

A hepatoprotective effect of an aqueous extract of pomegranate (*Punica granatum L.*) rind against acetaminophen treated rats

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Abstract

In ancient times Egyptians regarded pomegranate fruit as a symbol of fertility due to the round shape and abundant seeds .They used it to heal many ailments.

Pomegranate (El-Ruwmon) that fruit mentioned in our holy Quran not less than three times, that paid attention for its importance.

This investigation aims to evaluate the hepatoprotective activity of an aqueous rind powder extract of pomegranate (*Punica granatum L.*) in its human therapeutic dose against overdose acetaminophen.

Pretreatment of rats with 0.43g/KgB.W.of an aqueous rind extract of *Punica granatum* for 3 days before intraperitoneally (i. p.) injection of 0.5gm acetaminophen significantly reduced the acute elevation of serum aspartate aminotransferase (AST),serum alanine aminotransferase , lactate dehydrogenase (LDH)and alleviated the degree of liver damage after the i.p. injection of hepatotoxin. The group treated only with 0.5gm acetaminophen displayed significant increase in serum (AST),(ALT), (LHD)and liver displayed congestion of central and hepatic portal veins, vacuolization and ballooning also a lot of pyknotic nuclei were detected with many small necrotic areas of hepatocytes. Inflammatory cells inbetween hepatocytes and around the portal tract were observed. Some fatty droplets were scattered in the hepatocytes.

In conclusion aqueous extract of pomegranate peels possess a strong antioxidant capacity could ameliorate the damage occurred in liver by overdose acetaminophen

Introduction

Punica granatum fruit rind was traditionally used in many ways as medicine to treat diarrhoea and dysentery, even for children, for dyes, inks and tannins for leather (Duke and Ayensu, 1985).

Astringent properties of the fruit rind and fruit juice explain the antidiarrhoeal activity (Das *et al*, 1999). Several parts of the plant have been used as haemostatic, anathematic specially against tapeworms and as a remedy for diabetes (Satyavati *et al*, 1978). Further, a number of therapeutic actions of these material have been described including providing minerals to liver and assimilates vitamin A from food intake, reduce atherosclerotic lesion ,increase resistance to tuberculosis ,relieve, hypertension ,mental tension and hysteria. Using pomegranate in the powder form to treat nose bleeds, tooth paste and gum powder (Duck *et al*, 2002). The *Punica granatum* medicinal herb have been

reported to promote tissue healing (Chidambara *et al*, 2004), modulate host responses (Gracious *et al*, 2001) , has chemopreventive and adjuvant therapeutic potential for human breast cancer (Kim *et al*, 2002) , has an inhibitory effect on skin tumor development (Hora *et al.*, 2003),a chemopreventive agent against lung tumorigenesis (Castonguay *et al*,1994) and promote cell function and replication (Aleksperov, 2002)

Pomegranate rind is thought to provide natural antiviral(Zhang *et al*, 1995) ,antifungal (Dutta *et al* .,1998) and antibacterial benefits (Prashanth *et al*, 2001).

The rind contains tannins, anthocyanins, flavonoids ,pectins (Nozire and Serpil, 1993), ellagitannins (punicalin , punicalagin, granatin, gallagylidilactone, casurinin), pedunculagin, tellimagrandin , corilagin (Satomi *et al*, 1993), ellagic tannins, gallic, ellagic acids, ursolic acid

(Ben-Nasr *et al*, 1996) and catechin (Chidambara, *et al*, 2004)

This investigation aims to evaluate the hepatoprotective activity of an aqueous rind powder extract of pomegranate (*punica granatum* L.) in its human therapeutic dose against overdose acetaminophen

Material and methods

Plant material

200ml boiling distilled water were added to 3gm powder pomegranate peel, left it for 10 minutes and filtered. The filtrate was dried at 40-45°C in the incubator.

Animals

18 adult male albino rats weighing 120-150g were obtained from breeding in animal histology department in NODCAR. The animals were housed under good hygienic condition, diet and water excess

Experimental design

The animals were divided into three groups consisting of six animals. Group I, served as control, group II (over fasted animals) was given acetaminophen (0.5g/kg B.W. i. p.) and group III was given pomegranate aqueous extract (0.43g/kg B.W. i. p.) in a dose equivalent to human therapeutic dose (Paget and Barnes, 1964) for three consecutive days prior to the administration of acetaminophen.

Blood sampling

After 48 hours from acetaminophen administration, blood samples were collected from retro-orbital vein in all groups. The blood was allowed to collect at room temperature and serum obtained after centrifugation was used for determination serum aspartate aminotransferase, alanine aminotransferase (Reitman and Frankle, 1957) and lactate dehydrogenase (Raabo, 1963). Fresh liver samples were collected in formalin 10% and stained with E&H.

Statistical analysis

All data obtained were analyzed using student 't'-test according to Sendecor and Coebam (1969).

Results and Discussion

In the present investigation the hepatotoxic effects induced in rats by intraperitoneally injection with 0.5g/Kg B.W. (Buttar *et al*, 1976) significantly elevated serum levels of

AST, ALT, LDH (table,1) and (figs.1, 2 & 3) and liver revealed congestion and dilatation of central and hepatic portal veins, vacuolization and ballooning, a lot of pyknotic nuclei were detected with many small necrotic areas of hepatocytes, inflammatory cells in hepatocytes and around portal tract were observed and fatty droplets were scattered in the hepatocytes (figs.5, 6,7,8 and 9) compared to control (fig.4)

Necrotic areas of hepatocytes led to elevated serum AST and ALT. The significant increase in serum LDH is associated with increased lipid peroxidation which reflecting hepatic membrane damage (Morliere *et al*, 1991). The hepatic cellular damage and impaired liver function by paracetamol treatment may be as the result of lipid peroxidation with its numerous toxic oxygen free radicals on the biological membranes of the liver, and cause hepatocyte necrosis. The liver became unable to transport fat from and to the liver. Fat droplets appear to accumulate in the hepatocytes. Pretreatment of rats with 0.43g/Kg B.W. of an aqueous rind extract of *Punica granatum* for 3 days before intraperitoneally (i. p.) injection of 0.5gm acetaminophen significantly reduced the acute elevation of serum aspartate aminotransferase (AST), serum alanine aminotransferase, lactate dehydrogenase (LDH) (table,1) & (figs.1,2 & 3) and alleviated the degree of liver damage after the i.p. injection of hepatotoxin (fig.10)

Pomegranate rind aqueous extract exhibit strong antioxidant activity (Chidambara, *et al*, 2002), scavenging free radicals and ameliorate the damage occurred in liver by acetaminophen.

In conclusion aqueous extract of pomegranate peels possess a stronger antioxidant capacity could ameliorate the damage occurred in liver by overdose acetaminophen.

Table1: Effect of an aqueous extract of pomegranate on serum AST, ALT and LDH levels in rats treated with hepatotoxic dose of acetaminophen

Groups	Parameters		
	AST U/L	ALT U/L	LDH U/L
Control	39 ±1.5	24 ±2.1	150 ±1
acetaminophen	91***↑ ±4.2	80***↑ ±3.8	250***↑ ±2.3
pomegranate + acetaminophen	45 ±2.5	32 ±3	155 ±2.1

Number of rats in each group =6

***P<0.001

Fig1: Effect of an aqueous extract of pomegranate on serum AST levels in rats treated with hepatotoxic dose acetaminophen

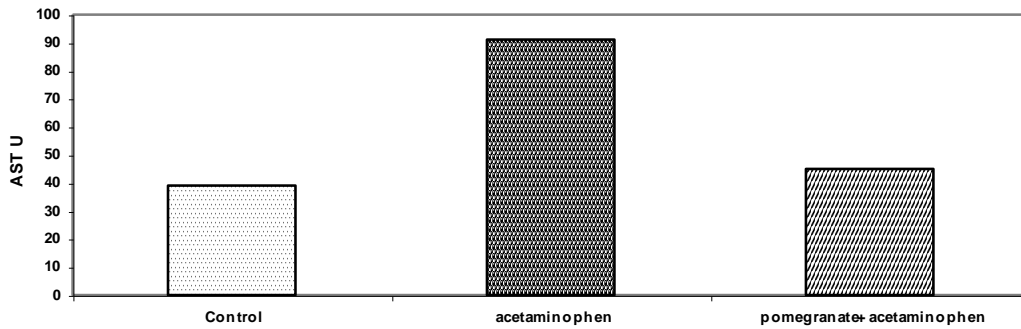


Fig.2: Effect of an aqueous extract of pomegranate on serum ALT U /L levels in rats treated with hepatotoxic dose of acetaminophen

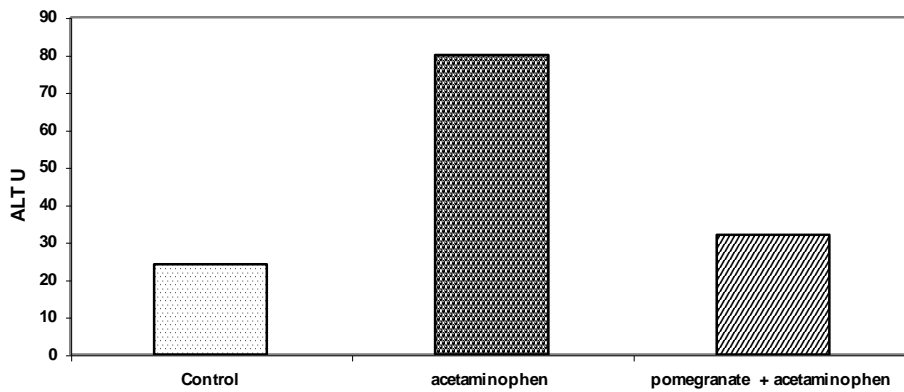
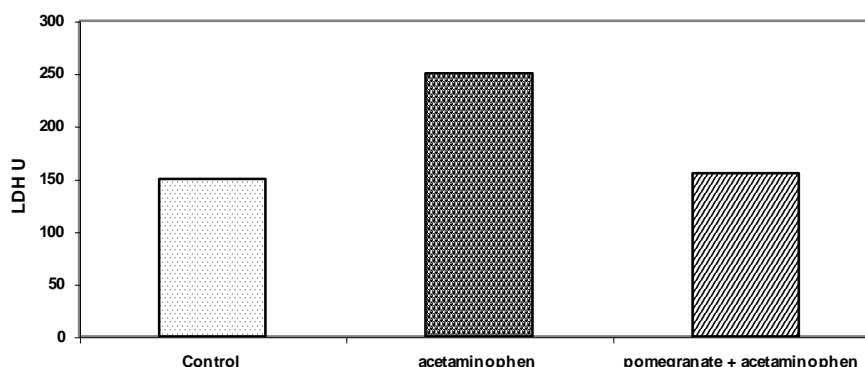


Fig.3: Effect of an aqueous extract of pomegranate on serum LDH UL levels in rats treated with hepatotoxic dose of acetaminophen



Legend of figures

Fig.4: Normal rat liver H&E x200

Fig.5: Liver of 0.5gm/Kg (i.p.) acetaminophen treated rat showing dilatation and congestion of central vein, dilatation of sinusoids, vacuolization and small areas of necrotic hepatocytes H&E x200

Fig.6: Liver of 0.5gm/Kg (i. p.) acetaminophen treated rat showing dilatation and congestion of portal tract with many inflammatory cells were appeared around it H&E x100

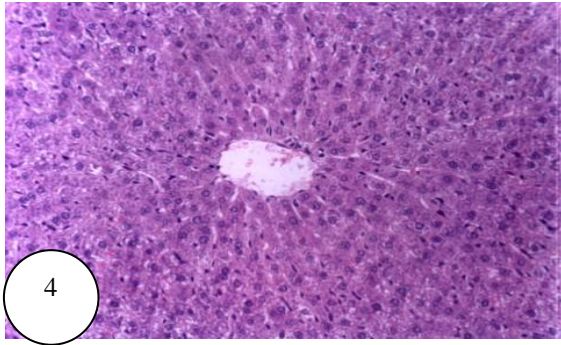
Fig.7: Liver of 0.5gm/Kg (i. p.) acetaminophen treated rat showing congestion of portal tract, inflammatory cells were appeared

around it and inbetween hepatocytes . Fatty droplets were scattered in the hepatocytes H&Ex400

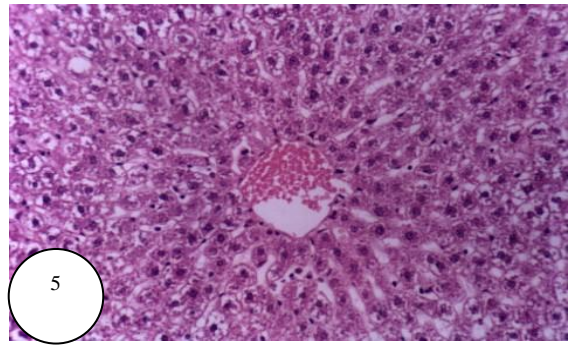
Fig.8: Liver of acetaminophen treated rat showing vacuolization and ballooning , a lot of pyknotic nuclei were detected with many small necrotic areas .

Fig.9: Liver of acetaminophen treated rat showing, a lot of inflammatory cells around the portal tract H&Ex400

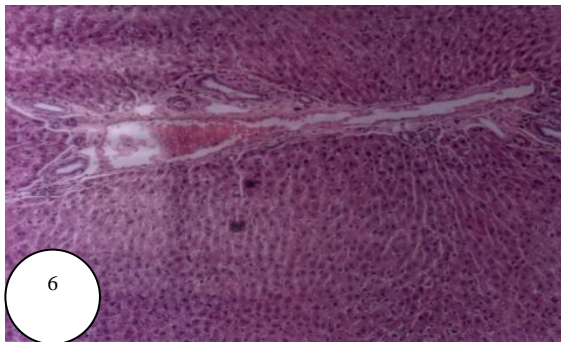
Fig.10: liver of 0.43g/kg B.W. i. p. pomegranate rind aqueous extract treated rats before (i.p.) injection of 0.5gm/Kg acetaminophen showing restoring the normal hepatic architecture and increased kupffer cells H&Ex400



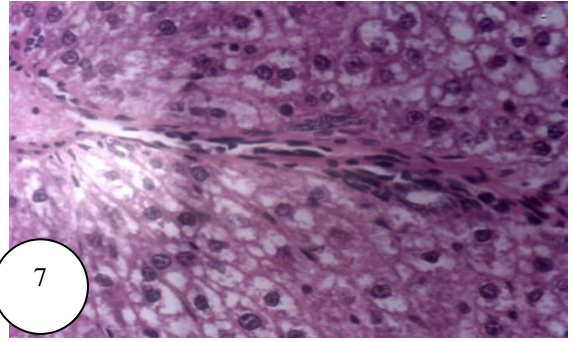
H & E X 200



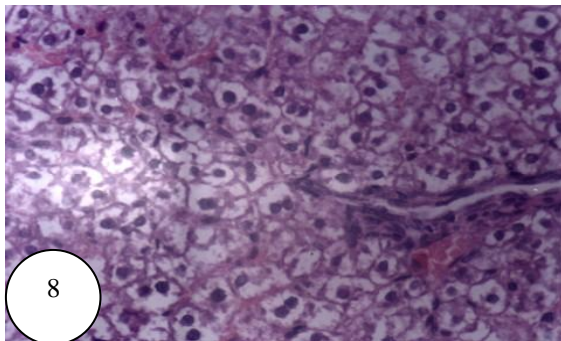
H & E X 200



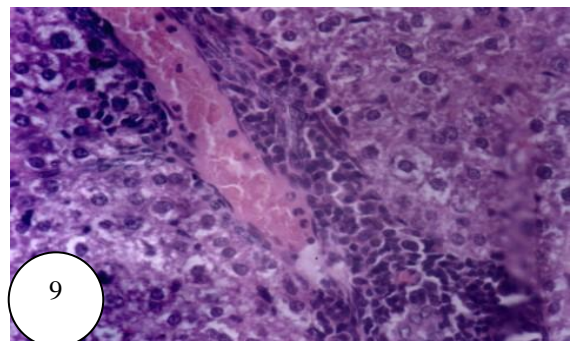
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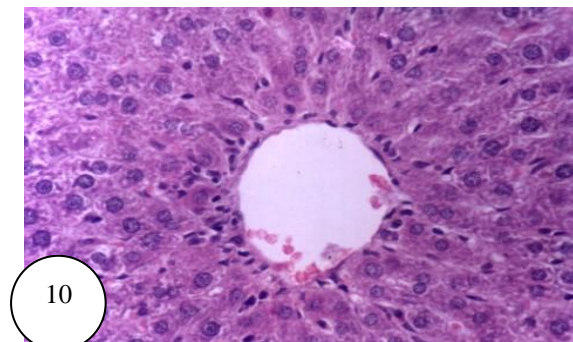
H & E X 400



H & E X 400



H & E X 400



H & E X 400

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**فاعلية تأثير المستخلص المائى لقشرة الرمان لحماية كبد الجرذان المعاملة
بأسيتامينوفين
إيناس على مهدى خليل
الهيئة القومية للرقابة والبحوث الدوائية**

إعتبر المصريون القدماء فاكهة الرمان كرمز للخصوبة ويرجع ذلك لتدويرها الشكلى وبذورها الوفيره وإستخدموها لعلاج كثير من الأمراض .
ويهدف البحث الى تقييم فاعلية المستخلص المائى لقشرة الرمان لحماية الكبد فبالجرذان المعاملة بأسيتامينوفين

ومعاملة الجرذان بجرعة 43 جم/كجم من وزن الجسم من المستخلص المائى لقشرة الرمان ثلاثة أيام قبل الحقن الغشاء البروتونى بأسيتامينوفين (5 جم / كجم) أدى الى انخفاض الأرتفاع فى مستوى مصل الخمائر الناقله لأمين وكذلك لكتات دى هيدروجيناز , وتخفيف درجة التلف فى الكبد والمجموعه المعامله بأسيتامينوفين 5 جم / كجم من وزن الجسم بأسيتامينوفين , وأظهرت أرتفاع فى مستوى الأنزيمات الناقله لأمين و لكتات دى هيدروجيناز و إحتقان وأ تساع فى الوريد المركزى والوريد الكبدى البابى وخلو خلايا الكبد من المحتوى السيتوبلازمى مع تتركز خلاياة وتحلل الأنوية وظهور خلايا التهابيه بين خلايا الكبد وحول المنطقه البابييه وأنتشار بعض الدهون فخلايا الكبد

ويستنتج البحث أن المستخلص المائى لقشرة الرمان له خاصية قويه مضادة لأكسدة حيث حد من التلف الحادث فى الكبد بأسيتامينوفين