

Amelioration of acetaminophen induced hepatotoxicity by methanolic extract of pomegranate peels in rats

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Abstract

Objective: To observe the ameliorating effect by methanolic extract of pomegranate peel in acetaminophen-induced hepatotoxicity.

Methods: The randomised controlled study was conducted from July 2013 to June 2014 at the University of Health Sciences, Lahore, Pakistan, and comprised rats that were randomly divided into three equal groups. Control group A was given normal saline (5ml/kg), whereas group B and C were given 750mg/kg acetaminophen intraperitoneally dissolved in normal saline (5ml/kg) on 1st day of experiment. From Day 2 till day 14, group A and B were given distilled water (5ml/kg), while group C was given 50mg/kg methanolic extract of pomegranate peel dissolved in distilled water (5ml/kg) orally. On day 15, blood was collected through cardiac puncture, and livers were removed and processed for histological examination.

Results: There were 24 rats weighing 175±25gm each. Each group had 8(33.3%) rats. Mean liver aspartate aminotransferase at the end of the experiment in groups A, B and C were 97.88±19.45, 148.25±16.48 and 96.13±17.95U/L, while alanine transaminase levels were 51.50±15.38, 96.75±10.91 and 49.63±12.08 U/L ($p < 0.05$ each) On histological examination of group B, the normal hepatic architecture was distorted with loss of classically arranged hepatic cords. Vascular congestion was present with centrilobular necrosis, marked by pyknotic nuclei and vacuoles.

Conclusion: Acetaminophen is hepatotoxic and methanolic extract of pomegranate peel ameliorated the hepatic picture probably because of its antioxidant properties.

Keywords: Acetaminophen, Pomegranate peel, Hepatotoxicity. (JPMA 66: 859; 2016)

Introduction

Acetaminophen, commonly known as paracetamol, is one of the most widely used over-the-counter analgesic and antipyretic for both children and adults.¹ Acetaminophen, an aniline, non-steroidal anti-inflammatory drug (NSAID) belongs to the para-amino phenol class and is an active metabolite of two other anilines, phenacetin and acetanilide which were both found to metabolise to paracetamol and P-phenatidine or aniline, respectively.²

Acetaminophen, when taken in toxic doses, can cause hepatic necrosis, nephrotoxicity and death in humans and experimental animal alike.³ Adult conventional dose of acetaminophen is 0.5-1gm every four to six hours with a maximum daily dose of 4gm.⁴ In children the dose depends on age and body weight which is less than 40mg/kg in 24 hours.⁵

More than 90% of acetaminophen, taken in therapeutic doses, is metabolised in the liver to phenolic glucuronide

and sulfate by glucuronyltransferases and sulfotransferases and is subsequently excreted in the urine.⁶ About 2% of the residual acetaminophen is excreted unchanged in the urine and about 5% to 10% gets metabolised by the cytochrome P450 to N-acetyl-p-benzoquinoneimine (NAPQI).⁶

With acetaminophen overdose, glucuronyltransferases and sulfotransferases get saturated, diverting the drug to be metabolised by cytochrome P450. This generates NAPQI in amounts that can deplete glutathione, which, if not replenished, results in the accumulation of NAPQI in the hepatocytes.⁷ Secondary to NAPQI-induced glutathione depletion and oxidative stress, lipid peroxidation takes place which leads to irreversible cell membrane injury and, hence, cell death.^{7,8}

Pomegranate (*Punica Granatum L.*) plant extract from its different parts possess antioxidant activity, which is highest among many foods.⁹ Chemically, the plant includes diverse polyphenol antioxidants, primarily ellagic acid and punicalagin.¹⁰ Peels of pomegranate contain high content of polyphenols such as condensed tannins and proanthocyanidins, anthocyanins (delphinidin, cyanidin and pelargonidin

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3-glucosides and 3, 5-diglucosides) and flavinoids which are referred to as antioxidants.¹¹ These compounds are known for their properties in scavenging free radicals and inhibiting lipid peroxidation.¹¹ Dried fruit peel is reported to exert diverse pharmacological functions with antioxidant activity and is used for diarrhoea and for the treatment of respiratory and urinary tract infections.¹² Parts or extracts of different parts of this plant had been used as anti-cancer, anti-bacterial, anti-diarrhoeal, anti-fungal and anti-ulcer remedy.¹³ Peel also has antifertility,¹⁴ hepatoprotective,¹⁵ cytotoxic,¹⁶ and hypoglycaemic activities.¹⁷ Studies report that the peel in particular possesses relatively higher antioxidant activity than other parts of pomegranate and, therefore, is a rich source of natural antioxidants.¹¹ Since hepatoprotective effect of methanolic extract of pomegranate peel (MEPP) had never been tried on acetaminophen-induced hepatotoxicity, the present study was designed to observe the acetaminophen-induced histological and functional changes in the liver of rat and the effect of MEPP on these changes.

Materials and Methods

The randomised controlled study was conducted from July 2013 to June 2014 at the University of Health Sciences, Lahore, Pakistan, and comprised healthy male albino Wistar rats.

The animals were divided through random balloting into three equal groups A, B, and C and were individually housed in stainless steel cages with wood shavings on the floor. The animals were fed on standard rat diet and water ad libitum. The animals were kept at 23±2°C and humidity (50±5%). The photoperiod was controlled at 12 hours. The experiment was started 4

days after acclimatisation of the animals.

Group A, control, 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 experiment. From day 2 till day 14, group B was given 5ml/kg distilled water, while group C was also given 50 mg/kg MEPP dissolved in 5ml/kg distilled water orally. On day 15, blood was drawn through cardiac puncture and serum was kept at -80°C for the assessment of liver enzymes aspartate aminotransferase (AST) and alanine transaminase (ALT). The animals were then sacrificed under chloroform anaesthesia and the livers were removed, weighed and small pieces of 3-5mm were excised and prepared for histological examination. Data were statistically analysed using SPSS 20. Comparison of variables was made using analysis of variance (ANOVA) followed by Post Hoc Tukey test. P<0.05 was considered statistically significant.

Results

There were 24 rats weighing 175±25gm each. Each group had 8(33.3%) rats. Mean liver AST at the end of the experiment in groups A, B and C were 97.88±19.45, 148.25±16.48 and 96.13±17.95U/L, while ALT levels were 51.50±15.38, 96.75±10.91 and 49.63±12.08 U/L (Table).

Histologically, normal hepatolobular characteristics were seen in control animals (Figure-1). In the histological preparations from group B, general hepatic architecture was disrupted with loss of classical arrangement of hepatic cords. The cell boundary of hepatocytes was not

Table: 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 liver enzymes of rats.

Parameters	Groups			Post Hoc Tuckey	p-value
	A Control (n=8) Mean±SD	B Acetaminophen 750mg/kg body weight i.p (n=8) Mean±SD	C Acetaminophen (750mg/kg) and MEPP (50 mg/kg) (n=8) Mean±SD		
Serum AST (U/L)	97.88±19.45	148.25±16.49		-50.38	.001
		148.25±16.49	96.13±17.95	52.13	.001
Serum ALT (U/L)	97.88±19.45		96.13±17.95	1.75	.99
	51.50±15.38	96.75±10.91		-45.25	.001
		96.75±10.91	49.63±12.08	47.13	.001
	51.50±15.38		49.63±12.08	1.88	.99

p-value ? 0.05 is statistically significant.

AST: Aspartate aminotransferase

ALT: Alanine transaminase levels.

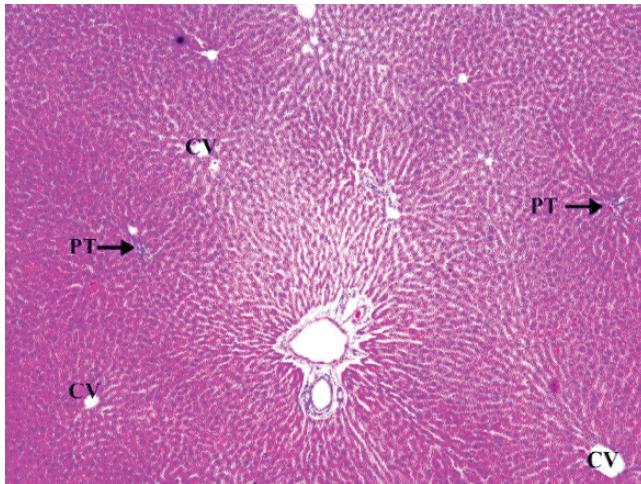


Figure-1: Photomicrograph of liver section from control group A showing classical hepatic lobule architecture with central vein (CV) and hepatocyte laminae or cords radiating to periphery of each lobule, Portal triad (PT) with portal vein, hepatic artery and bile duct. Haematoxylin and eosin (H&E) stain X100.

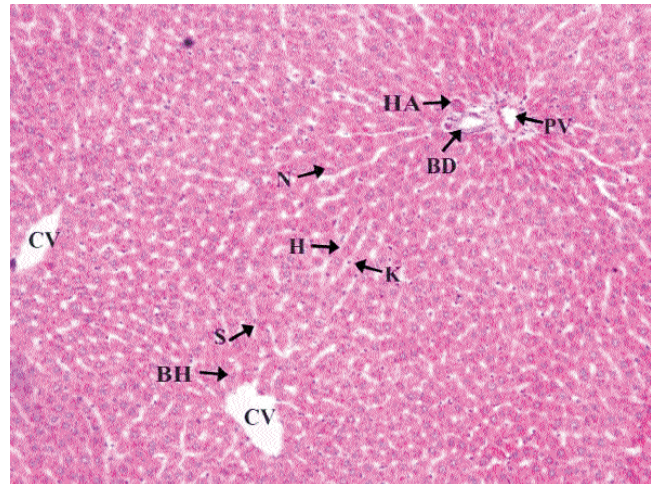


Figure-3: Photomicrograph of liver section from group C, showing classical hepatic lobule architecture, central veins (CV) and portal triad having portal vein (PV), bile duct (BD) and hepatic artery (HA). Hepatocytes (H) with conspicuous nucleus (N) are arranged as cords, Kupffer cells (K), sinusoids (S) and binucleated hepatocytes (BH). Hematoxylin and eosin (H&E) stain X200.

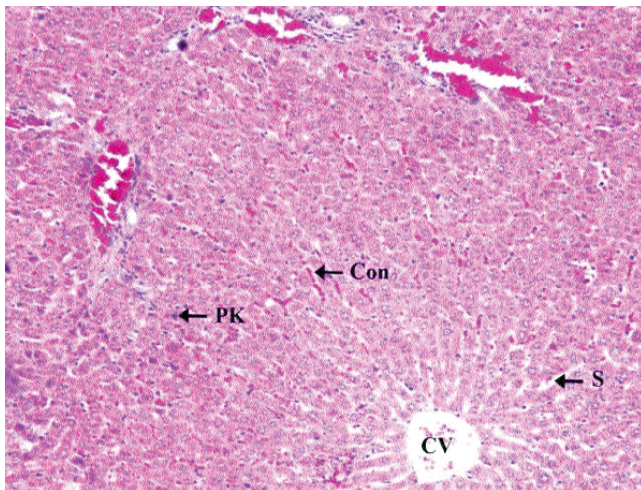


Figure-2: Photomicrograph of liver section from group B, showing loss of general architecture. Central vein (CV), blood (Con) in the Sinusoids (S) and other vascular spaces. Pyknotic nuclei (PK) can be seen at various places. Hematoxylin and eosin (H&E) stain X200.

clearly defined. Cytoplasm contained large number of vacuoles pushing the nucleus to one side. The nuclei were darkly stained, indicating pyknotic changes along with clumping of nuclear material. Nucleoli were not clearly seen in many hepatocytes. Necrosis was present in group B as evident by pyknosis. The liver parenchyma showed vascular congestion (Figure-2). In group C, general architecture of liver parenchyma was restored. There was hardly any evidence of damage to the organ; no vascular congestion, necrosis or inflammation was observed. Signs

of regeneration were present in group C as evident by cytoplasmic eosinophilia, prominent nucleoli, multinucleation and two cells thick liver cords (Figure-3) similar to those seen in control group A.

Discussion

The results showed that acetaminophen was toxic to liver as indicated by various histological changes and biochemical parameters. There are several reports showing that acetaminophen administration increased the lipid peroxidation, disturbing cell membrane integrity and releasing the liver enzymes into circulation.¹⁸⁻²¹ In the present investigation, it was observed that, in group B, serum levels of ALT and AST were significantly increased compared to those in group A ($p < 0.001$); this was indicative of damage to the hepatocyte plasmalemma as reported earlier.¹⁸⁻²¹

Histological examination of the liver preparations in the current study showed that the normal architecture of liver in group B was deranged and hepatocytes showed swelling (Figure-2). This loss of classical arrangement of hepatocytes due to ballooning was also previously reported after acetaminophen treatment of albino rats.^{22,23} In the current experiment, MEPP restored the general architecture of liver in group C (Figure-3). The current results corroborate previous study¹⁶ in which administration of a single dose of 2g/kg of carbon tetrachloride (CCl₄) caused disintegration and degradation of liver cell architecture and treatment with MEPP showed normal lobular pattern with well-

formed polygonal hepatocytes having conspicuous nuclei. Vacuoles were observed within the hepatocytes in the present histological preparations of the liver from group B which was indicative of degenerating hepatocytes and/or an increased deposition of fat.²⁴ It has been reported that the toxic metabolite of acetaminophen bound covalently to cell macromolecules; NAPQI, the toxic metabolite of acetaminophen was reported to be metabolised by cytochrome P-450 in liver and subsequently detoxified.²⁴

Hepatotoxicity had been reported to result as a mark of oxidative stress and free radical production.²⁴ It is also suggested that acetaminophen is responsible for hepatotoxicity in the current experiment by increasing oxidative stress in animals of group B and protection was provided by MEPP since the extract contained strong antioxidants and polyphenols such as condensed tannins and proanthocyanidins, anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3, 5-diglucosides) and flavinoids.¹¹ It appears that the ingredients present in MEPP restored hepatotoxic changes by decreasing the oxidative stress-induced toxicity of acetaminophen (Figure-3).

Evidences of regeneration were present in group C which was treated with MEPP (Figure-3). Literature reports that increased cytoplasmic eosinophilia, multinucleated hepatocytes (mostly binucleated), prominent nucleoli and two cell thick liver cords seen in group C animals are suggestive of regeneration.²⁵ Therefore, we can hypothesise that MEPP restored the toxic effects of acetaminophen when given orally. However, further detailed studies are needed to establish and confirm these effects.

Conclusion

The study showed acetaminophen-induced hepatotoxicity in rats as manifested by histological and functional changes; total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolisation, loss of cell boundaries and elevation in levels of AST and ALT. However, treatment with MEPP reversed these changes, showing its ameliorating effects. MEPP 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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Conflict of Interest: There is no conflict of interest.

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