Original Article

Acetaminophen Induced Hepatotoxicity and Effects of Pomegranate Peel Extract on Livers of Adult male Albino Rat

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ABSTRACT

Objective: To observe the effects of pomegranate peel extract on acetaminophen-induced hepatotoxicity.

Methodology: A randomized control study was conducted at the University of Health Sciences, Lahore, Pakistan. Twenty four rats weighing 175±25gm each were included in this study. Animals were divided into three groups; A, B & C by random balloting method. Group A served as control and was given 5ml/kg normal saline intraperitoneally. Group B & C were given on day 1 intraperitoneally 750 mg/kg acetaminophen dissolved in 5 ml/kg normal saline. Group A & B received 5 ml/kg normal saline orally from day 2 till day 14 of experiment whereas group C received pomegranate peel extract (PPE) 50mg/kg dissolved in 5ml/kg distilled water orally. After 14 days of experimental period animals were sacrificed and their livers were removed and processed for histological examination.

Results: Mean body weight at end of experiment in group B showed marked decrease as compared to group C. Histological examination of group B showed necrosis with vacuolar degeneration and a number of apoptotic bodies. Results of group C showed preservation of normal histological architecture.

Conclusion: Pomegranate peel extract due to its antioxidant property remediated the effect of acetaminophen induced hepatotoxicity.

Keywords: *Hepatotoxicity. Apoptotic bodies. Acetaminophen. Pomegranate peel.*

INTRODUCTION

Acetaminophen is a commonly used analgesic in adults and children worldwide but it is considered a potentially harmful drug due to its narrow therapeutic index.[1] Acetaminophen is a non-steroidal anti-inflammatory drug (NSAID), belonging to the para-amino phenol class and is an active metabolite of two anilines, phenacetin and acetanilide which were both found to metabolize to paracetamol and P-phenatidine or aniline, respectively.[2] Toxic doses of acetaminophen is known to cause hepatic necrosis, nephrotoxicity and death in humans and experimental animals.[3] Recommended dosage of acetaminophen in adults is 0.5-1gm four to six hourly with a maximum daily dose of 4gm.[4] Dosage in children is calculated according to age and body weight which is less than 40mg/kg/day.[5] Acetaminophen is 90% metabolised by sulfotransferases and glucuronyl transferases to phenolic glucuronide and sulfate in the liver which is ultimately excreted in the urine.[6] About 2% of the residual acetaminophen is excreted unchanged in the urine and about 5% to 10% gets metabolized by the cytochrome P450 to N-acetyl-p-benzoquinone imine (NAPQI).[6] Overdosage of acetaminophen causes saturation of glucuronyl transferases and sulfotransferases, diverting the drug to be metabolized by cytochrome P450, resulting in generation of large amounts of NAPQI hence depleting glutathione, resulting in the accumulation of NAPQI in the hepatocytes.[7] This produces NAPQI-induced glutathione depletion and oxidative stress leading to lipid peroxidation and irreversible cell membrane injury and cell death.[7,8].

Extracts of Pomegranate (Punica Granatum L.) plant from its different parts have high antioxidant activity.[9] This plant includes chemically diverse polyphenol antioxidants, primarily punicalagin and ellagic acid.[10] The high content of polyphenols are found in Pomegranate peels such as condensed tannins, anthocyanins, proanthocyanidins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3, 5-diglucosides) and flavinoids which are antioxidants.[11] These antioxidants are known for scavenging free radicals and inhibition of lipid peroxidation.[11] Dried peel of pomegranate is reported to have diverse pharmacological functions and antioxidant activity and is used for the cure of diarrhea, respiratory and urinary tract infections.[12] Different parts of this plant or its extract have been used as an anticancer, antibacterial, antidiarrhoeal, antifungal and antiulcer remedy.[13] Peel also has antifertility, hepatoprotective, cytotoxic and hypoglycemic effects.[14-17] Peels of pomegranate have been shown to possess relatively higher antioxidant activity than other parts of this plant and, therefore, are a rich source of natural antioxidants.[11] Since the hepatoprotective effect of methanolic extract of pomegranate peel (PPE) has never been tried on acetaminophen-induced hepatotoxicity, the current study was designed to observe the acetaminophen-

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induced histological changes in the liver of rat and the effect of PPE on these changes.

METHODOLOGY

At the University of Health Sciences, Lahore, Pakistan, a randomized controlled study was conducted on healthy male albino Wistar rats. The study was approved by the ethical committee of the university. The animals were divided through random balloting into three equal groups A, B, and C and were individually caged in stainless steel units with wood shavings on the floor. Standard rat diet and water ad libitum were fed to the animals and they were kept at 23±2°C and humidity (50±5%) with 12 hours day and night cycle. The experiment was started 2 days after acclimatization of the animals. The control Group A was given 5ml/kg normal saline intraperitoneally (IP) on the day 1 and then 5ml/kg distilled water orally from day 2 till day 14; groups B and C were given 750 mg/kg acetaminophen IP dissolved in 5ml/kg normal saline on day 1 of the experiment. From day 2 till day 14, group B was given 5ml/kg distilled water, while group C was given 50 mg/kg PPE dissolved in 5ml/kg distilled water orally. On the day15, under chloroform anesthesia, the animals were sacrificed and the livers were removed, washed and weighed. For histological examination 3-5mm pieces were excised and prepared.

Data was analyzed by using SPSS 20. Analysis of variance (ANOVA) was used to compare variables followed by Post Hoc Tukey test. $P<0.05$ was considered statistically significant.

RESULTS

Twenty four (24) rats were taken weighing 175±25gm with 8(33.3%) rats in each group. Mean body weight at the start and end of the experiment in groups A, B and C was 184±6.347 and 203.13±6.379gm, 183.25±9.099 and 174.50±8.229gm and 181.25±3.536 and 203.25±3.454gm respectively. Comparison of body weight of animals at the end of the experiment is shown in Table 1. Animals of group A and C remained healthy and active for the entire duration of the experiment whereas animals of group B showed exhaustion and lethargy.

Histologically, normal hepatolobular features were seen in control animals of group A (Figure 1).

In histological preparations from group B, general hepatic architecture was deranged; cells showed necrosis and were empty looking containing many vacuoles of varying sizes pushing the nucleus to one side. The liver parenchyma was also disrupted. A number of apoptotic bodies were also clearly seen in group B (Figure 2).

In group C, liver parenchyma showed restoration of classical architecture. There was barely any indication of damage to the organ; no necrosis, vacuoles or apoptotic bodies were observed. We also observed signs of regeneration in group C as evident by multinucleation (Figure 3) similar to those seen in control group A.

Table 1: Effect of single intraperitoneal (IP) injection of acetaminophen (group B) and the effect of methanolic extract of pomegranate peel orally for 14 days (group C) on the body weight of rats at the end of an experiment.

Parameters	Groups			Post Hoc Tuckey	p-value
Weight at end of experiment in gm	A Control (n=8) Mean±SD	B Acetaminophen 750mg/kg (n=8) Mean±SD	C Acetaminophen (750mg/kg) body weight i.p and PPE (50 mg/kg) (n=8) Mean±SD		
	203.13±6.38	174.50±8.229		28.625*	.009*
		174.50±8.229	203.25±3.45	-25.250*	.024*
	203.13±6.38		203.25±3.45	-.125	1.000

* p-value <0.05 is statistically significant.

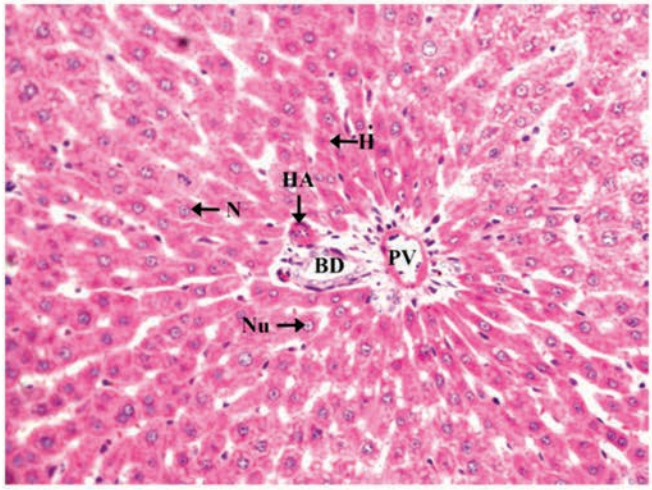

Figure 1: Photomicrograph of liver section from Group A, showing Portal triad consisting of portal vein (PV), bile duct (BD) lined by cuboidal cells and Hepatic artery (HA). Hepatocyte (H) with nucleus (N) and nucleoli (Nu) are also seen. H&E stain. X400

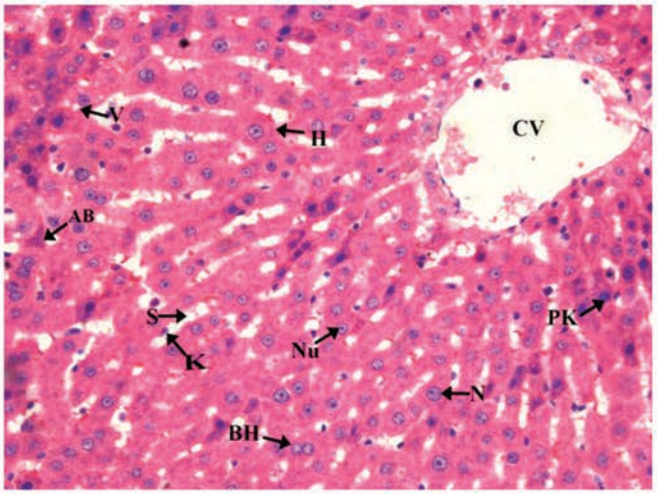

Figure 2: Photomicrograph of liver section from Group B, showing hepatocytes (H) with cytoplasmic vacuoles (V). Areas of necrosis (Ne) were also evident by fragmented (Fr) and pyknotic nuclei (PK) and apoptotic bodies (AB). H&E stain. X400

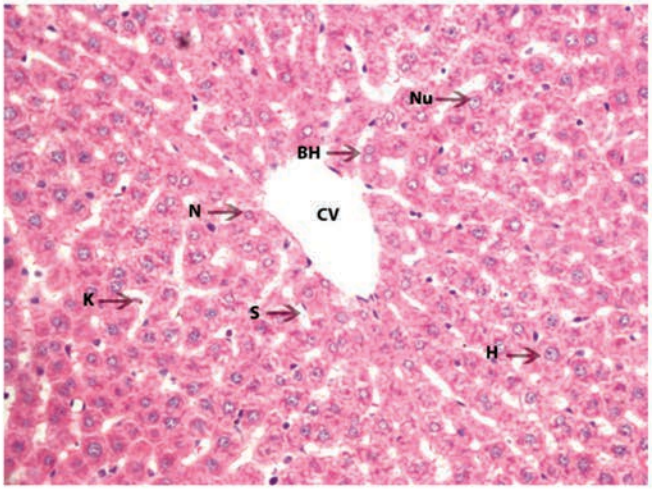

Figure 3: Photomicrograph of liver section from Group C, showing hepatocytes (H) having nucleus (N) with prominent Nucleoli (Nu) and central vein (CV). Kupffer cells (K) with prominent nuclei are seen projecting into the lumen of Sinusoids (S). Few binucleated hepatocytes (BH) are also present. H&E stain. X400

DISCUSSION

In the current investigation, we observed a statistically significant difference in the body weight of group B as compared to groups A and C at the end of the experiment. This was presumably due to decreased food intake and lethargy of the rats on account of experimental intervention, also supported by earlier work which reported a marked decrease in body weight of animals after acetaminophen injection; in the same experiment decrease in body weight was more in animals injected with a higher dose of acetaminophen.[18-20] Our results demonstrated that acetaminophen was toxic to the liver as evident by various histological changes. The normal architecture of liver in group B was disturbed (Figure-2). This loss of classical arrangement of hepatocyte was due to necrosis similar to previously reported effects after acetaminophen treatment of albino rats.[21,22] In the current experiment, PPE restored the general architecture of liver in group C (Figure 3). The current results corroborate previous study in which administration of a single dose of 2g/kg of carbon tetrachloride (CCl4) caused deranged hepatic architecture and treatment with PPE showed restoration of hepatic architecture.[16] In group B of the current study, many vacuoles were observed in the hepatocytes, which were indicative of degeneration.[23] It has been reported that the toxic metabolite of acetaminophen binds covalently to cell macromolecules; NAPQI, toxic metabolite of acetaminophen, was reported to be metabolized by cytochrome P-450 in the liver and subsequently

detoxified.[23] Hepatotoxicity had been reported due to oxidative stress and free radical production.[23] In the current experiment, it is also suggested that acetaminophen is responsible for hepatotoxicity of group B animals by increasing oxidative stress. In group C, protection was provided by PPE since the extract contained strong antioxidants and flavinoids and polyphenols such as condensed tannins and proanthocyanidins, anthocyanins (cyanidin, delphinidin, 3, 5-diglucosides and pelargonidin 3-glucosides).[11] It appears that the ingredients present in PPE restored hepatotoxic changes by decreasing the oxidative stress induced toxicity of acetaminophen (Figure 3). Evidence of regeneration was present in group C which was treated with PPE (Figure 3). Literature reports that hepatocytes which are multinucleated (mostly binucleated) with prominent nucleoli and two cell thick liver cords as seen in animals of group C are suggestive of regeneration.[24] Therefore, we can hypothesize that PPE restored the toxic effects of acetaminophen when given orally. However, it is recommended to do further comprehensive studies to establish and confirm these effects.

CONCLUSION

The present study demonstrated acetaminophen-induced hepatotoxicity in albino rats as manifested by histological changes; total loss of hepatic architecture with centrilobular hepatic necrosis, vacuolization and apoptotic bodies. However, treatment with PPE reversed these changes, showing its ameliorating effect. PPE deserves further intensive study as a protective and preventive agent since it has more potential being a rich source of antioxidants.

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