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Effects of Chronic Alcohol Ingestion on Visceral Organs in Albino Mice Experimentally Challenged with *Escherichia coli* Strain 0157:H7

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Abstract The aim of this study was to investigate the effect of chronic alcohol consumption on visceral organs when challenged with *E. coli* strain 0157:H7 using albino mice as an experimental model. Eight weeks old mice of both sexes (26.6 – 35.3g) were used in the study and were divided into 6 groups of 12 mice each using stratified random sampling method. Group 1 was given 10 % ethanol (V/V) in their drinking water. Group 2 received 20 % of ethanol. The third group received 30 % of ethanol while group 4 and 5 received 40 % of alcohol *ad-libitum* respectively. Group 6 served as control and received only water. The alcohol-receiving groups received ethanol for 3 weeks to establish a chronic state of alcoholism and Groups 1-4 were then challenged with *E. coli* strain 0157:H7 for 7 days. The mice were then humanely euthanized and dissected. The kidney, liver, stomach, intestine and spleen were collected and fixed in Boiun's fluid for 24 h and histopathology slides were prepared. The results showed that when the alcohol intoxicated animals were challenged with *E. coli*, about 90 % of the females in each of the different groups were died between 24-48 h. However, the females in group 5 (without *E. coli* challenge) survived. Postmortem examination of the animals showed that with increase in the concentration of alcohol in the groups, there was a concentration dependent decrease in the gross size of the kidneys. Gross finding from the carcass showed that there was a relative increase in the size of the stomach with increasing concentration of alcohol consumed. The stomach seemed translucent which was concentration-dependent. With an increase in the concentration, the stomach wall was more transparent. It was also observed that there was a decrease in the peritoneal fat with an increase in the concentration of consumed alcohol. Comparing the sizes of the spleen of the different groups, it was also observed that there was a gradual decrease in the degree of size enlargement of this hematopoietic/lymphatic organ with an increase in alcohol concentration in the presence of *E. coli*. Histopathology slides showed significant changes with increased necrosis on the spleen, liver and gastrointestinal tract (GIT) and other portal areas due to toxicity from secondary metabolic products of alcohol. The necrosis and quick death of the females was seen more in the alcohol challenged and *E. coli* infected groups. This was proposed to be as a result of hemo concentration and concurrent weakening of the lymphatic organs, allowing the toxins of the *E. coli* to act faster and destroy a larger number of cells. Chronic alcohol (ethanol) consumption has adverse pathologic effects on the spleen, liver, kidneys and gastrointestinal organs. Although alcohol is generally obtained from the fermentation of starch-containing food, its abuse and daily consumption causes damage to visceral organs and metabolisms in the body. Thus, when such a body is challenged with a pathogenic organism, there is less resistance to systemic entry of the cells, faster access to body cell due to the dehydration effect, and a quick necrotic time due to the toxins produced by such pathogenic organisms.

Keywords: *alcohol, ethanol, albino mice, Escherichia coli strain 0157:H7, spleen, liver, kidney, stomach*

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1. Introduction

Alcohol, especially ethanol is a by-product found in virtually all fermented sugar or starch containing foods. According to Stockley (1983), sugar fermentation into alcohol occurs regularly in nature through contact with air borne yeast. Throughout history, alcohol has been used socially for a lot of diverse purposes like calming feuds, giving courage in battle, sealing agreements, marriages

and festivals, seducing lovers and for religious and medicinal purposes (Stanton, 1996). The present day consumption of alcohol could be attributed to pressures that arise from work, family, peers or sometimes, false information from persons. Sometimes, alcohol consumption could also be recommended for the following reasons: it could be taken in moderate amounts to relieve anxiety and foster a feeling of euphoria (Valliant, 1983), increased blood HDL-cholesterol thus could antagonize cholesterol accumulation on the arterial walls, and lessen the risk of infarction (Langer et al., 1992), relieve constipation cases (Addolorato *et al.*, 1997). However, in all the above suggested conditions, it is only a moderate amount that is recommended. Alcohol has an addictive tendency, and thus moderation becomes a relative statement. When consumption becomes excess injurious effects like dementia (Oslin *et al.*, 1998), shrinkage of the brain owing to the loss of both white and gray matter (Kril and Halliday, 1999), elevation of the level of tissue plasminogen activator, a clot dissolving enzyme (Ridker *et al.*, 1994) and decrease in the likelihood of clot formation. Some 15 % to 20 % of idiopathic cases of idiopathic atria fibrillation may be induced by chronic alcohol use (Braunwald, 1997). Ventricular tachycardia may be responsible for the increased risk of unexplained sudden death that has been observed in humans who are alcohol-dependent (Kupari and Koskinen, 1998). These and many more conditions could emanate from the chronic consumption of alcohol.

In humans and animals, the occurrence of the disease condition Colibacillosis is common. Colibacillosis is caused by the pathogen *Escherichia coli*. The harmless strains of this organism are normally part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Bentley and Meganathan, 1982), and by preventing the establishment of pathogenic bacteria within the intestine (Hudault *et al.*, 2001; Reid *et al.*, 2001). However, sometimes these organisms leave the gut and relocate to other parts of the body, or in the gut, some strains develop traits that can be harmful to a host animal. These pathogenic strains typically cause a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world (Nataro and Kaper, 1998). More virulent strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immuno-compromised (Nataro and Kaper 1998; Hudault *et al.*, 2001).

In general, it is been shown that *E. coli* normally inhabits the gut area, but it is also known that alcohol has some eroding effect on the mucosal lining of the gut and some adverse effect on some organs. There is currently a dearth of information on the effects of alcohol consumption on *E. coli* infection either in human or animal models.

The objective of this work is to study the effects of alcohol consumption on visceral organs of mice experimentally infected with *E. coli* strain 0157:H7.

2. Materials and Methods

2.1. Preparation and Harvest of *E. coli* Strain 0157:H7

The bacteria were collected from an already identified and characterized stock (ECO 6) which has previously being maintained for research purposes in the Microbiology laboratory of the Department of Veterinary Microbiology and Pathology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. With a sterile wire-loop, some bacteria were streaked into a petri-dish containing Mac Conkey agar. A colony from here was harvested and sub-cultured on an Eosin Methylene blue agar to confirm that the colony was *Escherichia coli*. 100 ml of nutrient broth was prepared and 10 ml of the broth was put into 10 test-tubes. These were clogged with cotton-wool and autoclaved at 121 °C for 15 min. A colony of *E. coli* was harvested from the growth on the Eosin methylene blue agar growth and was introduced into the first test-tube. This was properly mixed and a 1 ml of the mixed solution was collected and transferred into the next test-tube. This process was used to achieve a serial dilution in all the 10 test-tubes. 20 ml of Mac Conkey's agar was poured into 10 universal bottles and these were autoclaved. After allowing to cool, 0.1 ml of the mixture in each of the above serially diluted test-tubes was introduced into each of the universal bottles, after a mild, yet thorough mixing, the mixture was poured into already sterilized Petri-dishes for growth of colonies. Twenty-four (24) h later, the plates were examined and colony growth was counted. The amount per dilution was used to ascertain the amount in the parent stock, and this was used to determine the volume for infecting the mice.

2.2. Animals and Experimental Design

Eight weeks old albino mice of both sexes (26.6 – 35.3g) were used in this study. The mice were sourced from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for 14 days before the commencement of the experiment and were fed with commercial feed (Vital feed®, Grand Cereal Oil Mill Ltd., Nigeria) and provided with clean water *ad libitum*. The albino mice were divided into 6 groups of 12 mice each using stratified random selection method, and were kept in clean cages in the Animal House of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Group 1 was given 10 % ethanol (V/V) in their drinking water. Group 2 received 20 % of ethanol solution. The third group received 30 % of ethanol while group 4 and 5 received 40 % of alcohol *ad-libitum* respectively. Group 6 served as normal control and received only water. The alcohol-receiving groups received ethanol for 3 weeks to establish a chronic state of alcoholism before groups 1-4 were then challenged with *E. coli* strain 0157:H7. Group 5 was not challenged with *E. coli*.

From the result obtained after the colony count, it was deduced that the parent stock contained about $2.3X10^8$ CFU/ml. 0.5 ml of the parent stock, which contains about 1.15×10^4 CFU and was used to challenge the mice intraperitoneally. All challenged animals were weighed 2 days after to evaluate the effect of the challenge on their weight. Seven days after the challenge, the mice in each group were humanely euthanized and dissected. The kidney, liver, stomach, intestine and spleen were collected and fixed in Boiun's fluid for 24 h and used for histopathology studies. Ethical conditions governing the

conduct of experiments with life animals were strictly observed (Ward and Elsea, 1977; Zimmerman, 1983; Anonymous 1996).

3. Results

3.1. Effect on General Behavioral Patterns

With an increase in the concentration of consumed alcohol, there was a corresponding increase in the excitation of the animals that is, being very restless. This observation was however not seen in the control group that was not receiving any dosing of alcohol. An increase in the concentration of the alcohol led to a corresponding decrease in the consumption of feed as the groups receiving alcohol were not able to finish their feed compared with before they started receiving the dose and subsequent challenge with *E. coli*. This however was not observed in the control group without alcohol. The groups that had a higher concentration of alcohol consumed less amount of fluid daily. Also, in those same groups, it was also noted that their litter remained dry. In the group that received 40 % alcohol, cannibalism was observed. This was seen in only this group of animals after they had consumed alcohol for about 3 weeks. When the animals were challenged with *E. coli*, about 90 % of the females in each of the different groups were lost between 24-48 h, leaving more of the males in each of the groups. It was also observed that the death was most imminent in the 40% group, and delayed further as the concentration reduced down to the 10% group. It was also observed that the animals in the 40% groups looked most shriveled and older than other animals used for the experiment. The skin of this group lacked any form of robustness although they had food supply ad-libitum.

3.2. Effects of Chronic Alcohol Ingestion on Stomach Size in Albino Mice Experimentally Challenged with *Escherichia coli* Strain 0157:H7

On postmortem examination of the animals, it was observed that there was a relative increase in the size of the stomach with increase in concentration of alcohol consumed. The stomachs did not appear bloated. However, they seemed as if they were translucent (lighter than the normal stomachs). This observation was not relative to a challenge to infection or not. The mentioned damage was an effect of alcohol as it was seen across all alcohol-intoxicated groups. (Figure 1).

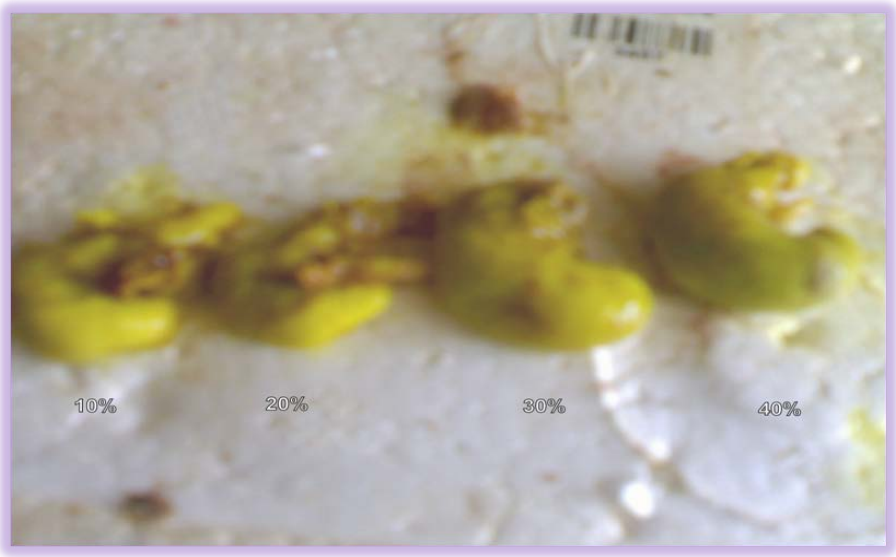

Figure 1. Gross photograph of the mice stomachs showing the variation in size with increase in alcohol concentration

3.3. Effect of Chronic Alcohol Ingestion on Kidney Size in Albino Mice Experimentally Challenged with *Escherichia coli* Strain 0157:H7

With the increase in the concentration of alcohol across the groups there was a concentration- dependent decrease in the gross size of the kidneys. This was seen as a shrinkage or atrophy in the size and possibly functions of this organ. It also was seen to be alcohol dependent and not due to the bacterial challenge (Figure 2).

3.4. Effect of Chronic Alcohol Ingestion on Spleen Size in Albino Mice Experimentally Challenged with *Escherichia coli* Strain 0157:H7

Comparing the sizes of the spleen of the different groups of animals, it was also observed that there was a gradual shrinking in size of this hematopoietic/lymphatic organ with an increase in alcohol concentration (Figure 3).

3.5. Effect of Chronic Alcohol Ingestion on Peritoneal Fat in Albino Mice Experimentally Challenged with *Escherichia coli* Strain 0157:H7

It was also observed that there was a decrease in the peritoneal fat with an increase in the concentration of consumed alcohol. For instance, on euthanizing the animals, Group 1 animals had more peritoneal fat than those in Group 4.

Figure 2. Gross photograph of the kidneys showing the various kidney sizes from the various alcohol exposed groups

Figure 3. Gross photograph of the spleen showing the varying sizes of the spleen with varying alcohol concentration

3.6. Histopathology of the Liver and Spleen

The histopathology slide shows liver section of Group 2 with central vein (V), areas of necrosis (N) and neutrophilic infiltration (arrows) at the centro-lobular area (Figure 4). Also, a liver section of Group 4 mouse showing severe areas of cirrhosis (C) and necrosis (N) around the portal triad (Figure 5). Spleen section of Group 1 mouse shows splenic nodules (N) with areas of lymphocytic depletion (D) around the central arterioles (A) (Figure 6).

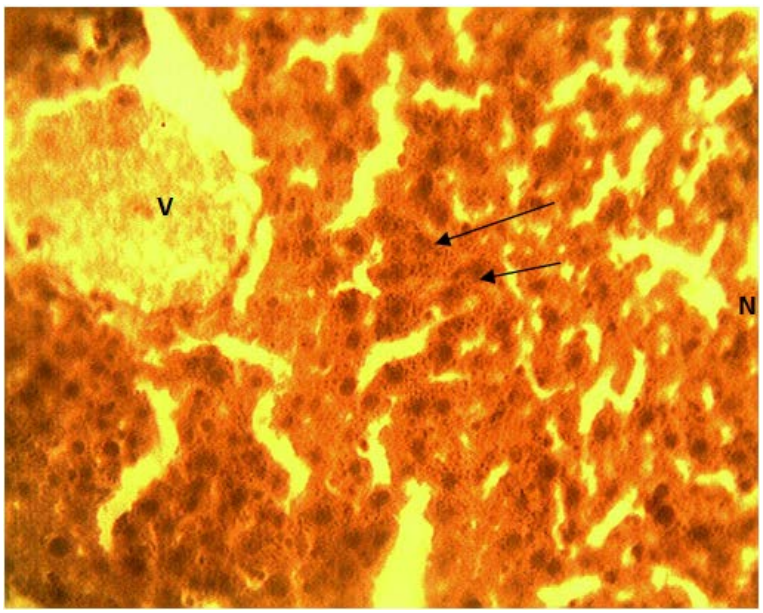

Figure 4. Liver section of 20 % ethanol exposed and *E. coli* challenged mouse showing central vein (V), areas of necrosis (N) and neutrophilic infiltration (arrows) at the centro-lobular area (H&E X400)

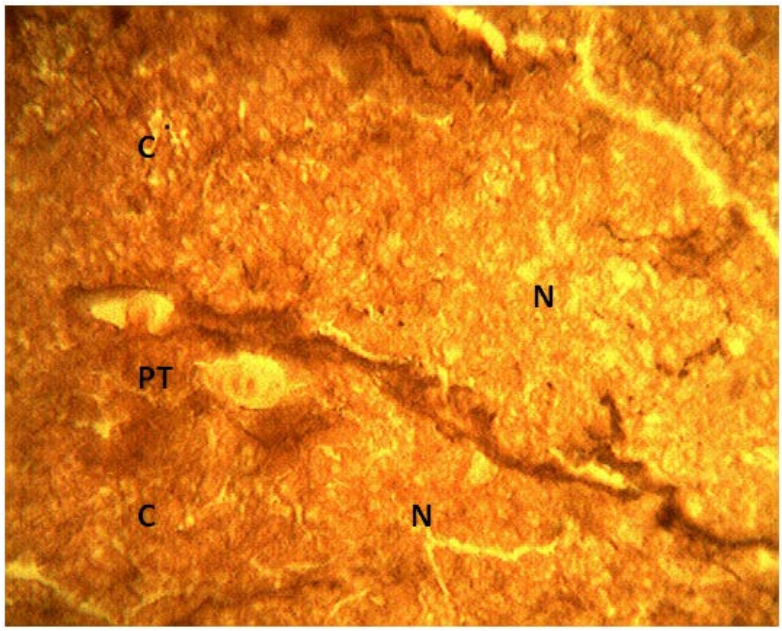

Figure 5. Liver section of 40 % ethanol treated mouse showing severe areas of cirrhosis (C) and necrosis (N) around the portal triad (PT) (H&E stain X400)

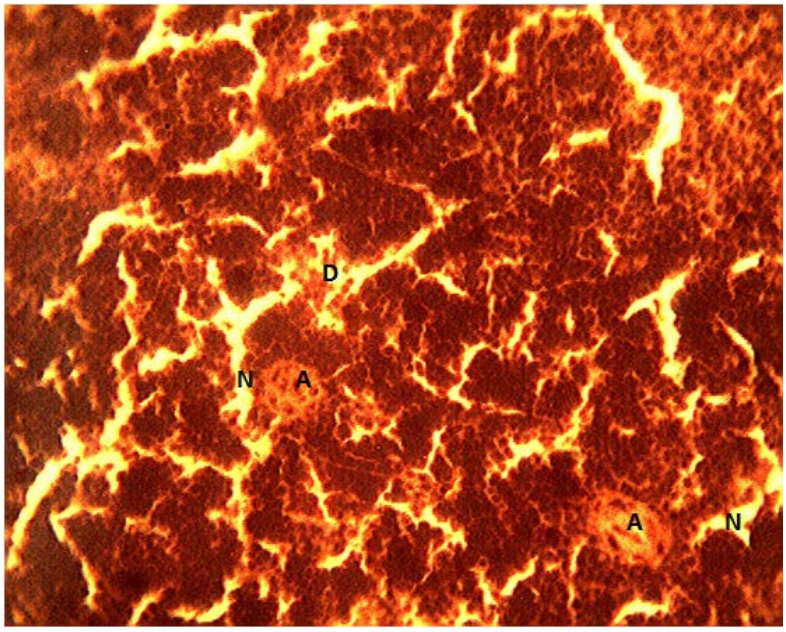

Figure 6. Spleen section of 10 % ethanol exposed and E. coli challenged mouse showing splenic nodules (N) with areas of lymphocytic depletion (D) around the central arterioles (A) (H&E stain X400)

4. Discussion

The observed excitation in the animals in this study is supported by prior findings of Valliant (1983). This is believed to be primarily due to the neuro-stimulatory effect of alcohol and was easily potentiated by *E coli* challenge which could have caused diarrhea, structural and functional changes in the small intestine and liver (Papa *et al.*, 1998). Also, the observed cannibalism among the 40 % alcohol-exposed group could be as a result of the shrinkage in the brain owing to the loss of both white and gray matter (Kril and Halliday, 1999). The frontal lobes are particularly sensitive to the damage by alcohol and the extent of damage is determined by the amount and duration of alcohol consumption. The shrinkage could have damaged the part of the brain responsible for recognition and olfactory not permitting the animals to decipher properly the difference between food and their fellow group mates (Volkow et al., 1994). Older alcoholics being more vulnerable than the younger ones (Pfefferbaum *et al*., 1998). The cause of death in the females in groups that were challenged with *E. coli* was not fully understood, but other findings related to this study have reported that female rats are less able to fight infection when intoxicated with alcohol than male ones (Spitzer and Zhang, 1996b; Li *et al.*, 1998; Spitzer, 1999). It is possible that gender differences in alcohol-related liver disease could be explained by gender differences in the breakdown and elimination of alcohol and its byproducts. This includes the resulting differences in acetaldehyde levels within the liver and the amount of alcohol that is metabolized in the stomach (first-pass metabolism) (Thomasson, 1995; Li *et al.*, 2000). Some

researchers have indicated that women break down less alcohol in the stomach than men, leading to higher blood alcohol levels and hence greater risk to the liver for a given dose of alcohol (Pozzato *et al.*, 1995; Baraona *et al.*, 2001). With an increase in the concentration of the alcohol in the groups, this could result to reduced life span (Ashley et al., 1977).

The results of this study also show that alcohol causes hemo concentration and this is more severe with an increase in the concentration of alcohol consumed. When such an animal is further challenged with septicemia, the dehydrated blood exposes more blood cells to the toxic effect of the infection. This brings about a more pronounced destruction of cell, tissues or organs affected. Necrotic tissues were seen more in the animals that consumed higher alcohol concentrations and were challenged with *E. coli*. This supports the assumption that the toxin released could elicit more necrotic damage due to the alcohol-induced dehydrated state. Here, the intestinal mucosa has flattened villi (not shown), and digestive enzyme levels are often decreased. The flattened villi and corresponding digestive enzyme levels could decrease the rate of peristalsis of food ingested, prolonging the time of absorption in the body and causing a reduction in the appetite of the animal, thus reducing the amount of food consumed by animals receiving alcohol (Baumgart et al., 2007). The differences in the size of the stomach (Figure 1) which were concentration dependent may be due to structural changes in the intestinal mucosa (Papa *et al*., 1998). The stomach seemed to have been chronically ulcerated making it void of a mucosa (not shown). Alcohol exacerbates the clinical cause and severity of ulcer symptoms. It appears to act synergistically with *Helicobacter pylori* to delay wound healing (Lieber, 1997a) which could be a replica of what is seen in the case of *E coli*. The stomach, being a component of the gastro-intestinal tract and, being the first organ to receive alcohol after the esophagus, will be normally eroded at high concentrations. It could also be as a result of increasing the pH of the stomach caused by the increased concentration of alcohol which finally deteriorates the mucosal lining of the organ (Baumgart et al., 2007).

The decrease in the gross size of the kidney was not fully ascertained but it could be as a result of shrinkage (Figure 2). Alcohol destroys the delicate balance of the ions and water in the body by altering the filtering ability of the kidneys. Studies have implied that acute alcohol consumption does not significantly change kidney-hemodynamics or sodium excretion in dogs, but these studies did not extend beyond 6 hours after alcohol ingestion nor involve challenge with *E. coli* (Heuvelink et al., 2002). In contrast, earlier studies that examined dogs for a longer period reported that a single dose of 3 g of alcohol per kg of body weight (g/kg) elevated plasma volume between 10 and 26 h following alcohol ingestion (Nicholson and Taylor, 1940). Although the exact mechanisms for how alcohol changes the kidney's ability to function are not clearly known, the changes in ionic concentrations have been studied in humans and in animals for many years. Kidney complications are even greater if a person has also been diagnosed with liver damage due to alcohol consumption (Meadows, 1999). Alcohol has been known to cause dehydration of tissues and increasing the water content of the blood which is eventually eliminated by the kidneys (Addolorato *et al.*, 1997).

The observed reduction in the peritoneal fat is thought to be caused as a result of a decrease in body glycogen as reported by Vernet *et al.* (1995). Muscle biopsies from heavy drinkers also reveal decreased glycogen stores and reduced pyruvate kinase activity (Vernet *et al.*, 1995).

The variation in the spleen size in the different concentrations could be attributed to a decrease in the activity in this organ (Figure 3). It was observed that 10 % presented more enlarged spleen edges than 20 %, which in turn was more enlarged than 30 % and 40 % respectively. An increase in the concentration of alcohol caused the lymphatic systems to shrivel and become less sensitive in the the presence of septicemia, and less active thus, increasing the capability of the infecting agent to induce more damage in the body (Figure 6).

The histopathological studies revealed areas of necrosis and neutrophilic infiltration which progressed further to cirrhosis to be due to an increase in the concentration of ethanol consumed which became severe with increase in concentration of the ethanol as seen in liver (Figure 4 and Figure 5) where alcohol produced a constellation of dose-related deleterious effects (Fickert and Zatloukal, 2000). The primary effects are fatty infiltration of the liver, hepatitis, and cirrhosis. Normal liver tissue is replaced by fibrous tissue. Alcohol can also affect stellate cells in the liver directly; chronic alcohol use is associated with a transformation of stellate cells into collagen producing, myofibroblast-like cells (Lieber, 1998; Szabo, 1999), resulting in the deposition of collagen around terminal hepatic venules (Worner and Lieber, 1985, Adachi et al., 1994). The histopathoogical hallmark of alcoholic cirrhosis is in the formation of Mallory bodies, which are thought to be related to an altered cytokeratin intermediate cytoskeleton (Denk *et al.*, 2000). A number of molecular mechanisms for alcoholic cirrhosis have been proposed. In non-human primate models, alcohol alters phospholipid peroxidation. Alcohol decreases phosphatidylcholine levels in hepatic mitochondria, a change associated with decreased oxidase activity and oxygen consumption (Lieber et al., 1994a, b). This liver damage observed led to the establishment of more necrotic lesions in *E. coli*-infected animals than in the non-challenged group. This could be that due to a loss of function of the hepatocytes permitted by a greater manifestation of invading pathogenic organisms. Acetaldehyde is thought to have a number of adverse effects, including the depletion of glutathione (Lieber, 2000), depletion of vitamins and trace metals, and decreased transport and secretion of proteins owing to the inhibition of tubulin polymerization (Lieber, 1997b). Acetaminophen-induced hepatic toxicity has been associated with alcoholic cirrhosis as a result of alcohol induced increases in microsomal production of toxic acetaminophen metabolites (Whitcomb and Block, 1994). Liver failure secondary to cirrhosis and resulting to impaired clearance of toxins such as ammonia also may contribute to alcohol induced encephalopathy. Alcohol may appear to increase intracellular free hydroxyl-ethyl radical formation (Mantle and Preedy, 1999), and there is evidence that endotoxins play a role in the initiations and exacerbation of alcohol induced liver disease. Hepatitis C

appears to be an important cofactor in the development of end stage alcoholic liver disease (Regev and Jeffers, 1999).

Rodent studies also show that animals are more vulnerable to infection after chronic or acute exposure to alcohol (Deaciuc, 1997; Cook 1998; Messingham *et al.*, 2002). This increase in susceptibility is equally dramatic in human patients who sustain traumatic injury (Smith and Kraus, 1988; Brezel *et al.*, 1988). Those who have consumed alcohol prior to their injury are six times more likely to die than are alcohol-free patients with comparable injuries (McGill *et al.*, 1995). The mechanisms responsible for this increased mortality are unknown, but it is thought that alcohol compromises the immune system's ability to quickly fight infection by unidentified invaders--a function of the innate immune system (Faunce *et al.*, 1997; Cook 1998; Messingham *et al.*, 2001).

5. Conclusion

From the concentration variance, increase in the concentration of alcohol causes more damage to visceral organs and in the presence of *E. coli*, there is little or no resistance to this damage. This damage by alcohol involves a combination of loss of function of some organs, decrease in glycogen content of tissues, destruction of epithelial cells in the gastrointestinal tract, and hemo-concentration of the animal system. Under this condition, any challenge encountered by the animal leads to death without much delay. Alcohol dehydrating effect damages the normal state of tissues, the more chronic this is, the more irreparable the tissues become. Further research should be done to elucidate the relationship between the reduced amounts of alcoholic fluid consumed, decreased urine excretion (oligouria) and other morphological changes in the anatomy of the animals with increasing variety of microorganisms on visceral organs.

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