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Histological study and Impact Assessment of Vitamin C on the Structural Changes of Mice Livers Induced by Ethanol

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Abstract

Ethanol have toxic effects on the microscopic structure of the adult mice liver and impact assessment for potential defense against these harmful effects of vitamin C. Twenty healthy female Swiss albino mice were randomly divided into four equal groups: Group A (control group): Animals were injected daily with normal saline for 4 weeks. Group B (ethanol treated group): animals were given a dosage of ethanol every day about 5g/kg b.wt for four weeks. Group C (ethanol+ vitamin C) (therapeutic group): Animals were ingested with ethanol ingestion in the same dosage as in group B one hour prior to vitamin C in a dose 12mg/Kg/b.wt for 4 weeks. Group D (ethanol+ vitamin C) (preventive group): Animals were ingested with vitamin C one hour prior to ethanol ingestion in the same dosage as in previous groups for 4 weeks. The ingestion was oral gavage using a feeding tube. By the end, the mice were killed by cervical dislocation (severing the spinal cord by applying the appropriate amount of pressure to the cervical (neck) vertebrae). After those livers removed, fixed in formalin 10% and processed for inspection under a microscope. The ethanol-treated group exhibited the highest total histological changes, reflecting severe hepatic injury characterized by steatosis, hepatocyte degeneration, necrosis, and inflammatory infiltration, while the preventive vitamin C group showed lower histopathological changes, indicating preservation of hepatic architecture with minimal fatty change and inflammation. Otherwise, the therapeutic group demonstrated intermediate scores, reflecting partial recovery of liver tissue with reduced but still detectable degeneration and inflammation. In conclusion alcohol abuse causes the severity of liver tissue damage and oxidative stress markers, The histological structure of the liver was preventively enhanced by the administration of vitamin C.

Keywords: Ethanol, Mice liver, Impact assessment, Vitamin C.

1. Introduction

Acute and chronic ethanol consumption is associated with increased incidence of alcohol liver disease (ALD). Both alcohol's direct activity and that of its first metabolite, acetaldehyde, are responsible for its metabolic effects, which can also be linked to

redox state changes [1]. Ethanol intensifies oxidative stress, lowers antioxidant levels, and speeds up the production of free radicals that enhancement of lipid peroxidation [2].

Alcohol excess is associated with a spectrum of disease ranging from simple steatosis through steatohepatitis to cirrhosis and, in some, hepatocellular carcinoma. Alcoholic steatohepatitis itself has a variable histological picture, but a constant feature is the presence of ballooning degeneration of hepatocytes [3]. Cells are protected against oxidation by the action of certain enzymes, vitamins, and other substances, known collectively as antioxidants [4].

Alcohol and its metabolites disturb the regulation of lipid and glucose metabolism, increasing the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), which further drives oxidative stress and inflammation, ultimately leading to cellular and liver damage. These interconnected pathways not only drive a range of chronic diseases, including metabolic liver disease, Type 2 Diabetes Mellitus, Cardiovascular Disease, and obesity, but also provide a clear roadmap for developing targeted therapeutic strategies [5].

The aim of this work is to evaluate the toxic effects of Ethanol on the microscopic structure of the liver of adult female mice and impact assessment for possible protective role of vitamin C against these toxic effects.

1. The Material and Methods

Both vitamin C powder and ethanol alcohol are used in the current study and are purchased from nearby pharmacies in Iraq. This study uses twenty healthy female Wister albino mice that are three months old and weigh between (25 – 30) gm. Before the trial began, these animals were housed in plastic cages with homogenized wood shaving for bedding and kept at a regulated ambient temperature (23–25C^{o}) with a 12-hour light/dark cycle. They were allowed to acclimate for a week. The animals had packed optimal food and unrestricted access to water. Four equal groups were formed from the animals.

Group A (control group): Animals were injected daily with normal saline for 4 weeks. Group B (ethanol treated group): Animals were ingested daily with Ethanol that was diluted to a concentration of 20% in a dose of 5g/kg b.wt for four weeks. Group C (ethanol+ vitamin C) (therapeutic group): Animals were ingested with ethanol one hour prior to vitamin C ingestion in a dose 12mg/Kg/b.wt in the same dosage as in group B for 4 weeks. The ingestion was oral gavage using a feeding tube. Group D (ethanol+ vitamin C) (preventive group): Animals were ingested with vitamin C one hour prior to ethanol ingestion in the same dosage as in previous groups for four weeks.

Following the completion of the fourth week of the experiment, the animals were

slaughtered, and their livers were removed and used for histological examinations. The specimens were fixed with 10% neutral buffered formalin and processed to produce paraffin slices with a thickness of 5 µm, that sectioned by microtome then stained with hematoxylin and eosin stains thereafter examined by light microscopic.

2. The Result

The current study demonstrated the strong therapeutic potential of vitamin C in treating ethanol-induced oxidative stress in the liver. the clinical and anatomical findings resulting from administration of ethanol in mice showed weight loss, loss of appetite, lethargy and reduced activity, rough or shedding fur and movement disorders (tremors, poor balance). while anatomical symptoms result in redness of limbs, liver enlargement, enlargement of spleen and kidneys, congestion of internal blood vessels.

The histological symptoms can be summarized as follows:

Group A (Control group): Histological results from a light microscopic examination did not differ from those observed in other normal tissues, liver tissue shows normal hepatic architecture. Hepatocytes appear polygonal with centrally located nuclei, arranged in radiating cords around the central vein. Sinusoids are clearly visible and free from degenerative or inflammatory changes, indicating normal liver parenchyma (Fig. 1).

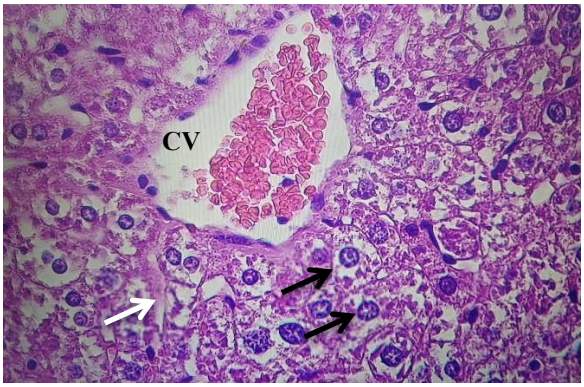

Figure 1: Photomicrograph of control group showed polygonal hepatocytes, central nuclei (black arrows), central vein (CV), sinuses channels (white arrow) .H&E.400X.

Group B (Ethanol group): Shows eosinophilic hyaline cytoplasmic inclusions within hepatocytes (Mallory bodies) and appearance of neutrophilic infiltration with ballooning of hepatocytes and nuclear changes (Fig. 2). Ballooning degeneration and disruption of the normal architectural organization (Fig. 3), impairment of endothelium-dependent relaxation with vascular damage (Fig. 4)

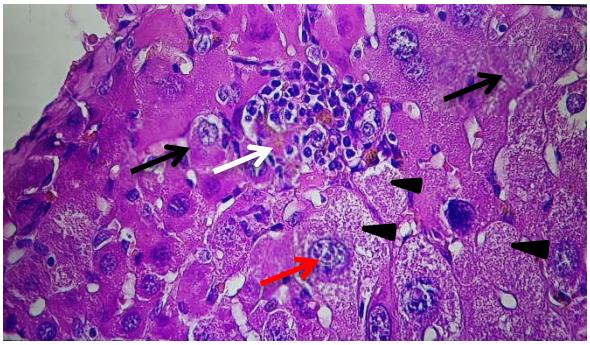

Figure 2: Photomicrograph of (Group B), showed ballooning of hepatocytes (black arrows) with Mallory bodies (arrow heads) and nuclear changes (red arrow), neutrophilic infiltration (white arrow). H&E.400X.

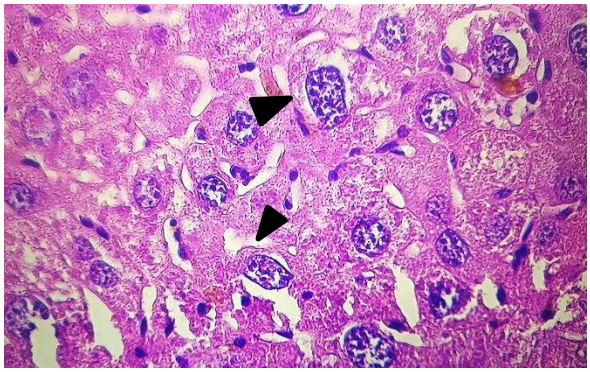

Figure 3: Photomicrograph of (Group B) showed Ballooning degeneration and disruption of the normal architectural organization (arrow heads). H&E.400X.

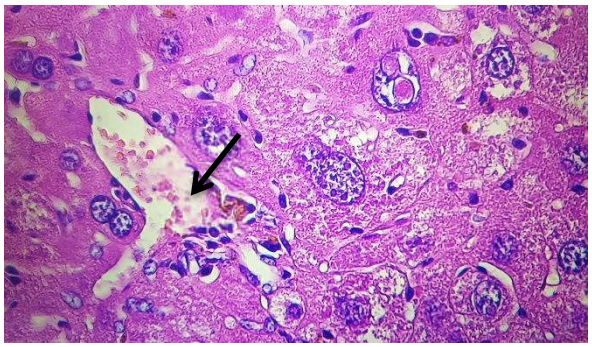

Figure 4: Photomicrograph of (Group B) showed impairment of endothelium-dependent relaxation with vascular damage of central vein (black arrow). H&E. 400X.

Group C (Therapeutic group): Shows partial disintegration and loss of hepatocytes, with cells appearing scattered and damaged (Fig.5). Partial appearance of Mallory bodies with approximately polygonal hepatocytes with central nuclei, healing ballooning degeneration, and normalization of the structural organization of the cells (Fig. 6)

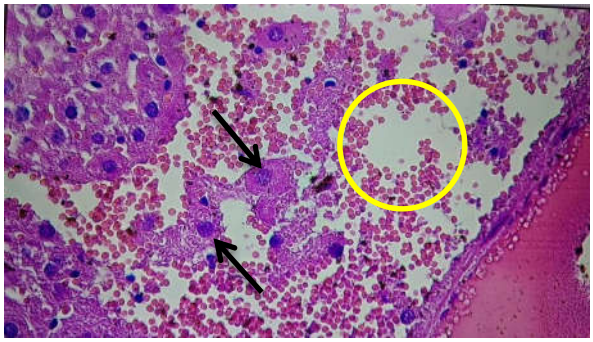

Figure 5: Photomicrograph of (Group C) showed scattered and damaged cells (black arrows) with loss of hepatocytes (circle). H&E. 400X.

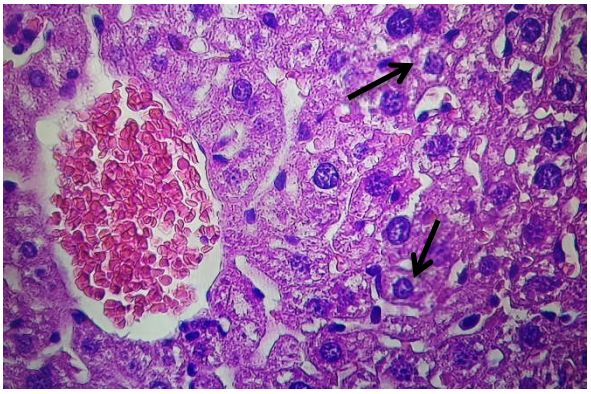

Figure 6: Photomicrograph of (Group C), showed healing of ballooning degeneration and polygonal cells (black arrows). H&E. 400X

Group D (Preventive group): showed hepatocytes are polygonal with centrally located nuclei, arranged in radiating cords around the central vein. Sinusoids are patent, with reduced fatty and degenerative changes compared to ethanol exposed tissue, lowers elevated inflammatory markers (Fig.7), endothelial swelling with congestion as well as thicken vascular walls with vascular dilation (Fig. 8).

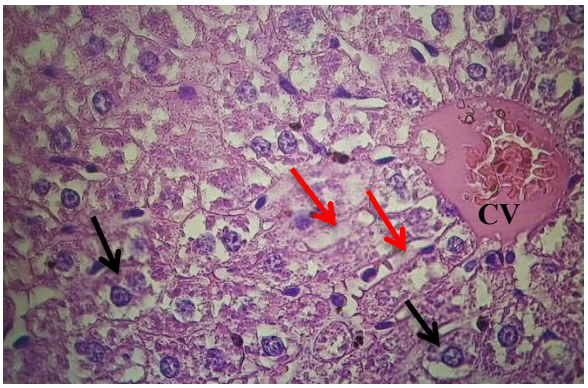

Figure 7: Photomicrograph of (Group D), showed hepatocytes with central nuclei (black arrows) arround central vein (CV). Visibility of Sinusoids (red arrows). H&E. 400X.

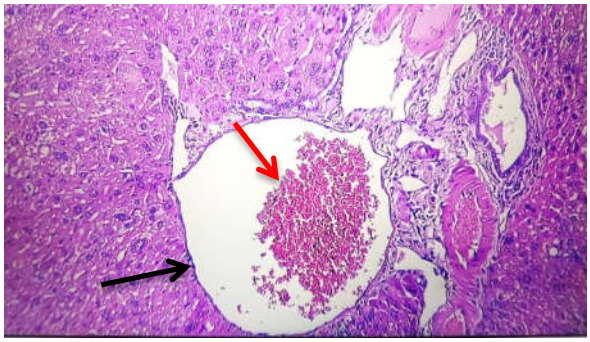

Figure 8: Photomicrograph of (Group D), showed endothelial swelling and thicken vascular wall (black arrow) with congestion (red arrow). H&E. 100X.

3. Statistical analysis

One-way ANOVA and LSD were used to compare the data, which were expressed as mean and standard error of mean. Statistical significance was defined as a P value greater than 0.05 [6].

Statistical Comparison of Liver in (Table 1) demonstrates that ethanol in (Group B) caused severe liver damage compared to the control group ($p < 0.001$). Vitamin C, whether therapeutic (Group C) or preventive (Group D), significantly reduced the damage ($p < 0.05–0.01$). Both approaches improved liver condition, but preventive treatment showed slightly stronger effects.

4- Discussion

The main advantage of this study is to clarify the degeneration of hepatocytes after exposure to factors that induce inflammatory reactions in the liver, and to demonstrate the dynamics of the process and its reversibility in hepatocytes through useful

Table 1: Statistical Comparison of Liver Damage Parameters Across Groups.

Group	Steatosis	Degeneration	Necrosis	Inflammation	Fibrosis	Total Score (Mean ± SD)	Interpretation
Control*	0	0	0	0	0	0.0 ± 0.0a	Normal
Ethanol*	3	3	3	3	2	14 ± 1 b	Severe damage
C (Preventive) **	1	1	0–1	1	0	3–4 ± 1 c	Mild damage
C (Therapeutic) **	1	2	1	1	0–1	5–6 ± 1 c	Moderate improver

*statistical significance was defined as $p < (0.01)$, ** $p < (0.05)$. Different small letters indicate significant effects between groups, while the same small letters indicate insignificant effects.

therapeutic strategies for a large proportion of liver cirrhosis cases, tis finding accords with results other study which mentioned the health problems motivated by many environmental pollutants [7]

As in the (Fig. 2-4) fibrosis and cirrhosis induced by ethanol. This agent causes direct injury to hepatocytes and trigger secondary inflammatory reaction in the hepatic parenchymal and nonparenchymal cells and result in fibrosis [8]. Ballooned hepatocytes by Mallory bodies (a granular and amorphous structures) that reflects a

response to oxidative stress were reported in this study and corresponding with other studies [9]. Polymorphonuclear neutrophils that caused by impairment of endothelium-dependent relaxation with vascular damage are involved primarily in the initiation of tissue injury in alcoholic liver disease, (ROS) The most prominent factors and proinflammatory mediators that involved in these processes, these findings that reported in other study mentioned that ALD cause noticeable rise in neutrophils and lymphocytes in the liver [10, 11].

Histological analysis in (Fig. 5, 6) confirmed that vitamin C effectively reduced oxidative damage and treated fat accumulation within hepatocytes. Even under continuous alcohol exposure, vitamin C improved hepatic architecture and restored normal tissue organization due to considered it as antioxidant as mentioned in other study that reported in extracellular fluids, vitamin C plays a crucial role as a free radical scavenger, capturing radicals and shielding biomembranes from peroxide damage [12, 13].

Histological analysis in (Fig. 7, 8) showed that the hepatocyte morphology, nucleus appearance, and radiolucent sinusoids were close to normal, with few degeneration changes, signs of inflammation and tightening vascular barrier due to vitamin C has pleiotropic pharmacological effects and demonstrates antioxidant qualities including scavenging reactive oxygen species and lowering oxidative stress that revealed in other studies by lowering fasting blood glucose in hypertensive and/or diabetic obese people, it may help reduce inflammatory diseases [14], whereas preventive vitamin C administration exhibited a slightly stronger inhibitory effect than therapeutic vitamin C administration [15].

The ethanol group recorded the highest damage score. However, both vitamin C groups showed a significant reduction. Interestingly, the preventive group had lower damage than the therapeutic group, as shown in the (Table 1) that demonstrates that the ethanol caused severe liver damage compared to the control group ($p < 0.001$), while vitamin C, preventive or therapeutic, significantly reduced the damage ($p < 0.05–0.01$). In summary, Vitamin C proved effective in mitigating ethanol-induced injury, with prevention being more beneficial. These findings also mentioned for non- alcoholic fatty liver disease [15].

4. Conclusion

In this study we concluded that alcohol abuse causes the severity of liver tissue damage, oxidative stress markers and many inflammatory diseases. Vitamin C supplementation improves the liver's histological structure, lowers oxidative stress, and repairs liver damage.

5. Acknowledgments

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