Evaluation of the hepatoprotective activity of an aqueous extract of olive leaves in male albino rats

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Abstract

Olive leaf extract is used for centuries in treatment of various medical purposes. This investigation aims to evaluate the hepatoprotective activity of an aqueous extract of olive leaves against overdose paracetamol.

18 rats were divided into three groups, control, 0.5 gm/kg B.W. intraperitoneally (i.p.) paracetamol treated group and rats were given 2.5 gm/kg BW i.p. of olive leaf aqueous extract for three consecutive days prior to the administration of paracetamol.

The results indicated that treatment with paracetamol caused a marked significant increase in serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) accompanied by severe 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. Meanwhile group treated by olive leaf extract followed by paracetamol displayed significant reduction in serum (AST), (ALT) and (ALP) associated with nearly normal hepatocyte architecture.

In conclusion, an aqueous extract of olive leaves has antioxidant property could protect against liver damage occurred by overdose paracetamol in male albino rats.

Introduction

Olive leaf extract is traditionally used to relieve fevers, malaria and in the treatment of mild to moderate hypertension (Weiss, 1988). More recently, it was found that olive leaf extract lowers blood pressure (e.g. systolic and diastolic) and dilate the coronary arteries surrounding the heart (Petokov and Manolov, 1972 & Khyyal, et al., 2002), inhibits the oxidation of LDL and the development of atherosclerosis (Visioli and Galli, 1994 & Somova et al, 2003), has antibacterial activity (Fleming et al, 1973), antimicrobial effect (Markin, et al, 2003), antiviral properties (Heinze et al, 1975), reduces blood sugar (Peirce, 1999), enhances the immune system (Kubo, et al, 1985), has hypouricemia effect (Department of Pharmacology and Toxicology, Society of Pharmaceutical Industries of Tunis, 1994), has antidiarrhea effect (Duke, et al, 2002) can provide effective and cheap source to treat hypertension, complicated by stenocardia and cardiac failure (Somova et al, 2003).

Olive leaf extract has a wide number of constituents, including 4 types of triterpenoid derivatives, namely (oleanolic acid, ursolic acid, uvaol and methyle maslinate) (Somova, et al, 2004) and several types of flavonoids (e.g. rutin, apigenin and luteolin) (Bruneton, 1995).

(Buttar et al, 1976) reported that hepatocellular damage and impaired liver function occurred following administration of single doses of 0.5 and 1 gm/kg B.W. of paracetamol in rats.

This investigation aims to evaluate the hepatoprotective activity of an aqueous extract of olive leaves against overdose paracetamol.

Materials and Methods

Plant material

Olive leaves were obtained from the trees cultivated in the front of Alexandria Library. The leaves were dried and well ground. 200 ml of boiling distilled water were added to 7 g powder olive leaves, left for 10 minutes and filtered. The filtrate was dried in an incubator at 35-40°C. The residue was given to rats in a dose
equivalent to human therapeutic dose (Paget and Barnes, 1964).

**Animals**

18 adult male albino rats weighing 120-150g were purchased from Helwan farm. The animals were housed under good hygienic condition 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 paracetamol (0.5g/kg B.W. i.p.) and group III was given olive leaf aqueous extract (2.5g/kg B.W. i.p.) for three consecutive days prior to the administration of paracetamol.

**Blood sampling**

After 48 hours from paracetamol 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 alkaline phosphatase (German Society for clinical chemistry, 1972).

Fresh liver samples were collected in formalin 10% and stained with E&H.

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

**Results and Discussion**

Table(1) displayed marked significant elevation in serum (AST), (ALT) and (ALP) in paracetamol treated group accompanied by congestion of central vein, vacuolization and small areas of many pyknotic nuclei were observed. Debris of cytoplasmic organoid were noticed with enlarged nuclei of endothelial lining of central vein. Inflammatory cells, fatty droplets were scatters in the hepatocytes. 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 unable to transport fat from and to the liver. Fat droplets appear to accumulate in the hepatocytes. Treatment of aqueous extract of olive leaves prior the administration of paracetamol preventing free radical generation and stopping the resulting damage occurred in the hepatocytes. Serum AST, ALT and ALP appear normal levels, and this was accompanied with normal architecture of hepatocytes and central vein, while few cells appeared faintly stained (fig.5).

The hepatoprotective effect of aqueous extract of olive leaves may be due to its antioxidant property (Peirce, 1999). The phenolic structure of olive leaf extract (Briante, et al., 2002) helps to reduce the free radicals which resulted from hepatotoxin paracetamol.

Farag et al.,(2003) reported that free and total polyphenolic compounds extracted from olive leaves are safe on serum (AST), (ALT).

In conclusion, an aqueous extract of olive leaves has antioxidant property which can protect liver damage occurred by overdose paracetamol in male albino rats.

**Legend of figures**

**Fig.2:** Normal rat liver H &E x 100
**Fig.3:** Liver of paracetamol treated rat showing congestion of central vein, vacuolization and small areas of many pyknotic nuclei were observed. Debris of cytoplasmic organoid were noticed with enlarged nuclei of endothelial lining of central vein. Inflammatory cells, fatty droplets were scatters in the hepatocytes. H &E x 400

**Fig.4:** Liver of paracetamol treated rat showing congested hepatic portal vein and dilated bile canaliculi with dilated sinusoidal spaces. H&E x 400

**Fig.5:** Liver of paracetamol and olive leaf extract treated rat showing normal architecture of hepatocytes and central vein, while few cells appeared faintly stained. H &E x 100
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Table1: Effect of olive leaf aqueous extract on serum enzymes in rats treated with hepatotoxic dose of paracetamol

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST (U/L)</td>
</tr>
<tr>
<td>Control</td>
<td>39 ±1.5</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>91***↑ ±4.2</td>
</tr>
<tr>
<td>Olive leaf + Paracetamol</td>
<td>45 ±2.5</td>
</tr>
</tbody>
</table>

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

Fig 1: Effect of olive leaf extract on serum enzymes in rats treated with paracetamol
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References
تقييم فاعلية المستخلص المائي لأوراق الزيتون لحماية الخلايا الكبدية
في ذكور الجرذان

أيناس علي مهدي خليل
الهيئة القومية للرقابة والبحوث الدوانية

يستخدم مستخلص ورق الزيتون في أغراض طبية متعددة من قرون مضت.
ويهدف هذا البحث لتقييم فاعلية المستخلص المائي لأوراق الزيتون لحماية الخلايا الكبدية في ذكور الجرذان.

وقد تم تقسيم عدد 18 فأر ثلاثة مجاميع، مجموعه ضابطة ومجموعه تعامل
بالحقن في الغشاء البروتيوني بالباراسيتامول بجرعه مقدره 1/2 جم/كلم من وزن
الجسم ومجموعه تحقن في الغشاء البروتيوني لمدة ثلاثة أيام متتاليه بمستخلص المائي
لأوراق الزيتون بجرعه مقدره 2.5 جم/كلم من وزن الجسم ثم تحقن في الغشاء
البروتيوني بالباراسيتامول بجرعه مقدره 1/2 جم/كلم من وزن الجسم وينتهي فتره
التجربة بعد يومين من حقن الباراسيتامول. وقد نتت عن هذه المعامله ارتفاع ذو دلالة
أحصائيه في مستوى الأنسيميات الناقل للأنمي وأرتفاع في مستوى الفوسفات القاعدى
وكان ذلك مصحوبا بأحجان شديد في الوريد المركزي والوريد الكبيدى البابى وخلو
خلايا الكبد من المحتوى السيتو بلازما مع تنكرز خلايا وختال الأنيوية.

وينضح من هذا البحث أن المستخلص المائي لأوراق الزيتون له خاصيه مضادة
للأكسدة وأظهر فاعليته تجاة حماية الخلايا الكبدية حيث اصبحت مقاربه للطبيعية
وانخفضت الأنسيميات الكبدية انخفض ذو دلالة إحصائيه.