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

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And Research

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) 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 diarrheoa 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 tumorgenesis (Castonguagy et al,1994) and promote cell function and replication (Alekperov, 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.
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(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

Results and Discussion

In the present investigation the hepatotoxic effects induced in rats by intraperitoneal 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 area 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 appears to accumulate in the hepatocytes. 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) (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.

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 Coebram (1969).
Table 1: Effect of an aqueous extract of pomegranate on serum AST, ALT and LDH levels in rats treated with hepatotoxic dose of acetaminophen

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>LDH U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>39 ±1.5</td>
<td>24 ±2.1</td>
<td>150 ±1</td>
</tr>
<tr>
<td>acetaminophen</td>
<td></td>
<td>91***↑</td>
<td>80***↑</td>
<td>250***↑</td>
</tr>
<tr>
<td>pomegranate + acetaminophen</td>
<td></td>
<td>45 ±2.5</td>
<td>32 ±3</td>
<td>155 ±2.1</td>
</tr>
</tbody>
</table>

Number of rats in each group = 6
***P<0.001

Fig. 1: Effect of an aqueous extract of pomegranate on serum AST U/L levels in rats treated with hepatotoxic dose of acetaminophen

Fig. 2: Effect of an aqueous extract of pomegranate on serum ALT U/L levels in rats treated with hepatotoxic dose of acetaminophen
A hepatoprotective effect of an aqueous extract of pomegranate on serum LDH U/L 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&E x400

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&E400

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&E400
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4. H & E X 200

5. H & E X 200

6. H & E X 100

7. H & E X 400

8. H & E X 400

9. H & E X 400

10. H & E X 400
References


فاعلية تأثير المستخلص المائي لقشرة الرمان لحماية كبد الجرذان المعامله باسيتامينوفين

إناس على مهدي خليل
الهيئة القومية للرقابة والبحوث الدوائية

يعتبر المصريون القدماء فاكهة الرمان كرمز للخصوبة ويرجع ذلك لتدويرها الشكلي والدوره الوفرة واستخدامها لعلاج كثير من الأمراض.
ويهدف البحث الى تقييم فاعلية المستخلص المائي لقشرة الرمان لحماية الكبد فيجرذان المعامله باسيتامينوفين

ومعاملة الجرذان بجرعة 43 جم/كلج من وزن الجسم من المستخلص المائي لقشرة الرمان ثلاثة أيام قبل الحقن الغشاء البروتوني باسيتامينوفين (5 جم/كلج) أدى إلى انخفاض الأرفاق في مستوى مصل الخمائر الناقل لامين وكذلك لكتات دي هيدروجيناز, وتخفيض درجة التلف في الكبد والمجموعه المعامله باسيتامينوفين 5 جم/كلج من وزن الجسم باسيتامينوفين, وأظهرت ارتفاع في مستوى الأنزيمات الناقل لامين ولكتات دي هيدروجيناز واحقان وأتساع في الوريد المركزي والوريد الكبدى البابى وخلو خلايا الكبد من المحتوى السيتو بلازمي مع تنكرز خلايا وتحل الأندية وظهور خلايا التهابى بين خلايا الكبد وحول المنطقه البابيه وانتشار بعض الدهون فيخلايا الكبد

ويستنتج البحث أن المستخلص المائي لقشرة الرمان له خاصية قويه مضادة لأكسدة حيث حد من التلف الحادث في الكبد باسيتامينوفين