Ethanol-Induced Hepatic and Renal Histopathological Changes in BALB/c mice

Snur MA Hassan1*, Azad K Saeed1 Adel Jabbar Hussein2
1.Department of Anatomy and Histopathology/College of Veterinary Medicine/Sulaimani University/Iraq
2.Department of Anatomy and Histology/ College of Veterinary Medicine/Basrah University/Iraq
* E-mail of the corresponding author: snur.amin@univsul.edu.iq

Abstract
This study was to investigate the histopathologic changes of different concentrations of ethanol on the mice liver and kidney. Forty albino mice of the Mus musculus species, BALB/c strain mice underwent this study and were divided into four groups; control, %20, %40 and %60 of ethanol administration groups. The mice of each group (%20, %40 and %60 of ethanol) were orally administered with 1ml of ethanol 4days/week for 3 weeks. Hematoxylin and eosin staining indicated development of mild to severe lesions in kidney and liver which included; In %20 of ethanol administration group there was mild lesion development; hydropic swelling in liver and swelling of kidney parenchyma while in %40 of ethanol administration group developed moderate changes; hydropic swelling of hepatocytes and kidney tubules with hyaline degeneration and in %60 of ethanol administration group produced severe lesion; focal macro and micro abscess in liver parenchyma and focal neutrophil infiltration within renal parenchyma and hyaline cast within renal tubules. Based on our study, it can be concluded that ethanol intoxication leads to a various disorders of the liver and kidney which arrange from mild to severe injury which was depended on the concentration of ethanol.

Keywords: Ethanol, Mice, Kidney, Liver, H&E stain.

1. Introduction
Alcoholic beverages have been used in human societies since the beginning of recorded history. The patterns of alcohol intake around the world are constantly evolving and alcohol is ubiquitous today (Das and Vasudevan, 2006). In recent times alcoholism has become a perennial and pervasive problem gradually among people (Weer, 2010; Mukherjee et al., 2007). Ethanol is a small molecule soluble in both water and lipids therefore it permeates all tissues of the body and affects the vital functions. Ethanol affects almost all organs of the body so excessive ethanol intake leads to a variety of gastrointestinal, neurologic, cardiovascular diseases and malignant tumor (Anderson, 1993; Bellentani et al., 1997; Mandyam et al., 2004). The volume of consumption as well as the patterns of drinking, especially irregular, heavy drinking has been shown to determine the burden of disease. In other words, the impact of the average volume of consumption on mortality or morbidity is partly moderated by the way of alcohol, which is consumed by the individual (Room and Mäkelä, 2000). It is believed that other factors such as genetic susceptibility and dietary intolerance may be co-factors in alcohol-induced damage (Norberg et al., 2003).

Ethanol is removed from the body mainly by metabolism in the liver. Approximately 90% of the alcohol is metabolized in the liver, while the gastrointestinal tract, lungs, and kidneys play only a minor role (Lieber, 1994). Within the liver, there are three enzyme systems involved in the metabolism or clearance of ethanol. These enzymes are; cytosolic alcohol dehydrogenase (ADH), catalyses reversible oxidation of ethanol to acetaldehyde. In normal conditions, it accounts for about 80-90% of ethanol oxidation and the third enzyme is cytochrome P450-2E1 (CYP2E1) which is normally of minor quantitative importance, but under situations of sustained exposure to ethanol it can be induced several-fold. Ethanol oxidation may produce pathogenic effects in several ways as a result of an altered redox state, toxic products made by the induced CYP2E1, and the direct cellular toxicity of acetaldehyde (Chrostek et al., 2005).

Chronic ethanol administration in rodents has been demonstrated to induce a number of hepatic changes, including statues, hepatocellular necrosis, inflammatory cell infiltration, terminal hepatic venular sclerosis and tumor development (Watabiki et al., 2000; Soffritti et al., 2002; Beland et al., 2005).

Alcohol-fed animals were found to have significantly reduced renal function, interstitial edema and renal hypertrophy, characterized by significantly increased absolute amounts of protein, fat and water (Kumar and Vasudevan, 2008). Our goal is to determine the histopathologic effects of different concentrations of ethanol in the mouse's body, which included liver and kidney.

2. Materials and Methods
2.1 Study area
This study was conducted in two different locations; the first part of the study which dealt with housing and ethanol administration to mice in the animal house of the Biology Department / Faculty of Science/Slemani University; the second part which included sacrificing and taking a biopsy from liver and kidney which were
then stained with Haematoxylin and Eosin (H&E), which was performed in the Medical Teaching Hospital/Slemani Governorate.

2.2 Animal model
Forty adult albino mice (*Mus musculus* species, BALB/c strain mice), (20 males and 20 females) were used in this experiment, their weights were ranged from 20-25 gms. They fed with standard pellet diet (Pico Lab) and provided water and ad libitum. Animals were housed under a controlled room temperature of about 22-25ºC and photoperiodicity of the 12 hours light/12 hours dark.

2.3 Experiment design
After obtaining the approval of ethics committee, the animals were assigned into four groups; Control group (n=10; 5 males and 5 females) which were not taken ethanol at all, a second group (n=10; 5 males and 5 females) which were taken 20% of ethanol, a third group (n=10; 5 males and 5 females) which were taken 40% of ethanol and the fourth group (n=10; 5 males and 5 females) which were taken 60% of ethanol. Each mouse from each group taken 1ml of ethanol 4 days/week for 3 weeks (2 days taken ethanol by 1 day off).

2.4 Sampling method
At the end of the experiment, the animals were euthanized using ketamine hydrochloride (100 mg/Kg) and xylazine. Tissue samples were taken from kidney and liver. Specimens were fixed in 10% formalin for at least 24 hours and then routinely processed. The tissues were paraffin embedded and sections of 5µm thickness were obtained and stained with haematoxylin and eosin to detect any abnormal lesions formed by ethanol administration.

3. Results and Discussion

**Histopathologic examination**

3.1 Control group

3.1.1 Liver
Microscopically, the liver showed normal histological structure; normal central vein and sinusoidal capillary size with no evidence of congestion or narrowing and normal hepatocyte without any changes in their cytoplasm and nucleus (Figure 1a).

3.1.2 Kidney
Microscopically, renal section showed normal histological structure of glomeruli and renal tubules, the glomerular basement membrane was thin and delicate, mesangial cellularity and matrix were within normal limits with no evidence of shrinkage or swelling. The tubules (convoluted tubules and Henle loop) which were lined by cuboidal epithelial cells had a normal luminous appearance. The interstitial tissue was devoid of inflammatory cells and hemorrhage (Figure 1b).

![Figure 1. A. Kidney show normal histological structure of tubules and glomeruli, B. Liver show normal central vein and hepatocytes structures (H&E stain, X400).](image)

3.2 Group of 20% Alcohol administration

3.2.1 Liver
Microscopically, liver showed central vein and sinusoidal capillary dilation, which were engorged with red blood cells, infiltration of neutrophil close to central vein (Figure 2a,c), hydropic swelling of hepatocytes in centrilobular zone, which was characterized by a large, pale cytoplasm became vacuolated and appeared moth-eaten like with a normally (central) located nucleus (Figure 2b-d), intracytoplasmic Mallory bodies (Mallory’s hyaline), which were consist of eosinophilic intra cytoplasmic inclusions look like a cord in degenerating hepatocytes (Figure 2b). This finding is compatible with those studies reported that alcohol affect protein turnover. Mallory body-like inclusions formed in hepatocytes, indicating that the ubiquitinated cytokeratins accumulated as a result of the inhibition of the proteasome by ethanol administration when oxidation of ethanol induced oxidative stress (Fleming and McGee, 1984; Bardag-Gorce et al., 2006). Accumulations of dark brown-olive green bile pigment in the liver parenchyma. Specifically, bile accumulations were located in the canaliculi, which were on the apical side of the hepatocytes (Figure 2d). This finding is in agreement with previous studies (Di Padova et al., 1982; Schwesinger and Kurtin, 1984), whom proved that ethanol leads to secretion of significant levels of unconjugated bilirubin into bile. The role of this mechanism in pigmentation remains to be determined.

3.2.2 Kidney
Microscopically, kidney showed enlargement of glomeruli that occupied all bowman's capsule, the lining epithelium of renal tubules (convoluted and collecting) showed swelling (large pale cytoplasm) that forming star shaped lumen while in some of the tubules their lumen were blurred with presence of slight hemorrhage in the interstitial connective tissue (Figure 3a-d). Same lesion in studies (Assadi, 1989; Epstein, 1997), who documented that acute ethanol administration alters renal sodium and potassium excretion which means electrolyte imbalance and finally cause swelling.

Figure 2. A. Congestion of central vein and sinusoidal capillaries, centrilobular infiltration of neutrophil as indicated by white arrow (H&E stain, X100), B. Mallory body (White arrows); intra cytoplasmic eosinophilic cord like appearance in degenerating hepatocytes, C. Hydropic swelling; pale cytoplasm become vacuolated and appeared moth-eaten (Black arrows) like with a centrally located nucleus, D. Bile pigment; accumulations of dark brown-olive green bile pigment in the canaliculi as indicated by black arrows (H&E stain, X400).
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3.3 40% of Alcohol administration group  

3.3.1 Liver  
Microscopically, the liver showed central vein dilation, severe hydropic swelling (ballooning degeneration) in the centrilobular zone. Ballooned cells were typically two to three times the size of normal hepatocytes, which were characterized by a wispy cleared and distending cytoplasm by excess fluid with centrally located nuclei (Figure 4a,b). Study of (Chen et al., 2009), who documented that the administration of 4g ethanol/kg BW three times/week for 10 weeks in mice, affected livers showed moderate to severe fatty infiltration with numerous vacuoles in hepatocytes while in our study mice administrated with ethanol for 21 days only. The study that done by (Tsukamoto et al., 1986), in his study on rat proved that taking a small amount for long duration produce typical lesion in the liver, He increased ethanol intake progressively from 32% up to 47% of total calories to maintain sustained intoxication for 30 to 120 days. Light microscopic examination of the liver revealed moderate to severe fatty infiltration in all of the ethanol fed rats, of which 14 had spotty or zonal necrosis in the centrilobular areas accompanied by polymorphonuclear and mononuclear cell infiltration. In addition, fibrosis were observed in association with the necrotic lesions or with large-droplet steatosis. These two studies used ethanol for long duration therefore, lesions were more severe than which produced in our study (only three weeks). Because, as (Lieber and Decarli, 1989), proved that even a small dose for long duration leads to persistence of blood ethanol at high levels and severe lesion development.

3.3.2 Kidney  
Microscopically, kidney showed vascular congestion and hydropic or ballooning degeneration in which the cytoplasm of renal tubule had multiple opaque vacuoles present within the cytoplasm with centrally located nuclei and some glomeruli underwent atrophy (Figure 5a-d), haemorrhage of interstitial connective tissue, present of abundant brightly eosinophilic proteinaceous material in the lumen of henle loops (hyaline cast) as in figure 5d. These finding are compatible with study (Omoto et al., 1997), who documented that one month ethanol (4 g/kg b.w./day) exposure showed swelling of glomerulus, thickening of the basement membrane of glomerulus, dilation of tubular lumen, swelling of tubular epithelial cell, hyaline droplet in tubular epithelial cell and basophilic tubule in the kidney.
Figure 4: A. Severe hydropic swelling in the centrilobular zone (H&E stain, X100), B. Ballooned cells have wispy cleared and distending cytoplasm appearance with eccentrically located nuclei (H&E stain, X400).

Figure 5. A. Renal vascular congestion and hydropic swelling of kidney (H&E stain, X40), B and C. hydropic degeneration; the cytoplasm of renal tubules has multiple opaque vacuoles with centrally located nuclei and atrophy of glomeruli, D. hyaline cast; brightly eosinophilic proteinaceous material in the lumen of henley loops (Black arrow) and interstitial haemorrhage as indicated by white arrow (H&E stain, X400).
3.4 60% of Alcohol administration group

3.4.1 Liver

Microscopically, the liver showed focal infiltration of neutrophils and necrotic hepatocytes, which were aggregated forming micro and macro abscess surrounded by thin layers of fibrous connective tissues with present of the large amount of pinkish exudate, vascular congestion, hepatocytes that present at the periphery of inflammatory showed swelling with sinusoidal hemorrhage (Figure 6 and Figure 7a-c). This indicates that a higher concentration of ethanol increase ethanol levels in the liver and in the blood, which stimulate the largest amount of free radical formation, increases circulating endotoxins and proinflammatory cytokines that produce severe injury, liver parenchyma and hepatitis that is in agreement with (Thurman et al., 1998; Hoek and Pastorino, 2004).

3.4.2 Kidney

Microscopically, kidney showed multifocal vascular congestion, focal infiltration of neutrophil and necrotic cells, haemorrhage inside glomeruli and interstitial connective tissue with pinkish exudate, the lining epithelium of convoluted tubules were blurred in most of them just only nucleus remain, present of abundant brightly eosinophilic proteinaceous material (hyaline cast) in the lumen of renal tubules and henle loops, epithelium of proximal and distal convoluted tubules contained massive cytoplasmic aggregation of hyaline droplet, in some of them cytoplasmic droplets were coalesced (Figure 8 and Figure 9a-c). Alcohol administration led to a significant increase in the level of protein oxidation in the kidney of rodent (Amanvermez et al., 2005), followed by constantly increasing levels of reactive oxygen species which is partly generated from acetaldehyde oxidation, may also contribute to the occurrence of oxidative stress and nephrotoxic of ethanol ingestion that was confirmed by (Rodrigo et al., 1997; Calabrese et al., 2001).

Figure 6. A-C. Focal infiltration of neutrophils and necrotic hepatocytes forming micro and macro abscess (White circles), large amount of pinkish exudate (Black circles) and vascular congestion (H&E stain, X100).
Figure 7. A and B. Macro abscess; aggregation of neutrophils (White arrows) and necrotic hepatocytes surrounded by thin layers of fibrous connective tissues with present of the large amount of pinkish exudate, C. Micro abscess; aggregation of neutrophils surrounded by thin layers of fibrous connective tissues with present of the large amount of pinkish exudate, sinusoidal haemorrhage (Black arrows) with swelling the hepatocyte (H&E stain, X400).

Figure 8. A and C. Focal aggregations of inflammatory cells (neutrophils as in figure B indicated by yellow arrow) in damaged area, vascular congestion (Grey arrow), present of hyaline cast in renal tubules (Black arrow), A and C (H&E stain, X100), B (H&E stain, X400).
3. Conclusion

- Ethanol intoxication leads to a variety of disorders of the liver and kidney which arrange from mild to severe injury.
- The severity of effects depends on the concentration of ethanol, when the concentration of ethanol increased, the effect would be severe.

References


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