Original Article

Alcohol-Induced Hepatotoxicity: Evaluation of Protective Effects of Vitamin C and Naproxen Based on Hematological and Histopathological Parameters in Rats

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ABSTRACT

Objective: To evaluate the protective effect of Vitamin C and Naproxen in Alcohol-induced Hepatotoxicity based on Hematological and Histological Parameters.

Study Design: Experimental Interventional Study

Place and Duration of Study: This study was conducted at the Anatomy Department of Al-Tibri Medical College and Hospital, Isra University Karachi from November 2018 to April 2019.

Materials and Methods: 60 albino rats were selected with an equal gender ratio and placed in three groups. Group A (Control Group) was given purified Ethanol for ten days, Group B (Prophylactic Group) was given Vitamin C and Naproxen prophylactically for seven days after which they were intoxicated with Ethanol for ten days, and Group C was simultaneously given Vitamin C, Naproxen, and Ethanol for ten days. Hematological and Histological parameters were then recorded, whereas data was analyzed using SPSS Version 24.0, and P-Value of ≤0.05 was considered statistically significant.

Results: Group A experienced a severe rise in hematological biomarkers, and a grossly damaged hepatic architectural change was also evidently observed histologically. Group B had also elevated hematological markers above the normal range but lower than Group A. Furthermore, they also had milder cellular damage compared to Group A. Group C produced excellent results by showing within standard range biomarkers and a remarkably near-normal hepatic architecture.

Conclusion: Hepatoprotective effects were observed in the rats' liver due to the antioxidant and anti-inflammatory effects of Vitamin C and Naproxen.

Key Words: Hepatocytes, Alcohol, Vitamin C

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INTRODUCTION

The use of alcohol is customary in many cultures, consumed as to enhance social well-being, relationships, and even health, however, alcohol abuse is common worldwide[1]. More than 50% of American consumes alcohol, with about 23.1% Americans taking part in heavy and binge drinking.

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Chronic alcoholism is said to be associated with 60 major types of diseases. This makes alcohol the third leading cause of disease and disability worldwide. Chronic alcohol use is associated with a spectrum of liver diseases ranging from steatosis, steatohepatitis to cirrhosis and hepatocellular carcinoma. The pathogenesis of acute and chronic alcohol consumption has various consequences in different cell types. This occurs because the production of reactive oxygen species (ROS) increases; simultaneously antioxidants productions are reduced[2]. ROS has always had substantial implications in many diseases, and one of them is acute and chronic alcohol treatment[(3,4)]. Alcohol represents the most common cause of chronic liver disease in most industrialized countries after hepatitis[5]. Ultimately this results in 2.5 million deaths worldwide due to alcohol, mostly due to alcohol liver disease (ALD). There is no definitive treatment available to treat the hepatotoxic effects of alcohol. However, studies have been conducted to see if certain substances can elicit a hepatotoxic effect.

Vitamin C is the redox form of Ascorbate and a physiological antioxidant that is various functions such as enhancing immune function, facilitating enteral uptake of iron, synthesis of collagen, catecholamine, carnitine, and improving tissue perfusion and oxygen, thereby mitigating organ dysfunction[6,7]. WHO lists it as an essential medicine, and studies suggest that its antioxidant effects can have a beneficial impact by protecting the liver from oxidative stresses[8,9]. Similarly, another commonly used therapeutic worldwide belong to the class of Nonsteroidal anti-inflammatory drugs (NSAIDs) called Naproxen. NSAIDs are the most commonly used class of analgesic agents, with 30 million users worldwide and over 100 million prescriptions being written out in the USA[10,11]. Naproxen was first introduced in 1976, and Naproxen sodium is approved in many countries for over the counter use. Naproxen, a member of the NSAIDs family, also inhibits prostaglandin synthesis by inhibiting the cyclooxygenase enzyme. Vitamin C and Naproxen are both essential medications used globally to treat various conditions; they both have a well-documented safety profile. With alcoholic intake also increasing in Pakistan and causing hepatotoxicity among chronic users, leading to alcohol liver disease, a study was conducted to assess Naproxen and sodium's protective effects on alcohol-induced hepatotoxicity.

MATERIALS AND METHODS

After seeking ethical approval from the concerned ethical committee, an experimental interventional study was carried out in the anatomy department of Al-Tibri Medical College and Hospital, Isra University Karachi Campus. Our study aimed to evaluate if there are protective effects of Naproxen and Vitamin C on alcohol-induced hepatotoxicity based on hematological and histological parameters. Animals were taken from the animal house of Al-Tibri medical college. Sixty health albino rats that weigh between 150-200 grams were selected, and all aged between 8-12 weeks. All of the albino rats were selected through a random sampling technique. The gender ratio of the albino rats was then kept at equal, after which we separated both of the genders during the study to prevent mating. The rats were all kept in plastic cages. All of the cages containing rats were kept in the anatomy department under controlled temperature (30^{o}C) with an equal light-dark interval (12/12 hour). The rats were all given a standard diet and water ad libitum. We purchased purified Ethanol 99.7%l, 500 mg Naproxen Tablet, and 500mg Vitamin C tablet from the local pharmacy located near the hospital for the experiment. Sixty albino rats were then divided into three groups, with each group consisting of 20 rats with an equal gender ratio. The following intervention was then carried out on each group:

Group A: Served as our positive control group and received purified Ethanol at 8ml/kg body weight for 10 days

Group B: we prophylactically gave vitamin C and Naproxen Sodium to this group at a dose of 100mg/kg and 5mg/kg, respectively, for 7 days, after which they were intoxicated with Ethanol for 10 days.

Group C: was simultaneously given Vitamin C, Naproxen, and purified Ethanol at doses 100mg/kg, 5mg/kg, and 8ml/kg, respectively, for 10 days.

All the rats were administered Vitamin C, and Naproxen Sodium between 9 am, and 11 am, while not being provided with any food during the night. Ethanol was given regularly after a one-hour interval through gastric gavage. After the completion of the dosing, all the rats were given anesthesia in a glass dissection. They were then euthanized, and the thoracoabdominal organs were exposed. Blood samples were then taken by carrying out an intracardiac puncture with 5CC syringes into tubes already labeled and having antisera present within them to detect serum levels of hepatic enzymes (ALT, AST, and GGT). The liver tissue was fixed using 10% formalin solution and cut into small pieces to create blocks. Fixed liver pieces were then processed in ascending alcohol concentration, cleared using xylene, and infiltrated using paraffin to make blocks. Rotatory microtome was used to cut thick sections floated in a water bath at 37°C for one minute. Tissues were then placed on a glass slide and fixed in the oven. Finally, the sections were then stained using hematoxylin and eosin for morphological and morphometric observation using a light microscope. Data were analyzed using the Statistical Package for social sciences (SPSS) Version 24. All variables and means were calculated, and results were expressed as mean ± standard error (Mean ± SE). Assessment of significant differences among groups was done using one-way ANOVA with post-hoc tukey's test and secondly student's test. The P-value was set at ≤ 0.05 to be statistically significant.

RESULTS

Figure 1: shows the Mean serum level of Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma Glutamyl transferase (GGT) in IU/L of rats in different groups.

Table 1: shows statistical analysis shows the Level of Significance between the different therapeutic groups

Figure 2: Shows Histopathological section of Liver taken from Group A

Figure 3: Shows Histopathological section of Liver taken from Group B

Figure 4: Shows Histopathological section of Liver taken from Group C.

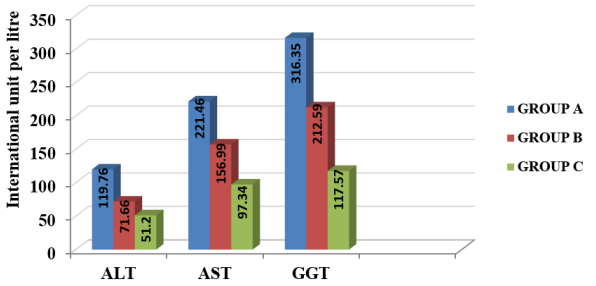

Figure No.1: Shows the Mean values of Serum ALT (Alanine transaminase), AST (Aspartate transaminase) and Serum GGT (Gamma Glutamyl transferase) IU/L among different therapeutic groups

Table No.1: shows the Level of Significance between the different therapeutic groups

Significance of Mean Difference between the Groups			
Comparison Between the Groups	Serum ALT	Serum AST	Serum GGT
A vs B	0.00	0.00	0.00
A vs C	0.00	0.00	0.00
B vs C	<0.001	0.00	0.00

One-Way ANOVA was applied followed by Post-hoc tukey's test
P=<0.05

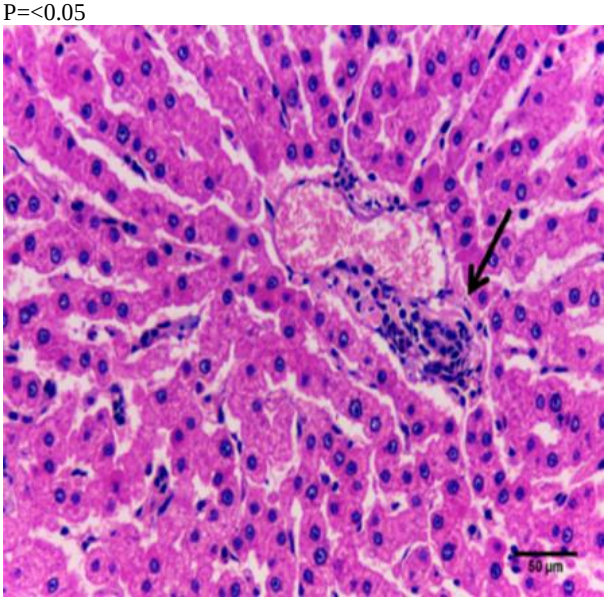

Figure No.2: Shows Histopathological section of Liver taken from Group A

Liver section of group (A) showing damaged hepatocytes. Markedly dilated sinusoids, highly increased number of inflammatory cells (Thick arrows), and necrotic debris. Congested portal vein (PV) and hepatic artery (HA). Abundant clear cytoplasmic vacuoles (Arrowheads) present. Hepatocytes plates were damaged.

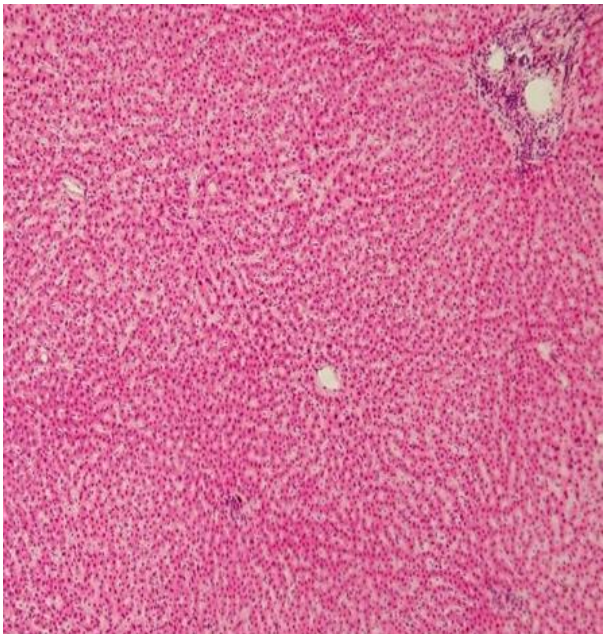

Figure No.3: Shows Histopathological section of Liver taken from Group B

Liver section of group (B) showing the mildly distorted hepatic architecture of lobule. Hepatocytes damaged and mild infiltration of immune cells (Thick arrows). Dilated sinusoids (Thin arrows) and portal vein (PV). Considerable numbers of clear cytoplasmic vacuoles (Arrowheads) are visible. Compared to group (A) figure is histologically better but in comparison to (C), it shows marked pathological changes.

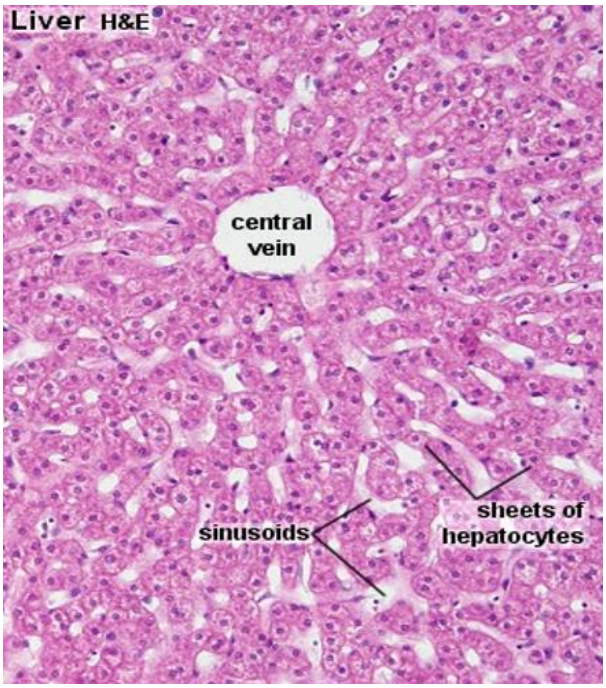

Figure No.4: Shows Histopathological section of Liver taken from Group C

Liver section of group (C) showing intact histologic arrangement. The hepatocytes (Thin arrows) are arranged as single-cell thick radiating plates surrounding the central vein (CV) with narrow sinusoids (Thick arrows) placed in between. Portal vein (Arrowheads) normal in diameter. No congestion and rare inflammatory cells are present. Almost absent cytoplasmic vacuoles. Much better histology in comparison to (B) and excellent in comparison to (A).

DISCUSSION

Alcoholic hepatitis is a prevailing complication not just in the west but all around the world and within our society. The liver becomes severely damaged, causes severe morbid complications. Consequently, 40% of individuals aren't even eligible for a liver transplant due to their chronic alcohol use. With its ability to neutralize free radicals and the potent anti-inflammatory effects of Naproxen sodium, Vitamin C was studied to see if it may have a hepato-protective effect.

We assessed the hepato-protective effects based on two parameters, Hematological and Histological. ALT, AST, and GGP are commonly used hepatobiliary markers; an increase in these enzymes indicates a hepatocellular injury or bile obstruction[12,13]. There was a remarkable rise in all of the hepatobiliary markers in group A, which also corresponds with the cells' histology. The rise is due to degeneration and necrosis of hepatocytes resulting in leakage of these enzymes into the blood; these findings are similar to another study of Zaidi et al. (2005), who also showed an increased level of transaminases after hepatocellular damage[(14)]. Compared to group B, the levels were significantly better than what was in group A but still above the normal ranges. This was because of the reduction in oxidative stress that was accomplished by the antioxidant effects of Vitamin C, thereby protecting the cells' vitality and minimizing cellular leakage. Similar results were also reported in another study[(15)]. However, the best results were produced by group C in which simultaneous infusion of both vitamin C and Naproxen lead to transaminase levels being marginally above the normal upper limits but within limits of normal values. Both therapeutic agents had a significant impact on keeping the levels of ALT in normal ranges.

Hepatocellular degeneration caused directly by Ethanol was observed in the liver of rats treated with alcohol for ten days. Vacuolar degeneration was also observed, along with changes in the generalized architecture of the hepatic lobules. These histopathological findings were also seen in other previous studies [(16, 17)]. Necrosis in the pericentral region was also visible along with an increased number of mononuclear inflammatory cells is also seen under the microscope. All these points towards severely damaged hepatic tissue seen in group A rats. Group B rats, on the contrary, showed milder damage as compared to group A. This probably has to do with the anti-inflammatory effect of Naproxen along with the antioxidant effect of Vitamin C halt severe hepatotoxicity caused by Ethanol. These findings are in line with other previous studies carried out by researchers[(18-20)]. Remarkably, the nearly standard architecture of hepatic lobule was observed in group C. Our results thus prove that there are hepato-protective effects of Naproxen and Vitamin C. However, further studies need to be carried out to see if other antioxidants (such as Vitamin A and E) or anti-inflammatory agents such as other members of the NSAIDs family or Steroids can replicate the same effects that were seen in our study.

CONCLUSION

Group B and C both had positive results and showed a significant difference when it came to hematological and histological parameters compared to group A. This was mainly down to the protective affection by Vitamin C and Naproxen. Due to their particular antioxidant and anti-inflammatory actions, these two agents synergistically produced a positive effect on the liver of rats, even in the presence of alcohol intoxication. Further studies on other agents can be carried out to see if they can deliver the same hepatoprotective effect as these two managed to do.

Author's Contribution:

Concept & Design of Study:	Raja Faisal
Drafting:	Nazia Qamar, Jamil Ahmed Siddiqui
Data Analysis:	Ghazala Panhwar, Syed Liaquat Ali and Shahid Korai
Revisiting Critically:	Raja Faisal, Nazia Qamar
Final Approval of version:	Raja Faisal

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Enoch MA. Genetic influences on the development of alcoholism. Current psychiatry reports 2013; 1;15(11):412.
2. Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. Archives Toxicol 2009;1;83(6):519-48.
3. Bai J, Cederbaum AI. Adenovirus-mediated overexpression of catalase in the cytosolic or mitochondrial compartment protects against cytochrome P450 2E1-dependent toxicity in HepG2 cells. J Biological Chemistry 2001; 276(6):4315-21.

4. Magdaleno, Blajszczak, Nieto: Events Participating in the Pathogenesis of Alcoholic Liver Disease. Biomolecules 2017;27; 7(1):9.
5. Singal AK, Anand BS. Recent trends in the epidemiology of alcoholic liver disease. Clinical Liver Disease. 2013;2(2):53.
6. Biesalski HK, McGregor GP. Antioxidant therapy in critical care—is the microcirculation the primary target?. Critical Care Med 2007;1;35(9):S577-83.
7. Shaik-Dasthagirisaheb YB, Varvara G, Murmura G, Saggini A, Caraffa A, Antinolfi P, et al. Role of vitamins D, E and C in immunity and inflammation. J Biol Regul Homeost Agents 2013; 27(2):291-5.
8. Xu P, Li Y, Yu Z, Yang L, Shang R, Yan Z. Protective Effect of Vitamin C on Triptolide-induced Acute Hepatotoxicity in Mice through mitigation of oxidative stress. Anais da Academia Brasileira de Ciências. 2019;91(2).
9. Son YS, Ullah HA, Elfadl AK, Chung MJ, Ghim SG, Kim YD, et al. Preventive effects of vitamin C on diethylnitrosamine-induced hepatotoxicity in Smp30 knockout mice, in vivo. 2018;1;32(1):93-9.
10. Singh G. Gastrointestinal complications of prescription and over-the-counter nonsteroidal anti-inflammatory drugs: a view from the ARAMIS database. Arthritis, Rheumatism, and Aging Medical Information System. Am J Therapeutics 2000;7(2):115-21.
11. Laine L. Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient. Gastroenterol 2001;1;120(3):594-606.
12. Toor HK, Sangha GK, Khera KS. Imidacloprid induced histological and biochemical alterations in liver of female albino rats. Pesticide Biochemistry and Physiol 2013;105(1):1-4.
13. Arfat Y, Mahmood N, Tahir MU et al; Effect of imidacloprid on hepatotoxicity & nephrotoxicity in albino mice. Toxi Reports 2014;1:554-561.
14. Zaidi SM, Al-Qirim, Banu N; Effects of antioxidants vitamins on glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver. Drugs RD 2005; 6(3):157-165.
15. Kaplan MM; Serum alkaline phosphatase-another piece is added to the puzzle. Hepatol 1986;6: 526-528.
16. Hirsova P, Ibrahim SH, Verma VK et al; Extracellular vesicles in liver pathobiology: Small particles with big impact. Hepatol 2016; 64(6): 2219-2233.
17. Sánchez-Valle V, Chávez-Tapia NC, Uribe M et al, Role of oxidative stress and molecular changes in liver fibrosis, Curr Med Chem 2012;19(28): 4850-60.
18. Bharrhan S, Chopra K and Rishi P; Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. Am J Bio Sci 2010; 2(1):51-62.
19. Cuce G, Cetinkaya S, Koc T et al; Chemo-protective effect of viamin E in cyclophophamide-induced hepatotoxicity. Chem Boil Int 2015;5: 232:7-11.
20. Zhu H, Long MH, Wu J, Wang MM, Li XY, Shen H, et al. Ginseng alleviates cyclophosphamide-induced hepatotoxicity via reversing disordered homeostasis of glutathione and bile acid. Scientific Reports 2015;2(5):17536.