ORIGINAL ARTICLE

Effect of Fructose in Acetaminophen Induced Liver Injury in Rats

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ABSTRACT

Objective: Low dose fructose was used in hepatotoxic rats to assess its hepatoprotective role. The objective of this study was to assess the effect of fructose on liver function using enzyme assays and morphologic changes.
Study Design: Quasi-Experimental study
Place and Duration of Study: Departments of Biochemistry, Pharmacology and Pathology, Army Medical College and National Institute of Health from Jan 2007–Jan 2008.
Methodology: One hundred and twenty healthy male Sprague-Dawley rats were injected Acetaminophen (APAP) (650 mg/kg) to induce acute hepatotoxicity, fructose (1g/kg) and *N*-acetyl cysteine (NAC) (1200 mg/kg) intraperitoneally. Blood samples ware taken after ten hours and serum was separated and centrifuged. Serum alanine aminotranferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase , albumin and total bilirubin were measured using kit method. Liver biopsy was taken to observe the necrotic changes.
Results: APAP had 200% elevation of serum ALT and AST ($p<0.01$). Serum alkaline phosphatase, bilirubin and albumin were insignificant as compared to controls in all the groups ($p>0.05$). Fructose and APAP co-administration (group III) had insignificant effect on serum ALT ($p= 0.6$) and AST ($p= 0.9$) as compared to APAP group ($p>0.05$). NAC (group IV) significantly decreased serum transaminases compared to groups II and III ($p<0.01$). Fructose did not reduce centrilobular necrosis produced by APAP, while NAC had significant cytoprotection in this animal model.
Conclusion: Low dose fructose (1g/kg) has no hepatoprotective role in acute APAP hepatotoxicity *in vivo* and NAC conferred hepatoprotection. Additional studies are needed to understand the combined interaction of fructose and APAP, as fructose is being extensively consumed by general population in form of commercial beverages.

Key words: Acetaminophen, hepatotoxicity, fructose, rats.

INTRODUCTION

Acetaminophen is a cost effective antipyretic and analgesic prescribed to all age groups injudiciously. It is presumed to be safe against gastric erosions however an intake of 7g will cause poisoning that may be fatal if not treated timely.[1] Although it is a leading cause of hepatic failure in the western world, the data in Pakistan are lacking, where most of the population in rural areas has limited access to tertiary care hospital.

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Therapeutic doses of APAP are metabolized by endogenous glucuronic acid, sulphate and reduced glutathione. A small amount of *N*-acetyl-*p*-benzoquinone imine (NAPQI) forms which is detoxified by glutathione.[2] Acute ingestion of 150-200 mg/kg (children) or 7 g total (adult) will cause excess production of NAPQI that will damage DNA, RNA and cellular proteins. It also damages mitochondria by creating pores in the inner mitochondrial membrane, called "mitochondrial permeability transition" (MPT). This initiates necrosis or apoptosis leading to cell death.[3]

Fructose in high doses cause obesity, hyperuricemia and metabolic syndrome.[4] A low dose of fructose has been found protective to hepatocytes against APAP toxicity in cell culture studies.[5-7] It decreased *N*- nitrosofenfluramine toxicity by providing more ATP by glycolytic pathway.[8] A cardioprotective role of fructose was found by Jordan and co-workers in ischemia –reperfusion injury.[9] Fructose improved neuronal function and studies are underway into neuroenergetics.[10]

In the light of biochemical observations and cytoprotective findings in vitro, the study was designed to evaluate hepatoprotective role of fructose in animal models of APAP toxicity.

MATERIALS AND METHODS

Drugs and reagents

Fructose of analytical grade (Panreact: QUIMICA;Spain.), Acetaminophen (APAP) of pharmaceutical grade (Provas: Sami pharmaceuticals®, Pakistan) and *N*-Acetylcysteine (Parvolex; Mayne Pharma® , Wellington, New Zealand) were purchased. Commercial kits for ALT, AST, ALP, bilirubin (Linear Chemicals, Spain) and serum albumin (Diamate Technology, Spain) were purchased from Hamza dealers.

Animals

One hundred and twenty healthy male Sprague-Dawley rats weighing (180-220 g; 9-12 weeks of age) were obtained from National Institute of Health (Islamabad, Pakistan). The protocol was approved by the ethical committee of Army Medical College. Animals were acclimated for one week at the animal house of Army Medical College. Rats were kept at 23-25°C and 12 hr light/12 hr dark cycle. Animals had free access to water adlibitum and chow, before initiation of any treatment.

Rats were randomly divided into four groups (n=30 each). Control (group I), APAP (group II), APAP+ fructose (group III) and group IV received both APAP and NAC. Animals were kept on a 16 hrs fast before start of experiment with free access to water. APAP was given in a dose of 650 mg/kg intraperitoneally.[11] after 10 hrs. Group III received fructose (1 g/kg) in three divided doses at 0.5 hr, 4 hrs and 8 hrs (i .p.) after APAP injection. Group IV received *N*-acetylcysteine (1200 mg/kg, i. p) as a single dose half hour after APAP.[12] The control group 1 received vehicle alone.

Blood was obtained via cardiac puncture under ether anesthesia 10hrs after drug administration. It was allowed to stand for half hour at room temperature to get serum. Serum was then centrifuged at 1000 *g* for 10 minutes at 4°C and subjected to spectrophotometry using auto analyzer (Vitalab Selectra-E, Netherlands).

The portions of liver were stored in 10% formalin and then embedded in paraffin. Microtome sections of 5µm thickness were prepared from liver samples and subsequently stained with hematoxylin-eosin.[13] These sections were examined for pathological findings. They were characterized as 0 with no sign; 1+ when only congestion was present; 2+ when vacuolar degeneration was seen and 3+ when predominantly centrilobular necrosis and inflammatory reaction was seen along with vacuolar degeneration.

STATISTICAL ANALYSIS

All the data were expressed as means ± SEM. Comparison between groups was performed by one-way analysis of variance (ANOVA) followed by multiple comparison post-hoc tests using software SPSS V 11. Differences were considered significant at $p < 0.05$.

RESULTS

Serum ALT

The serum ALT value was increased from (mean ±SEM) 46 ± 4 IU/L in control group to 107 ± 11 IU/L in group II (p=0.001). This value was 126 ± 18 IU/L in group III which was higher but insignificant as compared to group II (p=0.64). *N*-Acetylcysteine decreased these levels towards normal with a value of 77 ± 7 IU/ L in group IV as shown in figure1 (p=0.27).

Serum AST

In the control group, mean AST level was 175 ±11 IU/L. It increased to 364 ± 24 IU/L by APAP treatment (p=0.000). Fructose and APAP co-treatment resulted in serum levels of 353 ± 26 IU/L which was insignificant compared to group II (p=0.97). NAC treatment decreased the levels to 235 ±14 IU/L in group IV. It was significantly less when compared to both groups II and III (p<0.0001).

Serum Alkaline Phosphatase

The mean level of ALP in control rats was 161 ± 4 IU/L. Treatment with APAP, fructose and NAC gave values of 150 ± 5 IU/L, 147 ± 4 IU/L, and 144 ± 5 IU/L respectively. Comparison of group II with group I, III and IV was insignificant (p=0.36, 0.97, 0.85 respectively).

Serum Albumin

Mean serum albumin level was 41 ± 0.4 g/L in the control group. The levels were 40 ± 0.4 g/L in both the APAP and fructose treated groups. Treatment with NAC however decreased the value to 37 ± 1.6 g/L. There was no significant difference in the mean values among the groups.

Serum Total Bilirubin

Serum bilirubin levels were 1.7± 0.1 ìmol/L in the control group. APAP treatment produced insignificant change compared to control group with a value of 1.8 ± 0.1 ìmol/L. Fructose and NAC gave values of 1.7 ± 0.1 ìmol/L and 1.6 ± 0.1 ìmol/L respectively. There was no significant difference in the mean values among the groups.

Histopathological Findings

In group II, APAP produced centrilobular necrosis, congestion of the central vein and sinusoids of the liver. An inflammatory infiltrate was also seen as indicated in figure 1. Fructose and APAP (group III) produced similar changes along with vacuolar degeneration. However these changes were localized to focal areas of centrilobular necrosis as shown in figure 2. In group IV, NAC and APAP co-administration produced congestion of the sinusoids and central vein along with vacuolar degeneration while centrilobular necrosis was seen in only four rats. This indicates that NAC was hepatoprotective in this setting.

DISCUSSION

In this study acetaminophen in group II caused significant hepatocellular damage as evident by increase in the serum transaminases of up to 200% during acute liver injury.[14] The rise in serum ALT, AST, and ALP in Sprague Dawley rats was comparable with other studies. A higher value of ALT 607 ± 32 IU/L and AST 1178 ± 18 IU/L respectively has been reported with APAP given for twenty four hours against ten hours exposure in our study.[15-17]

There was no significant elevation of serum ALP, albumin and bilirubin in group II in our study, however a dose of 2 g/kg APAP produced a significant toxicity in another study. There was rise in ALP to 216 ± 9 IU/L and total bilirubin to 17 µmol/L after twenty four hours.[18] Serum albumin in our control group was 41 ± 0.4 g/L which has already been reported in Sprague Dawley rats.[19] Fructose has been shown to protect hepatocytes in hypoxia and anoxia in cell culture studies.[5-7] The serum ALT of 126 ± 18 IU/L and AST of 353 ± 26 IU/L, serum ALP, albumin and bilirubin clearly indicates that fructose did not protect against APAP toxicity *in vivo*.

N-Acetylcysteine is an antioxidant which functions to regenerate glutathione. This decreases free radical damage and consequent cellular injury.[20] Serum ALT and AST levels in this group were 77 ± 7 IU/L and 235 ± 14 IU/L respectively (Table 1). It is significantly less as compared to both groups II and III ($p<0.05$). Serum ALP, albumin and bilirubin were 144 ± 5 IU/L, 37 ± 1.6 g/L and 1.6 ± 0.1 µmol/L respectively albeit not significant when compared to group II and III. Another study has reported ALT and AST of 41.1 ± 5.6 IU/L and 60.7 ± 9.6 IU/L respectively in albino wistar rats.[21] They administered NAC intramuscularly for eleven days in a dose of 150 mg/kg while evaluating the drug cyclosporine A. When NAC was administered before inducing carbon tetrachloride toxicity in another study, it yielded certain beneficial effect. But similar to our results they were not able to reverse hepatic membrane damage completely.[22]

The histopathological findings as indicated in table I confirm the APAP toxicity. The rise in serum enzymes by fructose and APAP is in accordance with the microscopic picture as shown in figure 1 and 2. It appears from the results of our study that fructose has different effects in cell culture studies and *in vivo*. Fructose did not decrease APAP toxicity while NAC protected from centrilobular necrosis in twenty six out of thirty rats.

The protection by fructose in cell culture studies was shown due to more ATP production via glycolysis. One of the proposed mechanisms is that it by passes the rate limiting enzyme, phosphofructokinase I and stimulates pyruvate kinase. It also phosphorylates faster than glucose because Km of fructokinase is less than glucokinase.[7,8] In this study the dosing of fructose was done every four hours to maintain a constant supply of ATP, based on the work of Latta et al. They showed that initial depletion of ATP by fructose 1-phosphate recovers by anaerobic glycolysis at 4 hrs.[23] Fructose would protect when the rate of production of ATP via glycolysis overcomes the rate ATP consumption during initial fructose 1-phosphate formation. It appears that insufficient ATP was generated to sustain ATP dependent membrane functions evident by raised transaminases in group III.

The results from this study are more relevant to the human conditions, in typically consumed concentrations and in typically consumed form in contrast to other animal studies. We find strong recommendations on this in recent literature.[24] These findings have implications, both specific to acetaminophen and can be interpreted in context of a general toxic liver injury. Although fructose did not protect from acute Acetaminophen liver injury, the fact that it did not increase liver toxicity may show that it can be safely taken by patients of liver injury. Recent evidence is growing in favour of a dose and duration dependent effects of fructose, and our study has similar conclusions.[25] This consideration is important in defining nutrition policy and consumer perceptions in the present era when the general public knows a lot on dietary sugars through media.

The understanding of pathophysiology of APAP is developing into newer concepts of apoptosis and MPT. Although a limitation of this study is lack of simultaneous measurement of ATP, apoptosis and MPT, however, this study does show that additional *in vivo* studies are needed on the role of different doses and duration of fructose in APAP toxicity.

CONCLUSION

This study demonstrated that a low dose fructose (1g/kg) has no hepatoprotective role in acute APAP hepatotoxicity when compared with NAC. Additional studies are needed to understand the combined interaction of fructose and APAP as both are being extensively consumed by general population.

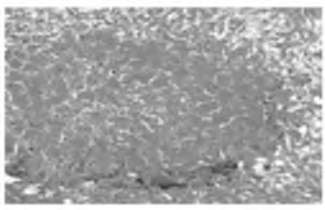

Figure 1: Photomicrograph (400 x magnifications) of liver showing necrotic area in group II rats treated with acetaminophen only.

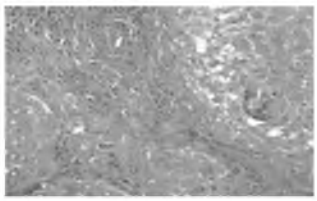

Figure 2: Photomicrograph (400 x magnifications) of liver showing necrotic area on right in group III receiving both acetaminophen and fructose showing similar centrilobular necrosis.

Table I: Liver function enzymes and histopathological findings with fructose and NAC in acute APAP toxicity

Group (n=30)	Serum liver enzymes (Mean ± SEM)					Histopathology			
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Albumin (g/L)	Bilirubin (µmol/L)	0	1+	2+	3+
I Control	46 ± 4	175 ± 11	161 ± 41	41 ± 0.4	1.7 ± 0.1	28	2	-	-
II APAP	107 ± 11	364 ± 24	150 ± 5	40 ± 0.4	1.8 ± 0.1	-	-	-	30
III APAP+ fructose	126 ± 18**	353 ± 26**	147 ± 4	40 ± 0.4	1.7 ± 0.1	-	-	-	30
IV APAP+NAC	77 ± 7*	235 ± 14*	144 ± 5	37 ± 1.6.	1.6 ± 0.1	-	-	26	4

** p>0.05 when group II is compared to group III which shows insignificant change.
* p< 0.05 when group IV is compared to groups II and III and indicates hepatoprotection.
0: No sign; 1+ congestion; 2+ vacuolar degeneration; 3+ vacuolar degeneration, centrilobular necrosis, and inflammatory reaction

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