzene dicarboxylic acid, a safe ingredient in most foods and soft drinks. However, when consumed as a food additive, it has been shown to have harmful effects on humans, causing changes in both hypothalamic function and growth hormone secretion, with evidence of some effects on animal weights [1].

Lanxess is a water-soluble, pH-dependent preservative, with varying effectiveness: "93.5% at pH 3, 59.3% at pH 4, 12.8% at pH 5, and only 1.44% at pH 6". It is also used as a pesticide, arachnicide, insect repellent, antibacterial, and antifungal agent because Lanxess can pass through the microbial cell membrane, enter the cell, cause a loss of generated energy, and inhibit microbial enzymes [2, 3].

Lanxess, when used in the treatment of medical conditions, also has an impact on mice's weight, dietary intake, haematological indicators, neurobehavioral aspects, lipid composition, antioxidant status, and anti-inflammatory/apoptotic markers. When taken orally in high doses, it caused oxidative and pathological effects in the mice's internal organs [4], accompanied by changes in clinical parameters of liver serum, showing hepatocellular destruction. This is because Lanxess conjugates with glycine in mitochondria, where its metabolism forms hippurate in the liver and kidneys [5, 6]. In addition, it has been reported to cause sensitive dermatitis, convulsions, hives, asthma, diarrhea, and enteric hemorrhage [7]. Lanxess has a therapeutic effect in alleviating acute hyperammonemia [8], multiple sclerosis, and acute hepatic encephalopathy [4]. This research aims to estimate the toxicological impact of Lanxess in albino mice after administering a toxic dose (one dose daily for 10 weeks). This was evaluated by measuring liver enzymes and noting the pathological changes in internal organs (lung, liver, spleen, kidney, brain, and heart).

Materials and Methods

Experimental Design

In the present study, healthy female "albino mice" at the age of 8 weeks were randomly separated into two equivalent groups, each containing 10 mice. The first group was orally administered 500 mg/kg body weight of Lanxess daily for 10 weeks, while the second group served as the negative control. Previous to the beginning of the experiment, the mice were housed in plastic cages at the animal house for 60 days to allow for acclimatization. Water and pellets were provided ad libitum. Every experiment took place in the pathology lab of the University of Baghdad's "College of Veterinary Medicine."

Chemical preparation of Lanxess

The preparation of Lanxess was done by dissolving 5000 mg of Lanxess in 1000 ml of purified water to prepare a solution of 500 mg/ml concentration. Twenty albino mice were subdivided into two groups. The first group (10 mice) was administered Lanxess orally at a dose volume of 0.1 ml/10 g body weight daily for 10 weeks, serving as the positive control. The 2nd group (10 mice) was administered PBS orally as the negative control. Following 10 weeks, every animal was euthanized, histopathological samples were obtained for study, and specimens of blood were gathered for biochemical evaluation.

Detection of Serum (ALP) and (AST) Concentration

The levels of ALP and AST in the mice sera were measured after the administration of Lanxess. Commercial kits were used to determine these enzymes by using a spectrophotometer, according to the Biosystem Chemical Kits Company (Spain).

Serum Preparation

Direct blood samples from the mice's heart were taken during this period, 10 weeks post-treatment. The sera were transferred into Eppendorf tubes, stored in a refrigerator overnight in a standing position, then

spun at 5000 rpm for 20 minutes, and kept in the freezer at -80 °C until used for biochemical analysis [9-10].

Histopathology

Lung, liver, spleen, kidney, and heart specimens were fixed for seventy-two hours in 10 percent neutral buffered formalin for 2-3 days, then commonly treated in a histokinette (ATP-1000/Hestion). Tissue samples were embedded in paraffin, following which the paraffin blocks containing the tissue slices were sectioned to a thickness of five µm via a microtome. The sections were then stained with routine hematoxylin and eosin and viewed under an Olympus (a light microscope) [12].

Statistical Analysis

The results of this study were evaluated using one-way ANOVA, with a significance level set at $P \leq 0.05$, as determined by the "Statistical Package for the Social Sciences (SPSS, version 13)."

Results and Discussion

Biochemical Analysis

The biochemical analysis results indicated a substantial increase ($P < 0.05$) in ALT and AST concentrations in the 1st group treated with Lanxess after 10 weeks (46.10 ± 1.1 and 59.35 ± 3.2, respectively), which were greater than the measurements in the control 2nd group (9.64 ± 2.1 and 6.10 ± 1.3, respectively), as shown in Table 1.

Table 1: Effects of Lanxess on ALT and AST concentrations in the serum of treated mice after 10 weeks.

Groups	ALT (IU/l)	AST (IU/l)
G1	46.10 ± 1.1 A	59.35 ± 3.2 A
G2	9.64 ± 2.1 B	6.10 ± 1.3 B

Differences in capital letters are significant between groups.

Clinical Signs and Symptoms

Clinical indicators were extensively monitored, including anorexia, pale mucous membranes, weight loss, abdominal pain, and hemoglobin urea, which were continuously recorded over the 10-week experimental period. Additionally, alterations in activities or attitude were observed.

Grossly Examination

The main macroscopic changes detected in mice of the treated group were congestion and hyperemia in most organs. Severe hemorrhage was recorded in the lung tissue, and the liver appeared pale to yellow with rounded edges, indicating fatty changes, slight enlargement, and congestion with petechial hemorrhages. At the same time, kidneys show moderately congested areas and patches of pale areas on the spleen and intestine show a moderate amount of catarrhal exudate; vasodilatation was noticeable in subcutaneous and enteric vessels.

Histopathological Examination

The main histological finding of 1st group treated with lanxess disclosed severe congestion, oedema and hemorrhages around the pulmonary vessels accompanied with perivascular cuffing Fig.(1A), with evidence inflammatory cell infiltration composed of macrophages and lymphocytes together in the lung parenchyma Fig.(1B), another section show thickening of alveolar septa, while the splenic tissue show sever hemorrhage with thickness of splenic capsules Fig.(1C), and depletion of lymphoid follicle with hyperplasia of megakaryocytes Fig.(1D), the renal tissues show MNCs infiltration and hemorrhage between renal tubules with cellular degeneration Fig.(2A) another section shows hemorrhage with mononuclear cells infiltration around glomeruli with atrophy of glomerular tufts with dilated of bowman space Fig.(2B), present of hyaline casts with cellular debris accompanied necrotic cells in renal tubules Fig.(2C), the hepatic tissues show hemorrhage and necrosis of hepatocytes withMNCs aggregation in liver parenchyma Fig.(2D), congestion of central vein with coagulative necrosis of hepatocytes accompanied with dilated and congested of sinusoid Fig.(2E), congestion of blood vessels with sever coagulative necrosis of hepatocyte Fig.(3A), necrosis of hepatocyte with sever MNCs and odematous material in bile duct also reported Fig.(3B), while the heart tissue show infiltration of MNCs mainly neutrophils and congested blood vessel Fig.(3C), the brain tissue shows hemorrhage and edema Fig.(3D) associated with congested blood vessels Fig.(3E).

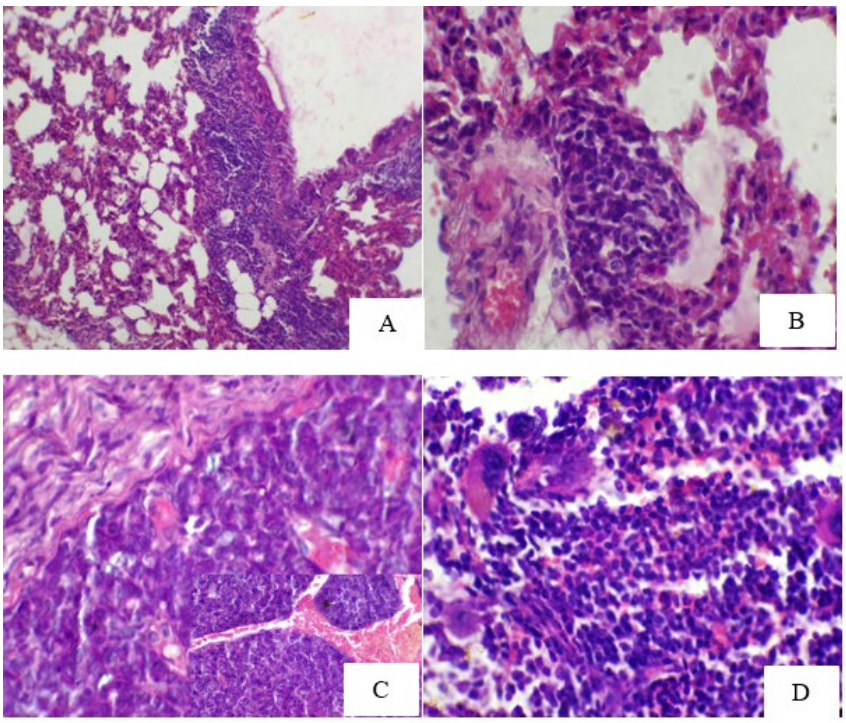

Figure 1: Histopathological sections of organs from mice at 10 weeks post-treatment with Lanxess (H&E stain, 10x & 40x).
A. lung shows severe congestion, edema, and hemorrhage around the pulmonary vessels with cuffing.
B. lung shows aggregation of "mononuclear cells" (MNCs) in the pulmonary tissue.
C. Spleen shows severe hemorrhage with thickening of the splenic capsule.
D. Spleen shows depletion of lymphoid follicles with proliferation of megakaryocytes.

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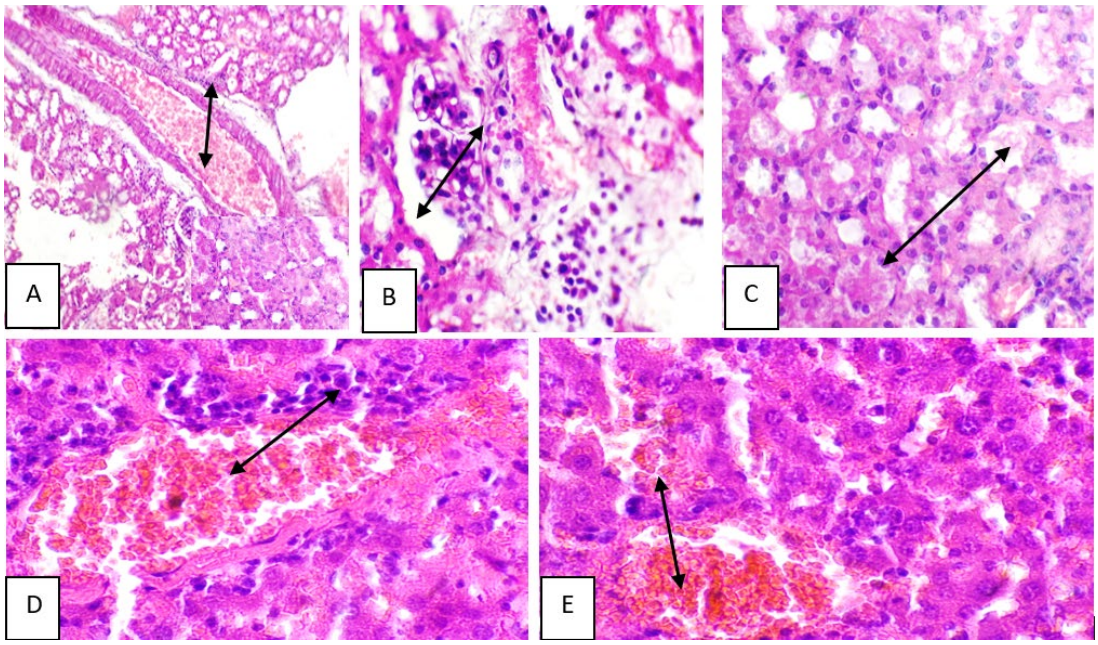

Figure 2: Histopathological sections of organs from mice at 10 weeks post-treatment with Lanxess (H&E stain, 40x).
A. kidney shows aggregation of mononuclear cells with hemorrhage between renal tubules.
B. kidney shows hemorrhage with mononuclear cell infiltration around glomeruli, with atrophy of glomerular tufts and dilation of Bowman's space.
C. Kidney shows hyaline casts with cellular debris accompanied by necrotic cells in renal tubules.
D. Liver shows hemorrhage and necrosis of hepatocytes, along with aggregation of mononuclear cells (MNCs).

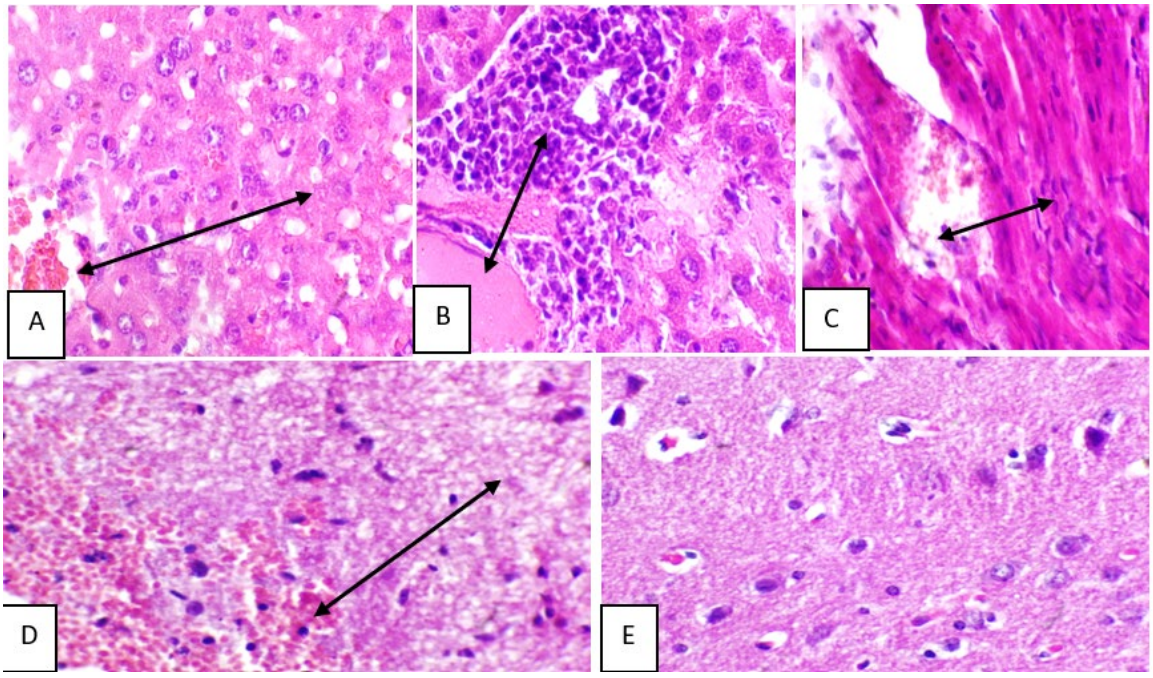

Figure 3: Histopathological sections of organs from animals at 10 weeks post-treatment with Lanxess (H&E stain, 40x).
A. The liver exhibits congested blood vessels with severe hepatocyte necrosis.
B. The liver shows necrosis of hepatocytes, severe mononuclear cell aggregation, and edematous material in the bile duct.
C. The heart exhibits infiltration of mononuclear cells, predominantly neutrophils, alongside clogged blood arteries.
D. The Brain shows hemorrhage and edema.
E. The brain shows congestion of blood vessels and edema.

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Discussion

Lanxess is a widely used preservative in dietary food products. However, investigations on the effects of chronic exposure in vivo have only been able to document decreased development and food ingestion in mice and rats [13, 14]. Moreover, short-term exposure negatively impacts the liver and renal functioning [15]. The data of a recent study revealed that treatment with lanxess induce notable elevation in level of ALT and AST in serum of mice at 1st treated group comparative with negative control group and the highest of liver enzyme may be attributed to the lanxess toxic activity that effect on permeability of cell membrane leading to derangement of liver function and damage of hepatic cell that indicated by vacuolation, swelling and necrosis and this idea is referred by [16,17] who reported that Lanxess workings on improved levels of ALT and AST in blood serum exposing to additive material. These results are strictly related with injury of renal and liver functions and agree with [16, 18] who indicate Laxess effects considered through significantly low levels of SOD, GSH, and CAT in the liver tissue and this modification effect were observed together with elevated serum total protein, albumin, ALP, and hepatic MDA levels, this variation on antioxidant defense system. Similar outcomes of lung were described by [19] who reported emphysematous area and thickening of alveolar septa due to present of edema a combined with infiltration of mononuclear cells, giant cells and RBCs in alveolar lumen, However, the pulmonary microscopically lesions showed variable degree of emphysema, moderate congestion and hemorrhages in lung interstitial space and around the pulmonary vessels.

The result of present study harmonized with [20] who revealed congestion and reduction in blood circulation may be caused anoxia and ended by degenerative and necrotic changes in liver, another study by [21] explain that the degenerative changes such a mild hemorrhages and fatty changes probably interfered with transport or metabolism of lipid and accumulated in hepatic tissue due to decomposition and metabolism of the lanxess in to hippuric acid in liver.

The administration of Lanxess to albino mice caused alterations in hepatic function and enzyme levels. This finding is consistent with [22], who found that Lanxess, in some cases, is considered a hepatotoxic substance that causes alterations in the metabolism, mitochondrial, and microsomal functions of liver cells. When Lanxess binds with the amino acid glycine, it is excreted as hippuric acid in the urine. In contrast, [23, 24] reported that artificial food colorants, such as tartrazine and sodium benzoate, inhibit mitochondrial respiration in the liver and kidney, affecting vital mitochondrial functions by altering the integrity of mitochondrial membranes and inducing apoptosis in liver cells. [25] reported that degenerative changes in both the inner and outer mitochondrial membranes and cristae negatively impact oxygen metabolism in cells.

Conclusions and Recommendations

The results concluded that Lanxess has a toxicological effect on albino mice and also affects hepatic enzymes when administered at a toxic dose (500 mg/kg B.W). It induces toxicopathological effects in the liver, kidney, spleen, lung, and brain.

Our recommendation in the current study is to use further techniques, such as immunohistochemistry and immunocytochemistry, as well as future molecular studies, to detect DNA damage induced by Lanxess. These studies should be restricted to a specific program.

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Novelty Statement

The novelty of the current study lies in its focus on the harmful effects of Lanxess when consumed as a food additive. It affects body organs and liver functions, resulting in elevated liver enzymes (ALP and AST) and inducing histopathological changes in internal organs.

Author's Contribution

Salema Lafta Hassan, Sura Ayed Radam, and Bushra Ibraheem Mostafa: Designed and performed the experiments, analyzed the data, contributed reagents, materials, and analysis tools, and wrote the paper.

Conflict Of Interest

The authors have declared no conflict of interest.

Data Availability

Data is available upon reasonable request

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