Research Journal of Biological Sciences 7 (9-12): 350-354, 2012
ISSN: 1815-8846

Acute and Chronic Toxicity of Iranian Jaft an Oak Fruit Component

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Abstract: Oak (*Quercus* sp.,) fruit has external and internal layers and the internal layer of the fruit is known as Jaft in some parts of Iran. Acute toxicity (LD_{50}) was estimated by the intraperitoneal (i.p.) and oral (p.o.) routes. For the sub-chronic study, 20 adult Wistar rats were randomly divided into 4 groups (1-4) comprised of 5 animals each. Animals received Jaft extract daily by gavage at doses of 250, 500 and 1000 mg kg^{-1} for 28 days. At the end of the experiment, blood was collected for hematology and biochemical tests. For histopathological study, liver was dissected and prepared slides with 4-6 mm thick. LD_{50} of the Jaft extract in p.o. and i.p. route was more than 5000 and 3000 mg kg^{-1}, respectively. All hematological parameters, with the exception of White Blood Cell (WBC) count, did not significantly differ in the experimental groups compared to the control group. No statistically significant differences were observed in serum cholesterol, triglyceride, blood urea nitrogen, Alkaline Phosphatase (ALP), Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxaloacetate Transaminase (GOT) levels in the treatment groups when compared to the control group. These results suggest that the aqueous Jaft extract could be used safely in humans, particularly by the oral route.

Key words: Acute toxicity, chronic toxicity, aqueous extract of Jaft, biochemical parameters, hematological parameters

INTRODUCTION

The oak (*Quercus* sp.) fruit has external and internal layers and the internal layer of this fruit is known as Jaft. The color of Jaft turns from yellow to brown after exposure to light due to oxidation. Jaft is a product of *Quercus* sp., which is widely distributed in the Northern and Central parts of Iran (Khosravi and Behzadi, 2006). Of the many Oak species, *Quercus brantii* is widely grown in Yasuj Iran. According to Iranian indigenous information, this plant is traditionally used for problems; such as, gastropathies (Khennouf *et al*., 1999), acute diarrhea, inflammation, burns, cuts (Konig *et al*., 1994) and cancer.

Quercus species exhibit an extensive range of biological and pharmacological potentials such as antioxidant and anti-lipid peroxidation activities which may justify the therapeutic use of this plant. Such activities may be due to the presence of polyphenol and tannin compounds (Khennouf *et al*., 2010; Rocha-Guzman *et al*., 2009). Oak fruit is also used as a food in both fresh and processed forms. Oak fruit also used for handcraft preparation, leather tanning and sheep and goats fodder (Rivas-Arreola *et al*., 2010). In spite of the extensive use of this plant in Southern Iranian folk medicine, its potentially toxic properties have not been previously studied.

The present study was performed to estimate the biochemical and hematological toxicities of an aqueous extract of Jaft after acute and sub-chronic oral administration in rats. The overall purpose of this study was to acquire knowledge on the safety of Jaft and to offer guidance for choosing a safe amount for its use in traditional medicine.

MATERIALS AND METHODS

Collection and extraction of plant material: Fruit of *Quercus brantti* were collected in and around the Yasuj hills in September, 2010. Identification and authentication was performed by the Department of Botany, Faculty of Agriculture, Yasuj University. A voucher specimen (HMRC-J 11/09/2011) was deposited in the Herbal Medicinal Research Center, Yasuj Medical Science University, Iran. To make a powder, fruit bodies were washed, shade-dried and the internal layer (Jaft) was collected. This layer was then ground to a fine powder (20 mesh) using a mill (Restsch Ultra Centrifugal Mill and Sieving Machine, Haan, Germany).

The aqueous extract was prepared by extracting 300 g of the powdered material with 2000 mL of Distilled Water (DW) by the maceration method for 24 h. The solvent was collected and the obtained residues were further extracted twice. The solvent extracts were pooled and combined, filtered through Whatman No.1 filter paper

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and concentrated and dried using a rotary evaporator (Heideolph model 4000; Germany). The dried extract was kept in the refrigerator for further studies.

Acute toxicity studies: About 36 adult male Wistar rats were chosen from the rat colony. Animals were maintained in a controlled environment (22±2°C, 65-70% humidity and a 12 h light/dark cycle) and nourished with a standard laboratory diet (Pars, Iran Ltd., Tehran, Iran). The experiment was conducted according to the ethical guideline recommended by the Animal Ethical Committee.

Acute toxicity (LD_{50}) was estimated via the intraperitoneal (i.p.) and oral routes (p.o.) in 2 separate groups. Each group consisted of 18 male rats and was further sub-divided into 6 groups with 3 animals in each group.

The 1st group which served as a control, received a single dose of Distilled Water (DW) (1 mL kg^{-1}) via p.o. or i.p. routes. Groups 2-6 were treated with single doses of Jaft aqueous extracts ranging from 1000-5000 mg kg^{-1} for the p.o. and 1000-3000 mg kg^{-1} for the i.p. route.

Animals were observed for general behavior and signs of toxicity for a period of 14 days (Twaij *et al*., 1983). All animals had free access to tap water and conventional laboratory rat diets. The LD_{50} of the extract was estimated using the method of Miller and Tainter (Ghosh, 1984; Yerra *et al*., 2005).

Sub-acute toxicity studies: For sub-chronic studies, 20 adult Wistar rats (200-250 g each) were randomly divided into 4 groups (1-4), each consisting of 5 animals. The 1st group served as the control and was given DW orally. Groups 2-4 received Jaft extract daily by gavages at doses of 250, 500 and 1000 mg kg^{-1}, respectively for 28 days. Changes in body weight were recorded weekly by a sensitive balance during the dosing period. Animals were observed for clinical signs and mortality each day from the first day of dosing (D0) to the last day of expriment (D28).

At the end of study, animals were exsanguinated under diethyl ether anesthesia. Blood samples were collected by heart puncture in 2 tubes with or without sodium heparin for hematology parameters and serum biochemistry tests. Serum samples were stored after separation by centrifuge and hematology assay were analyzed immediately after blood collection.

Determination of hematological and biochemical parameters: The serum was analyzed for blood glucose, Blood Urea Nitrogen (BUN), total cholesterol and triglycerides, Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT), Alkaline Phosphatase (ALP), total proteins and albumin. Analyses were performed using standard kits (Pars Azemon company kits, Tehran, Iran) by RA-1000 analyzer (USA). Complete Blood Count (CBC) including Hemoglobin concentration (Hb), White Blood Cell counts (WBC), Red Blood Cell count (RBC), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and Platelet count (PLT) was analyzed on hematology samples by an auto analyzer (System H1; Bayer Diagnostics).

Gross and histopathological examinations: Macroscopic examination of all organs was performed. The wet organs; such as, the kidneys, liver and spleen of each rat were isolated and weighed. The relative organ weight of each animal was calculated as:

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Rat body weight on the last day of dosing (g)}} \times 100$$

For histopathological study, liver was preserved in 10% formalin and prepared slides with 4-6 mm thick. The slides were stained using haematoxoylin-eosin and examined microscopically for histopathology damaged with light microscope.

Statistical analysis: Comparisons among different groups were performed by analysis of variance using the ANOVA and post-hoc tests. Results are expressed as mean±SD. p-values <0.05 were considered significant.

RESULTS

Acute oral toxicity studies revealed a lack of mortality even at the highest dose (5000 mg kg^{-1} body weight) in all of the groups after 14 days, suggesting that the LD_{50} of Jaft is >5000 mg kg^{-1}. Furthermore, Jaft did not induce any abnormal clinical symptoms or any changes in the appearance of urine or stool color in any of the animals. Acute toxicity testing showed that the LD_{50} of the Jaft extract in the i.p. and p.o. procedures were >3000 and >5000 mg kg^{-1}, respectively.

Variable changes were recorded in the body weights of animals in all groups. The control rats acquired weight during of treatment whereas weight loss was recorded in rats received 500 and 1000 mg kg^{-1} of Jaft extract in the first half of the treatment period. Surprisingly, the rats received 250 mg kg^{-1} acquired weight in a way like to that of the control rats. However, these changes in the body weights of treated rats were not significantly different from those of the controls (Fig. 1).

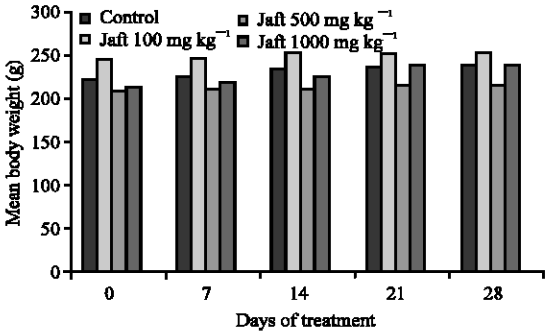

Fig. 1: Mean body weight changes in rats fed with aqueous extract of Jaft by gavage for 28 days; about 250 mg kg^{-1} treatment group rats gained weight during of the treatment compare to control; however, these body weights were not significantly differences from control

No significant changes were observed in the relative weights of the livers, spleens or kidneys of treated rats compared to the control group. An insignificant increase in kidney weight was observed at 500 and 1000 mg kg^{-1}. However, an insignificant dose-dependent decrease was observed in the relative liver weights compared to controls (Table 1).

Treatment with 1000 mg kg^{-1} of Jaft extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control.

Hematological parameters were not significantly different in the experimental groups compared to the control group ($p>0.05$) with the exception of WBC. Furthermore, all values were within normal reference ranges. A dose-dependent increase in WBC count was observed in the experimental groups when compared to the control group ($p<0.05$) (Table 2, 3).

Furthermore, in all experimental groups a dose-dependent but not statistically significant decrease in GPT activity was observed on the 28th day of treatment. However, GOT activity decreased at 250 mg kg^{-1} but increased in a dose-dependent manner at 500 and 1000 mg kg^{-1} dose levels (Table 4). A statistically insignificant dose-dependent increase in both serum cholesterol and triglyceride concentrations was observed in all treatment groups compared to the control group. Although, differences in BUN concentrations were not initially statistically significant, they later increased at 250 and 500 mg kg^{-1}; however, no change was observed at the 1000 mg kg^{-1} dose of Jaft extract. No significant differences were reported in blood sugar, total protein and albumin levels (Table 4, 5).

Table 1: Effects aqueous extract of jaft on the relative organ weights of rats (g 100 g^{-1} body weight)

Groups	Liver	Spleen	Kidneys
Control	4.13±0.28	0.45±0.03	0.72±0.02
Jaft 250	4.01±0.39	0.47±0.03	0.69±0.02
Jaft 500	3.94±0.23	0.40±0.03	0.75±0.04
Jaft 1000	3.76±0.09	0.48±0.02	0.77±0.01

Mean±SD are given; n = 5 for statistical significances; $p<0.05$

Table 2: Hematological parameters in rats given jaft aqueous extract by gavage (Liver Function Test: LFT) for 28 days

Group	Hb (g dL^{-1})	PCV (%)	MCV (fl)	MCH (pg)
Control	14.50±0.50	41.0±1.22	52.57±1.9	25.87±4.70
Jaft 250	15.45±0.67	42.7±2.00	54.60±1.4	20.70±0.94
Jaft 500	14.20±0.49	39.2±3.40	53.50±1.6	19.40±1.50
Jaft 1000	13.95±1.25	42.32±1.5	52.90±1.9	20.15±3.80

Hb = Hemoglobin concentration; PCV = Packed Cell Volume; MCV = Mean Cell Volume; MCH = Mean Cell Hemoglobin; Mean±SD are given; n = 5 for statistical significances; $p<0.05$ compared with control group

Table 3: Hematological parameters in rats given jaft aqueous extract by gavage for 28 days

Groups	RBC ($\times10^{12}$)/l	WBC ($\times10^{9}$)/l	PLT($\times10^{9}$)/l
Control	6.94±0.27	7850.0±180.2	607.00±17.5
Jaft 250	7.27±0.17	8544.0±478.2*	581.00±25.7
Jaft 500	7.11±0.53	8712.0±162.0*	598.00±13.1
Jaft 1000	7.20±1.56	9276.0±276.0**	612.75±31.3

RBC = Red Blood Cell count; WBC = White Blood Cell count; PLT = Platelet count; Mean±SD are given; n = 5 for statistical significances; $p<0.05$; *$p<0.05$; **$p<0.01$, compared with control group

Table 4: Effects of intake of Jaft aqueous extract by gavage on rat biochemical indices (Liver Function Test: LFT) for 28 days

Groups	SGOT (IU L^{-1})	SGPT (IU L^{-1})	ALP (IU L^{-1})	TP (g dL^{-1})	ALB (g dL^{-1})
Control	30.50±0.95	26.92±3.60	75.8±2.05	6.04±0.48	3.150±0.24
Jaft 250	29.30±2.40	22.86±3.14	79.5±2.64	5.88±0.34	3.204±0.30
Jaft 500	31.80±4.70	25.80±4.50	79.3±1.87	5.68±0.37	3.064±0.18
Jaft 1000	36.75±1.40	23.00±4.12	86.7±3.59	6.40±0.22	3.642±0.22

GOT = Glutamate Oxaloacetate Transaminase; GPT = Glutamate Pyruvate Transaminase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin; Mean±SD are given; n = 5 for statistical significances; $p<0.05$

Table 5: Effects of intake of Jaft aqueous extract by gavage on rat biochemical indices for 28 days

Groups	BS (mg dL^{-1})	BUN (mg dL^{-1})	Chol (mg dL^{-1})	TG (mg dL^{-1})
Control	59.88±0.0	15.820±3.03	74.48±7.7	57.76±2.65
Jaft 250	59.38±3.5	18.152±2.80	75.56±8.4	58.12±1.11
Jaft 500	62.64±6.1	19.140±2.65	79.76±6.0	58.94±2.74
Jaft 1000	58.18±4.2	15.620±1.86	80.10±5.8	59.78±2.26

BS = Blood Sugar; BUN = Blood Urea Nitrogen; Chol = Cholesterol; TG = Triglyceride; Mean±SD are given; n = 5 for statistical significances; $p<0.05$

DISCUSSION

Toxicological studies are carried out in different experimental animals to forecast the toxicity and safety of drugs and herbal products for human consumption.

The LD_{50} which is the index of acute toxicity, is not considered a biological constant because studies from different laboratories have revealed variations in LD_{50} values (Lorke, 1983). Factors such as sex, age, genus, strain, diet and environment temperature, can affect LD_{50} values (Inden and Flury-Roversi, 1981).

Although, medicinal plants may produce various biological activities in humans, few are recognized regarding their toxicity. In this study, the results of the acute toxicity testing revealed a lack of mortality in all of the groups, indicating that Jaft exerts protective effects sub-sequent to oral administration. The aqueous extract of Jaft was nontoxic in rats when administered by p.o. and i.p. routes at doses up to 5000 and 3000 mg kg^{-1}, respectively.

In the sub-chronic toxicity study, the Jaft extract did not affect the body weight or behavior of the rats and caused no significant changes in their food intake or utilization of food, regardless of dose. These observations indicate normal metabolism in animals dosed with Jaft and suggest that this extract did not retard the growth of rats at the oral doses administered.

The liver histology did not signify any indication of centrilobular degenerative changes, steatosis or necrosis at this dose level. Indeed microscopic examination of the liver did not detect any treatment-associated side effects.

This view is also supported by the fact that the relative weights of the liver and kidney did not show any sign of toxicity. Normal of total protein and albumin values in this study indicate of synthetic ability of the liver cell. All these results indicate a hepatoprotective potential of the extract.

The hematopoietic system is an important target for toxic substances (Pyszel *et al*., 2005) and is a significant indicator of the physiological and pathological condition in some organisms such as animal and man (Adeneye *et al*., 2006). In this study, hematopoiesis and leucopoiesis were not affected by Jaft. In addition, all biochemical parameters were within normal limits regardless of the dose of ingested Jaft. The transaminases GOT and GPT are hepatic enzyme markers to detect possible toxicity (Rahman *et al*., 2001) and damage to the liver parenchyma cells normally results in an increase in the levels of these enzymes (Shakti and Mitra, 2010). The lack of significant increase in the levels of GPT, GOT, ALP, cholesterol and BUN (El Hilaly *et al*., 2004) suggested that chronic intake of the Jaft extract did not alter the hepatocytes or the kidneys of the rats and furthermore did not change the normal metabolism of the animals.

The normal ALP activity observed in this study indicated that cholestasis did not occur at the dose levels because a rise in plasma ALP level is a characteristic finding in cholestatic liver disease (Kaneko *et al*., 2008). BUN, which is a marker of kidney function, was within the normal range in all experimental groups.

The histopathological findings supported the results obtained from hematology and biochemical parameters and safety of the Jaft extract on these organs.

The i.p. acute toxicity study showed that the LD_{50} of the extract is 3000 mg kg^{-1}, indicating that the aqueous Jaft extract is safe. The literature describes compounds with an i.p. LD_{50} >1000 mg kg^{-1} as non-toxic (Agaie *et al*., 2007). Whereas according to some literature guidelines, materials or substances with p.o. LD_{50} of 500-5000 mg kg^{-1} are regarded as virtually safe (Agaie *et al*., 2007). Because the Jaft extract had an LD_{50} of >5000 mg kg^{-1} via p.o. route, it may be safe for human consumption. Furthermore, administration of the Jaft extract did not induce mortality, gross lesions and pathological damages in rat organs suggest that the aqueous Jaft extract was safe, particularly when consumed by the oral route.

CONCLUSION

The present results suggest that administration of the Jaft extract has no adverse effects on liver and kidney function in rats and therefore, the aqueous extract may be safe for human consumption.

ACKNOWLEDGMENTS

Researchers are grateful to the Biochemistry Department and Iranian plant Medicine Research Center for making their facilities available for this investigation.

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