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Evaluation of hepatoprotective effect of *Pistacia lentiscus*, *Phillyrea latifolia* and *Nicotiana glauca*

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Abstract

The hepatoprotective effect of the boiled and non-boiled aqueous extracts of *Pistacia lentiscus*, *Phillyrea latifolia*, and *Nicotiana glauca*, that are alleged to be effective in the treatment of jaundice in Jordanian folk medicine, was evaluated in vivo using carbon tetrachloride (CCl_4) intoxicated rats as an experimental model. Plant extracts were administrated orally at a dose of 4 ml/kg body weight, containing various amounts of solid matter. Only total serum bilirubin level was reduced by treatment with non-boiled aqueous extract of *N. glauca* leaves, while the boiled and non-boiled aqueous extracts of the *N. glauca* flowers were non effective. Bilirubin level and the activity of alkaline phosphatase (ALP) were both reduced upon treatment with boiled aqueous extract of *P. latifolia* without reducing the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Aqueous extract of *P. lentiscus* (both boiled and non-boiled) showed marked antihepatotoxic activity against CCl_4 by reducing the activity of the three enzymes and the level of bilirubin. The effect of the non-boiled aqueous extract was more pronounced than that of the boiled extract.

Keywords: *Pistacia lentiscus*; *Phillyrea latifolia*; *Nicotiana glauca*; CCl_4; Antihepatotoxic; Liver; Jordanian folk medicine

1. Introduction

Decoctions of *Pistacia lentiscus* leaves, *Phillyrea latifolia* leaves, and *Nicotiana glauca* leaves and flowers, are being currently used in Jordanian folk medicine to ameliorate jaundice. The decoction is prepared by boiling leaves or flowers of the plants for few minutes. Although methods of preparation vary slightly from group to group, the efficiency of these decoctions is claimed high.

P. lentiscus and *P. latifolia* are evergreen shrubs, which are widely distributed in the Mediterranean region (Encyclopaedia Britannica, 1990). On the other hand, *N. glauca* is an annual shrub, which originated from South America (Zohary, 1972) but recently it has started infesting large areas in the valley of Jordan during summer.

In the Palestinian area *P. lentiscus* is also used as a folk medicine to ameliorate jaundice. In the Mediterranean region, the use of *P. lentiscus* as an antibacterial agent was reported by several research groups (Magiatis et al., 1999; Ali-Shtayeh et al., 1998; Iauk et al., 1996). It was also found as a potent antiulcer agent (Al-Said et al., 1986).

In Spain, Mediterranean Europe, and North Africa, people have used infusions prepared from the leaves and fruits of *P. latifoliai* as an astringent, diuretic and for the treatment of mouth ulcers and inflammations (Diaz et al., 2001). *P. latifolia* also has an anti-inflammatory activity (Diaz et al., 2000). The use of *P. latifolia* to ameliorate jaundice is unique to Jordanian folk medicine.

N. gluaca is known as a highly toxic and teratogenic plant (Panter et al., 1999), it is used to treat burns and inflammatory diseases in Italy (Morel et al., 1998). The use of *N. glauca* as an anti-jaundice plant is also unique to Jordanian folk medicine.

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The present study was undertaken to evaluate the efficacy of the boiled and non-boiled aqueous extracts of these three plants against experimental liver damage inflicted by a reliable hepatotoxin, CCl_4. Boiled extracts were used to simulate the most used method by Jordanians to ameliorate jaundice. Whereas non-boiled extracts were used to detect whether boiling affects the activity of the decoctions or not.

2. Materials and methods

2.1. Chemicals

Bilirubin, ALP, ALT and AST kits were purchased from Randox, UK. CCl_4 was purchased from Pharmacos Ltd., UK.

2.2. Plant material

Leaves of both *P. lentiscus* and *P. latifolia*, as well as leaves and flowers of *N. glauca* were collected during July 1998 from Jerash, Ajloun and Jordan Valley, respectively. The plant specimens were authenticated by Professor Dawood Al-Eisawi from Biological Science Department, at the University of Jordan (Al-Eisawi, 1998). Voucher specimen of each plant was deposited at the herbarium of the University of Jordan under acquisition numbers 31, 32, 33, respectively.

2.3. Test animals

Male Wistar albino rats (4–6 weeks), 130–180 g were obtained from the Animal House at Jordan University of Science and Technology. They were housed individually in suspended screen wire cages in an air-conditioned room at 20 ± 3 °C, and maintained on tap water and standard diet ad libitum throughout the investigation, and were maintained on a 12-h dark and 12-h light cycle.

2.4. Preparation of boiled and non-boiled aqueous plant extracts

Fresh samples of the parts of the plants used in folk medicine in Jordan (leaves of both *P. lentiscus* and *P. latifolia*, as well as leaves and flowers of *N. glauca*) were homogenized 1:3 (w/v) in cold distilled water using a household blender on full speed for 1 min. This dose was not based on clinical trials, but similar to the method of preparation used by Jordanians. The homogenates were refrigerated overnight, then filtered through cheesecloth. The filtrates were centrifuged at 4000 rpm for 15 min at 4 °C then the supernatants were divided into two portions. One portion was boiled for 10 min and the other was left without boiling. The boiled and the non-boiled extracts were then concentrated using a steam of air at room temperature until the volume was one tenth of the original volume. Estimation of solid matter content was accomplished by bringing the samples to complete dryness.

2.5. Antihepatotoxic activity of extracts

Hepatotoxicity was induced with a (1:1) mixture of CCl_4 and olive oil, administrated intraperitoneally at a single dose of 2 ml/kg body weight. Rats were divided randomly into twelve groups of five. Four groups received boiled aqueous plant extract and the other four received non-boiled aqueous plant extracts. Two normal control groups and two intoxicated control groups were used for comparison. The normal control groups consisted of normal untreated rats, while all other groups were intoxicated intraperitoneally with 2 ml/kg body weight of CCl_4/olive oil mixture. Test groups were treated twice daily with 4 ml/kg body weight of the four plant extracts using intragastric tube for 3 days. The concentrated extracts contained various amounts of solid matter (Table 1). On the fourth day, the rats were intoxicated with CCl_4/olive oil mixture intraperitoneally, followed by two additional doses of plant extracts after 1 and 4 h of CCl_4 injection. Control group was administrated with normal saline instead of the plant extract. Blood samples were collected after 1 day of CCl_4 administration.

2.6. Assessment of liver function

Rats of all groups were anaesthetized with ether then blood was collected directly from the heart by heart puncture. Serum was separated by centrifugation at 3000 rpm for 10 min. Serum samples were immediately subjected to estimation of bilirubin level and the activity of liver enzymes detected in the serum. The level of total bilirubin and the activity of the enzymes; ALP, ALT, and AST were assayed according to the methods of Jendrassik and Groff (1938), Bergmeyer and Brent (1974), Reitman and Frankel (1957), Berger and Rudolf (1963), respectively.

Table 1
Solid matter content in 4 ml of the concentrated extracts

Sample	Weight of solid matter (g)/4 ml
P. lentiscus non-boiled	1.70
P. lentiscus boiled	1.946
P. latifolia non-boiled	1.184
P. latifolia boiled	1.017
N. glauca flowers non-boiled	0.947
N. glauca flowers boiled	1.05
N. glauca leaves non-boiled	0.848
N. glauca leaves boiled	0.811

3. Results

3.1. Effect of boiling on solid matter content of the extracts

Table 1 depicts that boiling of the extracts of *P. lentiscus* leaves, *P. latifolia* leaves and *N. gluaca* leaves and flowers did not affect solid matter content of the extract drastically. So, any difference in the effect would not be due to the concentration of the solid matter content in the dose, but rather to the nature of the solid matter.

3.2. Effect of non-boiled aqueous extracts

The activity of serum ALP, ALT, and AST and the level of bilirubin after the treatment with the non-boiled aqueous extracts of the three plants are shown in Table 2. The enzymes activity and the level of bilirubin were elevated markedly in the intoxicated control group compared with the normal control group. *P. lentiscus* non-boiled aqueous extract exhibited the best hepatoprotective activity by declining the activity of ALP ($P < 0.01$), ALT ($P < 0.05$), AST ($P < 0.01$) as well as the bilirubin level ($P < 0.05$).

The non-boiled aqueous extract of *N. glauca* leaves reduced the level of bilirubin ($P < 0.05$), however, it failed to reduce the activity of any of the three enzymes; ALP, ALT, or AST. Treatment with the non-boiled aqueous extract of both *P. latifolia* and the flowers of *N. glauca* showed no hepatoprotective effect as reflected by the high measured liver parameters.

3.3. Effect of boiled aqueous extracts

The hepatoprotective action of the boiled aqueous extracts on hepatotoxicity induced by CCl_4 is summarized in Table 3. Again, *P. lentiscus* aqueous extract was the most potent in decreasing the activity of ALP ($P < 0.01$), ALT ($P < 0.01$), and AST ($P < 0.005$), and the level of bilirubin ($P < 0.05$). The hepatoprotective action of boiled aqueous extract of *P. lentiscus* was lower than that of non-boiled extract.

The boiled aqueous extract of *P. latifolia* also afford hepatoprotection by reducing the level of bilirubin ($P < 0.05$) and the activity of ALP ($P < 0.01$), but did not reduce the activity of ALT and AST. Neither the flowers nor the leaves of *N. glauca* showed any hepatoprotective effect.

Table 2
Effect of non-boiled aqueous extracts

Rat group	BRN (mg/dl)	ALP (U/l)	ALT (U/l)	AST (U/l)
−ve control	0.19±0.01	68±6	104±20	178±9
+ve control	0.40±0.04	145±11	727±38	817±35
P. lentiscus	0.21±0.07*	81±8**	169±25*	337±35**
P. latifolia	0.93±0.68	138±10	775±6	760±66
N. glauca (leaves)	0.18±0.02*	139±15	770±14	767±18
N. glauca (flowers)	0.96±0.06	137±8	745±46	784±48

The activity of ALP, ALT, and AST and the level of bilirubin in rats intraperitoneally injected with 2 ml/kg b.w. of CCl_4/olive oil after oral administration of 4 ml/kg b.w. of non-boiled aqueous plant extracts. BRN, bilirubin; −ve control, normal rats; +ve control, CCl_4-intoxicated rats; $n = 5$. Data are shown as mean±S.D.
*, $P < 0.05$.
**, $P < 0.01$.

Table 3
Effect of boiled aqueous plant extracts

Plant extract	BRN (mg/dl)	ALP (U/l)	ALT (U/l)	AST (U/l)
−ve control	0.16±0.01	67±13	156±33	166±23
+ve control	0.41±0.03	141±17	801±10	819±25
P. lentiscus	0.20±0.04*	83±15**	271±43**	453±101***
P. latifolia	0.19±0.04*	89±21**	632±142	813±40
N. glauca (leaves)	0.73±0.15	161±7	808±12	841±21
N. glauca (flowers)	0.68±0.06	126±7	790±34	771±20

The activity of ALP, ALT, and AST and the level of bilirubin in rats intraperitoneally injected with 2 ml/kg b.w. of CCl_4/olive oil after oral administration of 4 ml/kg b.w. of boiled aqueous plant extracts. BRN, bilirubin; −ve control, normal untreated rats; +ve control, rats intoxicated with CCl_4 without treatment; $n = 5$. Data are shown as mean±S.D.
*, $P < 0.05$.
**, $P < 0.01$.
***, $P < 0.005$.

4. Discussion and conclusion

The presence of jaundice is a cardinal feature of liver disease, and its presence usually signifies disturbance involving the hepatobiliary system (Higa, 2000). Carbon tetrachloride is a potent hepatotoxin, and a single exposure to it can rapidly lead to severe centrizonal necrosis and steatosis (Zimmerman, 1982). Damage to the structural integrity of liver is reflected by increase in the liver hepato-specific enzymes (ALP, ALT and AST) in the serum, because they are cytoplasmic in location and are released into circulation after cellular damage (Janbaz and Gilani, 1995; Venkateswaran et al., 1995).

Non-boiled extract of *P. latifolia* leaves did not affect the activity of the three liver enzymes, but increased the level of Bilirubin (Table 2). However, boiled aqueous extract of *P. latifolia* leaves, which resembles the traditional way of the decoction preparation, showed a reduction in the elevation of the level of bilirubin and the activity of ALP; induced by CCl_4; without affecting the elevation in the activity of ALT and AST (Table 3).

Boiling of the extract might have caused formation of new active substances; that were not present in the non-boiled extract, which caused the reduction of bilirubin and ALP. The activity of ALP rises in both hepatic and post-hepatic diseases, but the rise is usually greater in post-hepatic (Kaplan et al., 1995). Thus, boiled extract of this plant may be used in the treatment of obstructive jaundice where the activity of ALP is greatly elevated.

The non-boiled aqueous extract of the leaves of *N. glauca* reduced only bilirubin level without affecting the activity of ALP, ALT, or AST (Table 2), whereas the boiled and non-boiled extracts of the flowers did not cause a reduction in the level of bilirubin or in the activity of any of the three enzymes (Tables 2 and 3). Although the non-boiled extract of *N. glauca* leaves reduced serum bilirubin level and may be considered as a useful remedy in the treatment of prehepatic jaundice, yet it should not be used due to its high toxicity and teratogenicity (Russel et al., 1997; Panter et al., 1990).

The antihepatotoxic activity was observed maximally with *P. lentiscus* non-boiled aqueous extract and it reduced all four parameters significantly (Table 2). On the other hand, *P. lentiscus* boiled aqueous extract also showed a clear hepatoprotective effect, but to a lesser degree (Table 3). Accordingly, *P. lentiscus* non-boiled aqueous extract might be used in the treatment of hepatic jaundice.

The results obtained in the present study revealed an effectiveness of *P. lentiscus* in the treatment of hepatic jaundice in the rat, and thus it may be used as a promising potential treatment of human hepatic jaundice.

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