ORIGINAL ARTICLE
HEPATOPROTECTIVE EFFECT OF PRAZOSIN IS COMPARABLE TO N-ACETYLCYSTEINE IN ACETAMINOPHEN INDUCED HEPATOTOXICITY IN MICE

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Background: Autonomic nervous system modulates acetaminophen induced hepatotoxicity. The purpose of the study was to determine the hepatoprotective effect of α_1 antagonist (prazosin) and β_2 agonist (salbutamol) on acetaminophen induced hepatotoxicity in mice. **Methods:** This experimental study was conducted at Post Graduate Medical Institute, Lahore in which 50 adult mice were divided in to five groups. With the exception of normal control, hepatotoxicity was induced in all other study groups by giving single intraperitoneal injection of acetaminophen 300 mg/ kg. First and second groups served as normal and toxic control were given distilled water 6 ml/ kg while third, fourth and fifth experimental groups were given N-acetylcysteine (300 mg/ kg), prazosin (0.18 mg/ kg) and salbutamol (0.35 mg/kg) intraperitoneally at 2,4 and 8 hours after acetaminophen injection. Serum liver enzymes were analysed at 0 and 72 hours while histopathological finding were assessed at the end of study by using SPSS-20. **Results:** All the groups treated with toxic dose of acetaminophen showed significant increase in serum ALT, i.e., B (Toxic control 3372%), C (NAC treated 282%), D (Prazosin treated 582%), E(Salbutamol treated 3297%) and AST levels, i.e., B (Toxic control 2750%), C (NAC treated 230%), D (Prazosin treated 280%), E (Salbutamol treated 828%) with *p*-value <0.001. When this increase was compared between groups, the lowest increase in serum ALT and AST levels was observed in N-acetylcysteine and prazosin group with no significant difference. Similarly, experimental animals receiving prazosin and N-acetylcysteine had the lowest inflammation, degeneration and necrosis scores than the toxic control group in histopathological analysis of the liver with *p*-value<0.001. **Conclusion:** The hepatoprotective effect of prazosin is comparable to N- acetylcysteine against acetaminophen induced hepatotoxicity in mice.

Keywords: Hepatotoxicity; Acetaminophen; Prazosin; Salbutamol

INTRODUCTION

Acetaminophen due to its antipyretic and analgesic properties widely used as over the counter drug. It is quite safe at therapeutic doses, however its overdose either intentionally or unintentionally has a documented potential to cause acute hepatic injury. It is one of the most common causes of acute liver failure in United States and in United Kingdom and remains a significant health issue.[1] Moreover, its chronic use may cause hepatic damage in susceptible patients. Extreme of age, race, genetics, pre-existing liver disorders, concurrent hepatotoxic drugs, malnutrition, chronic alcoholism, metabolic and immunological factors contribute to this susceptibility.[2]

At therapeutic dose, acetaminophen is primarily metabolized in liver by glucuronide conjugation and sulphation. However small amount of acetaminophen is metabolized by cytochrome P450 (CYP450) generating *N*-acetyl-

p- benzoquinoneamine (NAPQI) which is rapidly detoxified by glutathione (GSH). When acetaminophen is given at high doses there is formation of more NAPQI with depletion of glutathione stores. NAPQI is very toxic to liver and kidney.[3, 4] N acetylcysteine which is usually given in acetaminophen overdose replenishes store of glutathione with detoxification of NAPQI. N-acetylcysteine is not entirely protective for acetaminophen induced hepatotoxicity; it is most effective if administered within 8 hours of acetaminophen overdose.[5]

Autonomic nervous system has a strong potential to modulate acetaminophen induced hepatotoxicity. Studies have shown that soon after acetaminophen over dosage there is intense sympathetic nervous stimulation induced vasoconstriction that leads to decrease hepatic blood flow and cellular hypoxia. These sympathetic changes are mediated by both Alpha- and Beta-adrenergic receptor.

Alpha-1 adrenergic receptor blockers (Prazosin) prevent hepatic congestion and decrease RBCs accumulation, thus improving liver perfusion and function.[6,7] In addition Beta-2 adrenergic receptor stimulation (Salbutamol) also modulates hepatic function and reduces liver cell apoptosis.[8,9] However, in above mentioned experimental studies alpha-1 receptor blockers or Beta 2 receptor agonist were given as pre-treatment in acetaminophen induced hepatotoxicity, while in practical life problem arises after exposure to toxic amounts of acetaminophen. Keeping in view all these observations this study was planned to observe effect of prazosin and salbutamol in small doses on treatment of acetaminophen induced hepatotoxicity and compare it with that of N-acetylcysteine

MATERIAL AND METHODS

This comparative experimental study was conducted at Post Graduate Medical Institute (PGMI) Lahore. Study approval was obtained from Ethical Review Committee. Adult healthy mice aged 7-8 weeks of both sexes, weighing 25–35 g were purchased from Veterinary Research Institute (Ghazi Road). They were kept in animal house at PGMI Lahore in iron cages, under hygienic condition with free access to food and water. The room temperature was maintained at 25± °C under a natural day night cycle. They received humane care according to criteria outlined in "Guide for care and use of Laboratory Animals".[10]

After one-week period of acclimatization all mice were randomly divided into five groups each containing 10 animals. Randomization was done by simple balloting method. With the exception of group A, all groups were given injection acetaminophen (GSK) intraperitoneally (300 mg/kg)[11] to induce hepatotoxicity at 0 hour after taking blood sample. Then groups A and B which served as normal and toxic control were given distilled water (6 ml/ kg) equivalent to volume of experimental drugs while group C, D and E were given N-acetylcysteine (Abbott) 300 mg/kg.[12] prazosin (Pfizer) 0.18 mg/kg and salbutamol (Getz Pharma) 0.35 mg/kg respectively. Prazosin and salbutamol doses were calculated from human dose.[13] All doses were given intraperitoneally at 2, 4 and 8 hours after acetaminophen injection.

A blood sample of 0.3–0.5 ml was taken by cardiac puncture under light ether anaesthesia on 0 hour and 72 hour using 3 ml disposable syringe. Blood samples were put in serum vacutainer and put at room temperature for 30 minutes for clotting. The centrifugation process was done at 2000 rpm for 10 minutes. Serum was separated and stored in serum cups at -20 °C.[14] to analyse AST and ALT levels

with commercially available kits (Diasys) using calorimeter (Slim).

At the end of experiment (72 hours) all mice were sacrificed and their liver was removed. They were fixed in 10% formalin solution for histological studies. Sections of liver were prepared for each mouse and slides were stained with eosin and haematoxylin. Histopathological changes like necrosis, inflammation and degradation were observed in 10 high power fields and mean was taken. These parameters were scored as follows

0= no change, 1= mild change, 2= moderate change, 3= severe change.[15]

The data were analysed using SPSS version 20. Shapiro Wilk test was used to check the normality of data. Data was normally distributed within groups. One-way ANOVA was used to observe group mean difference in ALT level, AST level and histological score among groups. For multiple comparisons, post hoc Tukey HSD test was used. Paired t-test was used to determine the mean change in ALT and AST levels from 0 hour to 72 hours within each group. Results were considered statistically significant when p-value was ≤0.05.

RESULTS

All the groups treated with toxic dose of acetaminophen showed significant increase in serum ALT, i.e., B (Toxic control 1386±324), C (NAC treated 153±62), D (Prazosin treated 286±64), E (Salbutamol treated 1260±208) and AST levels, i.e., B (Toxic control 1972±426), C (NAC treated 236±109), D (Prazosin treated 320±86), E (Salbutamol treated 1466±308) with p-value <0.001. When this increase was compared between groups, the lowest increase in serum ALT and AST levels was observed in N-acetylcysteine and prazosin group with non-significant difference. The highest rise in ALT and AST levels were seen in the control group and the experimental group of animals that received salbutamol, with difference among these two also non-significant Table-1) The effect of prazosin was further substantiated by the results of table-2 that showed that the experimental animals receiving prazosin and N-acetylcysteine had the lowest inflammation, degeneration and necrosis scores than the toxic control group in histopathological analysis of the liver after the experiment with p-value <0.001 The difference between N-acetylcysteine and prazosin in these parameters was also insignificant.

This was as opposed to the toxic control group and the experimental group receiving salbutamol both which showed very high inflammation, degeneration and necrosis scores; the difference among these two was insignificant. Figures 1–5 show liver parenchyma of normal, toxic and treated groups.

Table-1: Effect of NAC, prazosin and salbutamol on serum ALT and AST levels (Mean±SD) in acetaminophen induced hepatotoxicity in mice (n=10)

Group	ALT (u/L)				AST (u/L)			
	0 hr	72 hr	Increase	# *p*- Value	0 hr	72 hr	Increase	# *p*-Value
A (Normal control)	33±7	34±9	1±12	0.675	128±47	101±29	-27±47	0.101
B (Toxic control)	41±9	1427±329	1386±324[c]	< 0.001	72±19	2044±420	1972±426[c]	< 0.001
C (NAC treated)	54±12	207±57	153±62[d]	< 0.001	102±22	338±92	236±109[d]	< 0.001
D (Prazosin treated)	49±14	335±61	286±64[b d]	< 0.001	114±29	434±76	320±86[a d]	< 0.001
E (Salbutamol treated)	38±10	1298±208	1260±208[c]	< 0.001	177±56	1643±268	1466±308[c d]	< 0.001
p- value (ANOVA)			≤ 0.001				≤ 0.001	

Paired sample t-test. [a]*p*- value ≤ 0.05, [b]*p*- value ≤ 0.01, [c]*p*- value ≤ 0.001 vs normal control. [d]*p*- value ≤ 0.001 vs toxic control

Table-2: Effect of NAC, prazosin and salbutamol on inflammation, degeneration and necrosis score in acetaminophen induced hepatotoxicity in mice (n=10)

Group	Inflammation	Degeneration	Necrosis
A(Normal control)	0.0±0.0	0.0±0.0	0.0±0.0
B (Toxic control)	1.80±0.89[c]	2.0±0.0[c]	2.90±0.32[c]
C (NAC treated)	0.70±0.48[a d]	1.0±0.0[c d]	0.50±0.53[d]
D(Prazosin treated)	0.60±0.52[d]	1.2±0.42[c d]	0.40±0.52[d]
E(Salbutamol treated)	1.60±0.52[c]	2.2±0.42[c]	2.40±0.67[c]
p-Value (ANOVA)	< 0.001	< 0.001	< 0.001

[a]*p*- value ≤ 0.05, [c] *p*- value ≤ 0.001 vs normal control. [d]*p*- value ≤ 0.001 vs toxic control
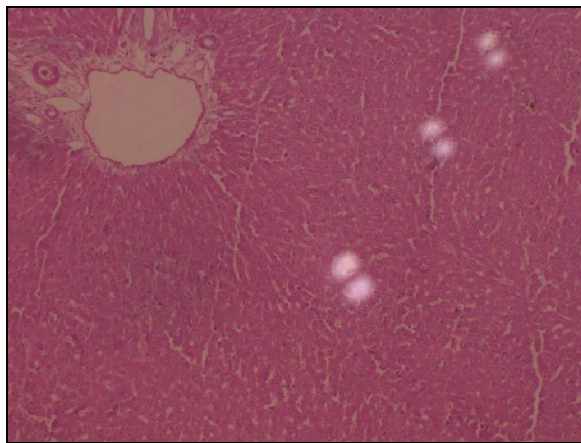

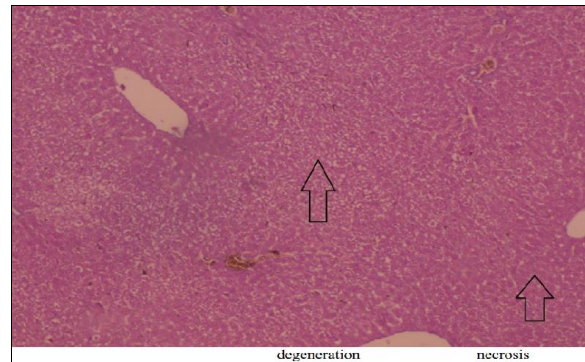Figure-1: Histology of normal liver architecture

Figure-3: Liver architecture showing mild necrosis and degeneration after administration of NAC
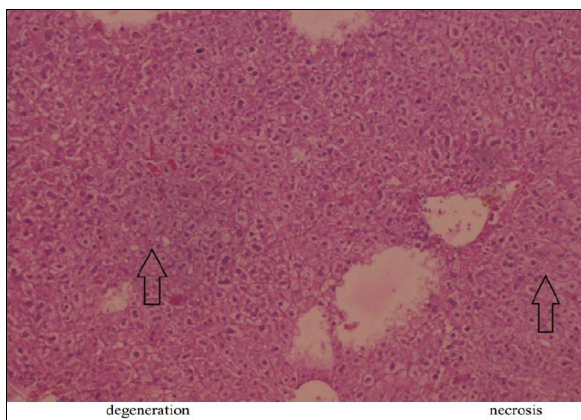

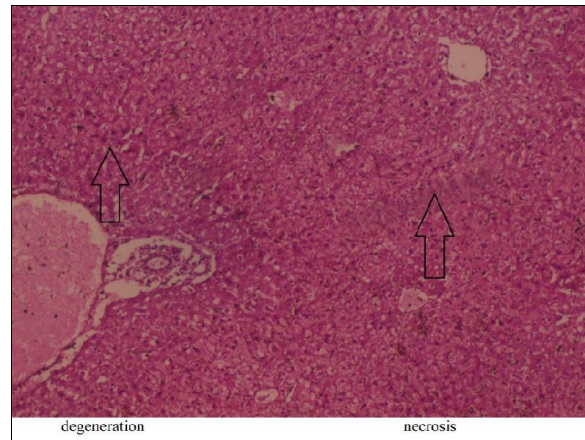Figure-2: Liver histology showing necrosis and degeneration after administration of acetaminophen

Figure-4: Liver histology showing mild necrosis and mild to moderate degeneration after administration of prazosin
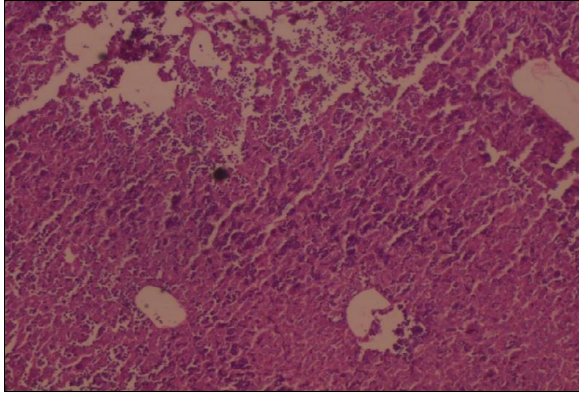
Figure-5: Liver histology showing severe necrosis after administration of salbutamol

DISCUSSION

In this experimental study we evaluated the effect of prazosin and salbutamol on acetaminophen induced liver injury. In our study serum ALT level from 0 to 72 hour was increased by 5% in group A (normal control), 3372% group B (toxic control), 282% group C (N-acetylcysteine treated), 582% group D (prazosin treated) and 3297% group E (salbutamol treated) respectively. Group C (N-acetylcysteine treated) showed less increase in ALT level which was in accordance with study carried out by Saito *et al.*[16] in which N- acetylcysteine was given one and a half hour after acetaminophen overdose. Group D, prazosin treated, also showed less increase in ALT level caused by acetaminophen overdose. In their study Randle *et al*,[7] showed that when given 30 minutes after acetaminophen overdose prazosin caused significant reduction in ALT level but insignificant reduction when given one hour after acetaminophen. The difference in result may be because we have given multiple doses of acetaminophen and they had used single dose. Other studies carried out by Clement and Williams[17] and Zubairi *et al*[18] showed protective effects of prazosin when given before acetaminophen overdose. Their results were less significant *p*-value ≤0.05 in Clement and Williams study and *p*-value ≤0.01 in Zubairi *et al.* study while in present study *p*-value was 0.001 versus toxic group; although they have given prazosin before acetaminophen administration. N-acetylcysteine and prazosin both reduced ALT serum levels but difference between two was not statistically significant. Salbutamol produced no effect on serum ALT level.

In our study serum AST level increased from 0–72 hour by 21% in group A (normal control), 2750% group B (toxic control), 230% group C (N-acetylcysteine treated), 280% group D (prazosin treated) and 828% in group E (salbutamol treated). Group C showed less increase in AST

value in accordance with study conducted by Acharya.[19] Group D (prazosin treated) revealed lower AST level in accordance with Zubairi *et al*[18] results. Their results are less significant with *p*-value <0.03 verses toxic control although they have given prazosin as a pre-treatment in their study. In present study prazosin caused less reduction in AST level as compared to N-acetylcysteine but statistically the difference was not significant. Salbutamol caused little lowering of serum AST.

At the end of the experiment histopathological examination of liver was conducted which demonstrated that both N-acetylcysteine and prazosin reduced inflammation caused by acetaminophen overdose. This improvement was parallel with results of Zubairi *et al*[18] who demonstrated that prazosin had reduced inflammation by interrupting early stages of toxicity. In present study prazosin caused more improvement in inflammation than N-acetylcysteine but the difference was not statistically significant. There was no effect produced by salbutamol on inflammation.

Comparison of degeneration in all the groups revealed that N-acetylcysteine and prazosin both improved degeneration in experimental animals with *p*-value <0.001. The improvement in degeneration caused by N-acetylcysteine was more than that caused by prazosin but difference between two was not statistically significant. Salbutamol had no effect on degeneration produced by acetaminophen overdose.

Necrosis was less as compared to toxic group by both N-acetylcysteine and prazosin with *p*-value <0.001. The effect produced by N-acetylcysteine is in accordance with Saito *et al*[16] who demonstrated attenuation of necrosis produced by NAC. The effect of prazosin was consistent with result shown by Randle *et al*[7] which showed improvement in necrosis. The improvement in necrosis caused by prazosin in this study was better than N-acetylcysteine but difference between two was not statistically significant. Necrosis produced by acetaminophen overdose was not affected by salbutamol.

Overall NAC caused a marked improvement in all parameters of liver injury but it failed to produce complete recovery. This is in accordance with other studies.[14,20,21] Prazosin also produced improvement in all aspects of liver injury. These results are better than other similar studies[7,18] although they used it for prevention of toxicity while in present study it was used for treatment. Salbutamol was used in this study because of the presence of beta cells in liver vasculature and ability of beta blockers to worsen acetaminophen induced

hepatotoxicity[22]. In other studies salbutamol reduced apoptosis of liver cells.[23] and promoted recellularization of liver.[24] but in the present study salbutamol failed to produce any improvement in liver injury.

CONCLUSION

Hepatoprotective effect of prazosin was comparable to N- acetylcysteine (NAC) against acetaminophen induced hepatotoxicity in mice. Prazosin effects were more on improving inflammation and necrosis while NAC effect was more on enzymatic level and degeneration. Salbutamol failed to show improvement in any parameter

AUTHORS' CONTRIBUTION

SS: Conceived the idea, designed and conducted the study, collected data, drafted the manuscript and did statistical analysis. MH: Search the literature, review the manuscript and preparing the manuscript. MNS: Analyzed & interpreted the data, manuscript editing and drafting. SC: Supervised the research and final editing of manuscript. All members hereby agree to take responsibility of work-and confirm that all questions related to the accuracy and integrity of the research have been properly and thoroughly resolved

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