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Full Length Research Paper

Antioxidant and hepatoprotective effects of the internal layer of oak fruit (Jaft)

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An aqueous extract of the internal layer of oak fruit (Jaft) was evaluated for *in vitro* antioxidant activity and hepatoprotective effects on carbon tetrachloride (CCl_4)-induced liver damage in rats. Thirty-six male Wistar rats were randomly divided into 6 groups. Groups 1, 2 and 3 served as negative controls and were given olive oil, distilled water, and Jaft extract (500 mg/kg), respectively. Group 4 served as the toxic group and received 1 ml/kg CCl_4 intraperitoneally as a single dose every 72 h. Groups 5 and 6 received oral aqueous extracts of Jaft at 250 and 500 mg/kg, respectively, 1 h after the injection of CCl_4 for every 72 h. Jaft doses of 250 and 500 mg/kg resulted in decreased serum enzyme and bilirubin levels ($P < 0.01$), which is an indication of hepatoprotection. CCl_4 induced a significant increase in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), and total bilirubin and a reduction of total protein ($P < 0.01$). Treating rats with Jaft extract significantly lowered these serum marker enzymes as compared to CCl_4-treated rats ($P < 0.001$). Aqueous extracts of Jaft at 250 and 500 mg/kg showed hepatoprotective potential and did not have toxic effects.

Key words: Antioxidant activity, hepatoprotective, enzyme markers, oak fruit.

INTRODUCTION

In spite of enormous scientific progress in hepatology field, hepatitis is one of the most liver-related condition with high mortality (Pang et al., 1992). There is a renewed interest in traditional herbal medicine for treating various hepatic diseases, because few reliable hepatoprotective medicines from natural sources are presently available (Shivkumar et al., 2006). Therefore, people show more interest in using indigenous herbal medicine for treatment of hepatic disorders.

Several plant and medicinal plant products are identified to have antioxidant activity with organ protective agent (Jyothi et al., 2007). Various species of oak plant, such as *Quercus resinosa, Quercus eduardii*, and *Quercus sideroxyla* leaves are used in herbal medicine for treating variety of diseases (Rivas- Arreola et al., 2010).

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Quercus brantii and *Quercus ilex* are widely distributed in the northern and central parts of Iran (Khosravi and Behzadi, 2006). The root bark of *Q. ilex* is used in Iranian folk medicine to treat gastropathies (Khennouf et al., 1999). A fruit decocted extracts of *Quercus* species are used to treat acute diarrhea, inflammation, burns, and cuts in traditional medicine (Konig et al., 1994).

The leaves and fruit components of several *Quercus* spp. have antioxidant activity which are used to treat diseases such as cancer in traditional medicine (Rocha-Guzman et al., 2007; Lee et al., 1992). The antioxidant and anti-lipid peroxidation activity of the *Quercus* spp. may be due to the presence of polyphenol and tannin compounds (Seddik et al., 2010; Rocha-Guzman et al., 2009).

Quercus spp. fruit is used for food in both fresh and processed forms, making of handcrafts, leather tanning, and fodder for sheep and goats (Luna-José et al., 2003), and for the past 50 years, oak fruit has been one of the major food components in the poorer classes of Yasuj

city.

The *Quercus* spp. fruit has external and internal layers and the internal layer is known locally as Jaft. Although, Jaft is usually yellow, its color changes to brown after exposure to light due to oxidation.

The aim of this study was to evaluate the antioxidant activity and hepatoprotective effect of the aqueous Jaft extract. carbon tetrachloride (CCl_4) was used to induce hepatotoxicity in a rat model (Parola et al., 1992). several different assay systems were also used to evaluate the *in vitro* antioxidant activity in different extracts of the Jaft.

MATERIALS AND METHODS

Fruits of the *Quercus* spp. were collected in and around the Yasuj hills, Iran, in September 2010. Samples were identified and a voucher specimen (HMRC-J 11/09/2010) was deposited in the Herbal Medicinal Research Center, Yasoj University of Medical Sciences, Yasouj, Iran. Fruits were washed, shade-dried, and the Jaft was removed and ground to a fine powder (20 mesh) using a mill (Restsch Ultra Centrifugal Mill and Sieving Machine, Haan, Germany).

Extraction

Plant materials were extracted with 70% (v/v) methanol, chloroform, and distilled water for preparation of methanolic, chloroform, and aqueous extracts, respectively, using a Soxhlet apparatus for 6 h. The extracts were collected and dried using a rotary evaporator (Hyedolph model 4000; Germany) and were kept in a refrigerator for further studies.

In vitro antioxidant activity and phytochemical component

The antioxidant activities of the extracts were tested using the following 4 assays: (1) phosphomolybdenum (PMB) (Prieto et al., 1999), (2) ferric-reducing antioxidant power (FRAP) (Benzie and Strain, 1996), (3) 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical (Von Gadow et al., 1997), and (4) trolox equivalent antioxidant capacity (TEAC) radical methods (Re et al., 1999). Furthermore, 2 phytochemical important compounds, such as, total phenols (Folin and Ciocalteu, 1927) and total flavonoids (Zhishen et al., 1999) were estimated.

Acute toxicity testing

Acute oral toxicity (LD_{50}) was estimated in 30 male rats, which were divided into 6 groups that comprised 5 animals matched for weight and size. The first group served as a control and received a single dose of distilled water (1 ml/kg) by the oral (p.o.) route. Groups 2 to 6 were orally treated with single doses of Jaft aqueous extracts ranging from 1000 to 5000 mg/kg. Animals were placed under observation for general behavior and signs of toxicity continuously for 1 h after the treatment and then intermittently for 4 h and thereafter over a period of 24 h (Twaij et al., 1983). The animals were also observed for abnormal signs and symptoms for 14 days. The acute toxicity LD_{50} was calculated. All animals had free access to distilled water.

Hepatoprotective activity

Thirty-six adult male Wistar rats (250 to 300 g) were selected for the present study and randomly divided into 6 groups (1 to 6) comprised 6 animals each. Animals were maintained in a controlled environment of 22 ± 2°C, 65 to 70% humidity, and 12 h light/dark cycle, and were fed a standard laboratory diet (Pars, Iran Ltd., Tehran, Iran).

Groups 1 and 2 served as negative controls and were given olive oil (1 ml/kg) intraperitoneally (i.p.) and distilled water (DW) 1 ml/kg p.o., respectively, in single daily doses for 28 days. Group 3, the extract control, received aqueous Jaft extract (500 mg/kg) in single daily p.o. doses for 28 days. Group 4, the toxic group, received CCl_4 (1 ml/kg) mixed with an equal volume of olive oil as a single i.p. dose every 72 h for 28 days. Groups 5 and 6, the protective groups, received aqueous Jaft extract (250 and 500 mg/kg, respectively) p.o. for 28 days 1 h after the injection of CCl_4 every 72 h. At the end of the 28th day, animals were exsanguinated under diethyl ether anesthesia.

Biochemical analysis

Blood samples were collected by heart puncture and the serum was centrifuged at 3000 g for 10 min and was used for biochemical tests. Biochemical parameters including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), conjugated bilirubin (CB), total proteins (TP), and albumin were determined in serum. Analyses were performed according to accepted local clinical chemistry standards using Pars Azemon local company kits.

Statistical analysis

All results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out by performing one way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests using Statistical Package for Social Sciences (SPSS) software version 13. Statistical significance was set at $P < 0.05$.

RESULTS

In vitro antioxidant activity

Aqueous and chloroform extracts showed the highest and lowest total phenol values, respectively (Table 1). The flavonoid content of extracts ranged from 31.3 to 83 mg rutin as a standard in each gram of extracts. The antioxidant activities of the extracts were determined as trolox equivalents (mmol trolox/g extract) using TEAC, DPPH, and PMB assays. The highest antioxidant potential was observed with methanol extracts in the TEAC assay (45.3 mmol trolox/g) followed by methanol extracts in the DPPH test (42.26 mmol trolox/g). The chloroform extract showed the lowest activity with a value of 25. The antioxidant activities varied from 832.7 to 1249 mmol Fe_2/g extract by the FRAP assay (Table 1).

Acute toxicity

The oral acute toxicity assessment did not reveal any abnormal clinical signs or mortalities in the surviving rats even at the maximum dose of 5000 mg/kg body weight after 14 days. LD_{50} values were calculated as >5000

Table 1. Measurement of *in vitro* total phenols, flavonoids, and antioxidant activity of different methods (DPPH, TEAC, PMB, and FRAP) of Jaft extracts, a product of *Quercus* spp. fruit.

Extract	T P[a]	Flavonoids[b]	DPPH[c]	TEAC[d]	PMB[e]	FRAP[f]
Chlorofom	79.3 ± 6	31.3 ± 5.5	25 ± 2.6	18.6 ± 3.05	1.55 ± 0.3	832.7 ± 11.4
Methanol	153 ± 6.24*	83 ± 1.05*	42.26 ± 3.8*	45.3 ± 5*	5.13 ± 1*	1249 ± 24*
Aqueous	152.7 ± 7.5**	67.7 ± 7.5**	33.16 ± 3.2**	43.6 ± 2.76**	6.06 ± 0.41**	1193.7 ± 20.1**

*Statistically significant difference versus chloroform ($P < 0.01$). **Statistically significant difference versus chloroform ($P < 0.01$). [a]Total phenolic (TP) content was expressed as mg gallic acid equivalents/g dried extract. [b]Flavonoid content was expressed as mg rutin equivalents/g dried extract. [c]Diphenyl Pyciryl hydrasyl (DPPH) was expressed mM trolox equivalent/g dried extract. [d]Trolex Equivalent Antioxidant Capacity (TEAC) was expressed as mM trolox equivalent/g dried extract. [e]Phosphomolybdynium (PMB) was express as mM trolox equivalent/g dried extract. [f]Ferric Reducing Antioxidant Power (FRAP) was express as mM SO_4 Fe equivalent/g dried extract. Values are expressed as mean ± standard deviation (n = 3).

mg/kg p.o., because 5000 mg/kg of Jaft was well tolerated in rats after 14 days.

Hepatotoxicity

No significant differences were observed in biochemical tests between control and extract groups (Tables 2 and 3). Lack of significant effects of Jaft in group 3 on biochemical tests indicates that Jaft extracts alone do not have any toxic side effects on liver functions. The effects of Jaft on serum marker enzymes are shown in Table 2. The results of this study show that the administration of CCl_4 produced hepatic damage and liver dysfunction. Liver dysfunction was confirmed by significant increases ($P < 0.01$) in the marker enzymes ALT, AST, ALP, and TB and a significant decrease in total protein ($P < 0.01$) level when compared with the control groups (Tables 2 and 3).

The levels of serum bilirubin and serum total protein are shown in Table 3. The level of bilirubin was significantly ($P < 0.05$) increased in group 4, whereas the total protein level was significantly decreased ($P < 0.01$).

Jaft extracts at doses of 250 and 500 mg/kg remarkably prevented CCl_4-induced hepatotoxicity in a dose-dependent manner as evaluated by enzyme markers (Table 2). Furthermore, treatment with Jaft extracts resulted in a significant ($P < 0.05$) decrease in plasma TB and an increase in the levels of total proteins (Table 3).

DISCUSSION

This experimental study evaluated the antioxidant and hepatoprotective activities of an aqueous extract of Jaft for the first time in an experimental liver toxicity model induced by CCl_4. This study also provided new scientific information on Jaft extracts in Iran.

This study have shown that methanolic and aqueous extracts of Jaft have potent antioxidant activities by using several different *in vitr*o antioxidant assays. The DPPH assay is commonly used to evaluate antioxidant or scavenging activities, whereas the TEAC method is useful for determining the antioxidant and radical-scavenging activities of plants. The PMB method is applied for estimation of antioxidant potential on the basis of reduction of Mo(6) to Mo(5) by the sample extracts. Finally, the FRAP assay is a quick and routine analysis that determines antioxidant potential according to ferrous ion formation (Chanda and Dave, 2009).

The antioxidant activity of most plant extracts is associated with their phenolic concentrations. Phenolic and flavonoid compounds, which are secondary metabolite compounds in plants, have potent antioxidant activity, because they contain a functional group with antioxidant and scavenging properties (Kessler et al., 2003). Aqueous Jaft extracts was selected for *in vivo* hepatoprotectivity assays, because the methanolic and aqueous Jaft extracts showed similar antioxidant activities.

The hepatotoxic effects of CCl_4 are mainly caused by peroxidation of membrane lipids and the presence of the trichloromethyl radical (Johnson and Kroening, 1998; Kaplowitz et al., 1986). In the present study, this hepatotoxicity was evidenced by significant ($P < 0.001$) increases in the levels of AST, ALT, ALP, and TB and a decline in the total protein level in CCl_4-treated animals as compared to controls. Hepatocellular injuries are measured by levels of AST and ALT in serum. The activity of ALT is normally predominant in the cytoplasm of hepatocytes, and its activity is elevated in hepatocellular inflammation and necrosis (Mayne, 1996).

The decreased serum transaminase activities in the experimental groups in this study indicates stabilization of the plasma membrane and hepatocyte protection in contrast to the damage caused by CCl_4 (Chandrashekar et al., 2004).

The insignificant effect of the Jaft extract on ALT, AST, ALP, total bilirubin, and total protein levels in group 3 reveals that Jaft does not have toxic side effects on liver functions. This finding is supported by our acute toxicity results. The decreased plasma protein levels in animals

Table 2. Effects of aqueous Jaft extract a product of *Quercus* spp. fruit on marker enzymes in CCl_4 induced hepatoxicity in rats.

Group no.	Treatment received	Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)
1	DW	1 ml/kg	66.8± 4	41.7± 2	83.5± 2.4
2	Olive oil	1 ml/kg	64.8± 3.1	39± 2.2	82±2.4
3	Extract	500	62.6± 5.6	40.3± 2.8	86± 5.1
4	CCl_4	1 ml/kg	198.3± 8.5[a]	160± 7.8[a]	175.3± 5.5[a]
5	Extract + CCl_4	250	169.1± 5.2[b]	131± 10.5[b]	159.1± 8.7[b]
6	Extract + CCl_4	500	142± 4[c]	123.3±4[c]	130 ± 5.1[c]

Statistically significant difference versus group 1 ($P < 0.001$). [b]Statistically significant difference versus group 4 ($P < 0.001$). [c]Statistically significant difference versus group 4 ($P < 0.001$). Values are expressed as mean ± SD of six animals per groups.

Table 3. Effects of aqueous Jaft extract a product of *Quercus* spp. fruit on serum bilirubin and protein levels in CCl_4 induced hepatoxicity in rats

Group no.	Treatment received	Dose (mg/kg)	TP (g/dl)	ALB (g/dl)	TB (mg/dl)	DB (mg/dl)
1	D.W.	1 ml/kg	6.53 ± 0.27	3.35 ± 0.2	1.06 ± 0.19	0.28 ± 0.07
2	Olive oil	1 ml/kg	6.46 ± 0.33	3.31 ± 0.3	1.11 ± 0.18	0.27 ± 0.08
3	Extract	500	6.03 ± 0.16	3.21 ± 0.23	0.917 ± 0.19	0.27 ± 0.1
4	CCl_4	1 ml/kg	4.05 ± 0.26[a]	1.44 ± 0.15[a]	3.63 ± 0.36[a]	1.33 ± 0.1[a]
5	Extract + CCl_4	250	4.48 ± 0.11[b]	2.03 ± 0.12[b]	2.21 ± 0.12[b]	0.90 ± 0.1[b]
6	Extract + CCl_4	500	4.6 ± 0.37[c]	2.45 ± 0.1[c]	1.5 ± 0.09[c]	0.77 ± 0.12[c]

[a]Statistically significant difference versus group 1 ($P < 0.001$). [b]Statistically significant difference versus group 4 ($P < 0.001$). [c]Statistically significant difference versus group 4 ($P < 0.01$). Values are expressed as mean ± SD ($n = 6$).

treated with toxin (Group 4) could be related to alterations in protein and free amino acid metabolism and their biosynthesis in the liver (Rivarola and Balegno, 1991).

Administration of CCl_4 caused a significant ($P < 0.001$) decrease in total protein as compared to control animals. In the groups treated with 250 and 500 mg/kg of Jaft extract, total proteins were increased as compared to the CCl_4-treated group ($P < 0.05$).

The greatest ALP activity normally occurs in liver, bone, and kidney tissues. However, increased serum levels of ALP occur during pathological states due to increased biliary pressure and cholestasis. Estimation of ALP activity in serum is an indicator of hepatocyte function (Muriel and Garcipiana, 1996). Furthermore, bilirubin is a heme end product that increases in hemolytic anemia, hepatobiliary disease, and necrosis (Raghavendren et al., 2004). The observed decrease in serum bilirubin after treatment with Jaft extracts in the CCl_4-induced liver-injury model indicates the efficacy of this extract in normalizing the functional status of the liver. A significant ($P < 0.001$) and dose-dependent restoration of enzyme marker levels (ALT, AST, and ALP) was observed upon administration of Jaft extracts.

Herbal drugs used for liver protection contain diverse chemical components such as phenols, flavonoids, organic acids, lignin, essential oils, monoterpenes, alkaloids, and coumarins (Gupta and Misra, 2006). In this study, we observed for the first time that treatment with Jaft extracts normalizes and improves the biochemical markers of CCl_4-induced liver injury in rats. This hepatoprotection might be due to the antioxidant activity of the total phenolic compounds in the Jaft extract.

Conclusion

We concluded that the aqueous Jaft extract exhibits significant hepatoprotective activity and may be safely used to treat liver ailments.

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