Original Article

Preventative Role of Vitamin C and Naproxen in Alcohol-Induced Liver Toxicity on the Basis of Gross Parameters: A Comparative Study in Rat Model

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ABSTRACT

Objective: To evaluate the preventative role of Vitamin C and Naproxen in Alcohol-induced liver toxicity by evaluating the gross parameters in albino rats.
Study Design: Experimental Study
Place and Duration of Study: This study was conducted at the Anatomy Department of Al-Tibri Medical College and hospital, Isra University Karachi Campus from Jan 2018 to Nov 2018.
Materials and Methods: 60 albino rats with equal gender distribution were selected based on the probability random sampling technique and placed in three groups of 20 again with an equal gender distribution. Group A was given only given purified Ethanol for 10 days and served as our positive control group, Group B was given Vitamin C and Naproxen Prophylaxis for 7 days after which they were intoxicated with Ethanol for 10 days, and Group C was simultaneously Administered Vitamin C, Naproxen, and Ethanol for 10 days. The initial body weight and final body weight, along with the absolute and relative liver weight of all the test subjects were recorded, and data analysis was conducted using SPSS 21.0 with P-Value ≤0.05 considered to be Statistically Significant.
Results: Group A animals experienced a weight loss; Group B did not experience a substantial weight loss, while Group C experienced a weight gain. No significant difference in body weight was seen between the three groups; however, there were significant differences in absolute and relative liver weight between all three groups. Group A rats experienced an increase in body weight, whereas the Weight of the liver in Group C animals remained constant.
Conclusion: The antioxidative and anti-inflammatory effects of Vitamin C and Naproxen demonstrated hepatoprotective effects on the liver, protecting it from alcohol-induced liver injury.
Keywords: Naproxen, Prophylaxis, liver toxicity

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INTRODUCTION

Alcohol is a widely consumed beverage around the world. Furthermore, it is also used in the preparation of different medications apart from being a part of many people's diets globally. Although alcohol is widely consumed in all parts of the world, its consumption has been shown to be associated with various health outcomes[(1,2)].

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Alcohol usage is a global issue, with estimates indicating it to be the world's third-largest risk factor, causing 60 different injuries and diseases. It is said that the chronic harmful use of alcohol results in nearly 2.5 million deaths annually, with most of them due to Alcohol liver disease (ALD) [(3).] Alcohol represents the most common cause of chronic liver disease in most of the industrialized countries, after hepatitis C[(4)]. Furthermore, obesity, female sex, genetic factors, and smoking can further module the host susceptibility in developing ALD [(5)]. The development of reactive oxygen species leading to oxidative stress is the primary pathogenesis associated with ALD [(6)]. Moreover, Link has also been suggested when it comes to inflammation and hepatic steatosis, contributing to alcohol liver disease [(7)].Vitamin C is an essential medicine listed by the World Health Organization and is known to be one of the most effective antioxidant agents available at our disposal [(8)]. Studies have shown that vitamin c, using its antioxidant effects, has helped in protecting the liver from oxidative stresses [(9, 10)].At the same time, Naproxen is a member of non-steroidal

anti-inflammatory drugs and is used for treating pain and inflammatory conditions. Naproxen inhibits prostaglandin synthesis by inhibiting the prostaglandin G/H synthase or COX enzyme[11]. As alcohol is a widely used medication and causes liver toxicity, we conducted a study to see if there is any preventative effect of Vitamin C along with the commonly used medication Naproxen in Alcohol liver toxicity.

MATERIALS AND METHODS

The interventional study was conducted by the Anatomy Department of Al-Tibri Medical College and hospital, Isra University Karachi Campus from Jan 2018 to Nov 2018 after taken an ethical approval of concerned institute. Total 60 locally bred and healthy male albino rats weighing 180-200 grams were selected based on a random sampling technique. The animals were kept in plastic cages, with each cage occupying 5 rats. The cages were kept under controlled atmospheric temperature (30°C) with regular 12:12 hours light-dark intervals. All of the rats selected for the study were given a normal rat diet and water ad libitum. We purchased Ethanol, 500 mg Naproxen tablet, and 500mg vitamin C tablets from the pharmacy store of the hospital. The selected 60 albino rats were then divided into 3 groups on the bases of therapy, with each group comprising of 20 rats.

Group A: Served as our positive control group and received purified Ethanol at 8ml/kg body weight for duration of 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, as well as purified Ethanol at doses 100mg/kg, 5mg/kg, and 8ml/kg, respectively, for duration of 10 days.

Rats were administered vitamin C and Naproxen Sodium between 10 am, and 11 am, while being kept on no food overnight. Ethanol was given to at one-hour interval through gastric gavage. All the rats were weighed using an electronic balance before the study's commencement, and repeated weight measurements were taken every 3 days. Once the study concluded, the rats were weighed once again for the final time before being euthanized under anesthesia. A midline incision was conducted to expose the thoracolumbar organs so that careful excision of the liver can take place. We then further calculated the absolute Weight of the liver once again and calculated the Relative Weight of the liver using the following formula:

Relative Weight of the liver =

$$\frac{\text{Weight of the liver (gm)} \times 100}{\text{Final body weight of liver (gm)}}$$

Once we had all the gross parameters (body weight, absolute Weight of liver, relative Weight of liver) data were analyzed using SPSS Version 21.0. All variables were expressed as mean ± standard error to judge the differences among the groups. Statistical analysis was done using ANOVA with posthoc Tukey's test and secondly student's test. The P-value of ≤0.05 was set to be considered statistically significant.

RESULTS

Figure 1: shows Mean initial and final body weight in grams among different therapeutic groups

Table 1: shows statistical analysis of the difference in body weight within the same group

Figure 2: shows Mean weight of Liver in grams among different therapeutic groups

Table 2: shows the statistical difference in absolute liver weight of rats (gm) of the different groups

Table 3: shows statistical analysis of the difference in relative liver weight (gm) of the different groups.

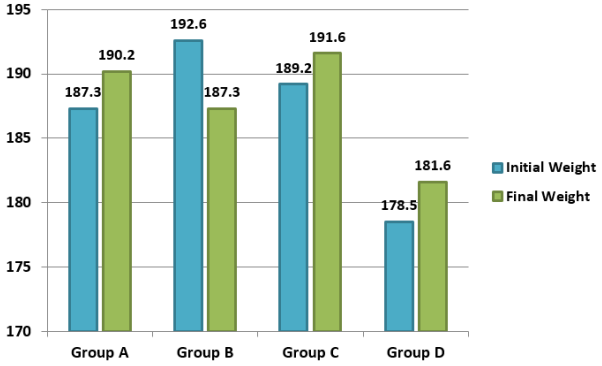

Figure No.1: Shows Mean initial and final body weight in grams among different therapeutic groups

Table No.1: Shows Comparative difference in body weight within the group

Comparison among groups	Difference (in grams)	P-value
final wt.vs initial wt. in group A	-2.47 ± 2.21	0.272
final wt.vs initial wt. in group B	-1.86 ± 0.46	0.001*
final wt.vs initial wt. in group C	1.34 ± 0.15	0.001*

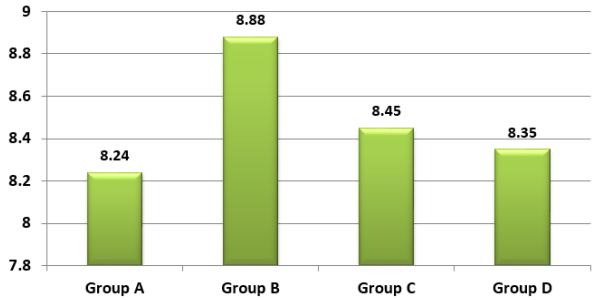

Figure No.2: Shows Mean Liver weight (gm) among different therapeutic groups

Table No. 2: Shows the Mean difference in the absolute liver weight of rats (grams) of the different groups.

Comparison	Parameters	Difference of mean	P-value
A vs. B	Positive control vs.Vitamin C & Naproxen (prophylactic)	0.27	<0.007*
A vs. C	Positive control vs.Vitamin C, Naproxen& Ethanol (simultaneous)	1.53	<0.001*
B vs. C	Vitamin C & Naproxen (prophylactic) vs. Naproxen& ethanol (simultaneous)	1.25	<0.001*

P<0.05 considered significant using Tukey's HSD test

Table No.3: Shows statistical analysis of the difference in relative liver weight (in grams) of the different groups

Comparison	Statistical parameters	Difference of mean	P-value
A vs. B	Positive control vs.Vitamin C & Naproxen (prophylactic)	0.26	<0.006*
A vs. C	Positive control vs. Vitamin C, Naproxen& Ethanol (simultaneous)	0.96	<0.002*
B vs. C	Vitamin C & Naproxen (prophylactic) vs. Naproxen& ethanol (simultaneous)	0.71	<0.001*

P<0.05 considered significant using Tukey's HSD test

DISCUSSION

The liver is the metabolic house of our body. It is crucial that it is protected and not overloaded with toxins that may damage it consistently, thereby causing impairment in detoxification, metabolism, and production of essential body proteins. Alcohol is notorious for damaging the liver and causing alcoholic hepatitis and fatty liver disease in individuals with chronic alcohol consumption. Treatment is usually not comprehensive, and in most scenarios, the best probable outcome is to perform a liver transplant. The drugs used in our study may prove to have a beneficial effect on the liver. The oxidative stress caused by the free radical can induce hepatotoxicity; however, the potent antioxidant effect of vitamin C produces a hepatoprotective effect on the liver.

Similarly, Naproxen is also an essential medication used to reduce liver inflammation caused due to alcohol. It is also a benefit that Naproxen has very minimal side effects can is a very safe medication to prescribe. We used both Vitamin C and Naproxen and assessed if they may have any protective action when it came to alcohol-induced liver toxicity. To assess their positive impact, we studied the gross parameters of rats. In Group A animals, we saw a reduction in the final weight of the rats, a finding very similar to what Rao et al. 2009 also showed in this study on gut barrier dysfunction and by Haouas et al. 2014 when studying the hepatotoxic effects of lead[10,11]. The cause of the weight loss in Group A can be linked to the inflammation due to alcohol intake. The prophylactic activity of naproxen and Vitamin C prevented any substantial weight gain in Group B. However, while evaluating Group C rats; we noticed a weight gain, similar to what Osfor et al. 2010 that went onto report an increase in body weight in copper and lead-induced hepatotoxicity[12].

We used absolute and relative liver weights to assess liver weight. Relative liver weight calculation is necessary for the comparison of the findings in our study. Group A showed an increase in liver Weight. Similar finding were seen in another study conducted in 1993[13]. The increase in Weight of the liver can be associated with hypertrophy of the cells rather than hyperplasia of these hepatocytes[14]. Group B animals showed a reduction in both absolute and relative liver weight. This has to do with the anti-inflammatory effects of Naproxen as well as the antioxidative effect of vitamin C. Another study conducted, showed that the antioxidant and anti-inflammatory capacity bearing substance such as maltol caused reduction in the liver weight as well[15]. Group C showed the most successful results, as the liver weight was almost healthy. This finding is similar to what was seen in the study conducted by Soylu et al 2006[15]. Antioxidants can have a very beneficial effect on the liver, and so can anti-inflammatory effects. This is what was seen in our study and can be supported by the study done by Zhao et al. 2016 in which he showed how the genistein and puerarin could alleviate alcohol liver injury through potential antioxidants and anti-inflammatory effects[16]. Overall the best results can be seen in Group C as both the agents were infused alongside alcohol in rats; therefore, the group displayed a more beneficial outcome. The best results could be interpreted from Group C rats, as during intoxication with Ethanol, there was also the administration of both Vitamin C and Naproxen, which helped provide a hepatoprotective effect on the organ. Group B, because of the prophylactic administration, also showed some positive

results. However, they were not as better as compared to what was seen in Group C animals.

We only studied and compared the gross parameters; more parameters need addressing to attain a better idea of how Vitamin C and Naproxen can help protect the liver. Future studies can be done on biochemical analysis and histological assessment to see how Vitamin C and Naproxen can attenuate the harmful effects of alcohol on the liver.

CONCLUSION

Our study showed that both Group B and C in which Vitamin C and Naproxen showed better results when it came to body weight and liver weight than group A, indicating the protective effects of Vitamin C and Naproxen. This is due to the antioxidant and anti-inflammatory effects of these two agents. However, further studies need to be carrying out and evaluation of other parameters concerning liver such as biochemical and histological is required to see the protective effects of these two agents on the liver.

Author's Contribution:

Concept & Design of Study:	Raja Faisal
Drafting:	Amanullah Khokhar, Shagufta Memon
Data Analysis:	Ghazala Panhwar, Syed Liaquat Ali and Farheen Hameed
Revisiting Critically:	Raja Faisal, Amanullah Khokhar
Final Approval of version:	Raja Faisal

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

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