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ACUTE TOXICITY OF *OPUNTIA FICUS INDICA AND PISTACIA LENTISCUS* SEED OILS IN MICE

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Abstract

Opuntia ficus indica and *Pistacia lentiscus* L. seeds are used in traditional medicine. The objective of this study was to investigate the toxicity of the fixed oil of *Opuntia ficus indica* and *Pistacia lentiscus* L. seeds in mice through determination of LD_{50} values, and also the physicochemical characteristics of the fixed oil of these oils. The acute toxicity of their fixed oil were also investigated in mice using the method of Kabba and Berhens. The fixed oil of *Pistacia lentiscus* and *Opuntia ficus indica* seeds were extracted and analyzed for its chemical and physical properties such as acid value, free fatty acid percentage (% FFA), iodine index, and saponification value as well as refractive index and density. LD_{50} values obtained by single doses, orally and intraperitoneally administered in mice, were respectively 43 ± 0,8 ;[40.7- 45.4] ml/kg body wt. p.o. and 2.72 ± 0,1 ;[2.52–2.92] ml/kg body wt. i.p. for *Opuntia ficus indica* ; and 37 ± 1 ;[34.4 – 39.8] ml/kg body wt. p.o. and 2.52 ± 0,2 ;[2.22 – 2.81] ml/kg body wt. i.p. for *Pistacia lentiscus* respectively. The yields of seed oil were respectively calculated as 20.25% and 10.41%. The acid and free fatty acid values indicated that the oil has a low acidity

Key words: *Opuntia ficus indica L.* ; *Pistacia lentiscus L.* ; seed oil ; acute toxicity.

Introduction

Pistacia lentiscus (L.), a member of the Anacardiacea family, typifies the Mediterranean regions and it is commonly dispersed in Algeria over the entire littoral zone(More and White, 2005; Seigue, 1985). The majority of the traditional products is the virgin fatty oil from *Pistacia lentiscus* seeds. The *Pistacia lentiscus* seed oil composition and its chemical characteristics were investigated. The seed oil composition of *Pistacia lentiscus* fruits was studied during the maturation period, the fruit are globulous berries first red then black at maturity (Yahya, 1992 ; Iserin, 2001). These authors concluded that *Pistacia lentiscus* seed oil was rich in polyunsaturated fatty acids and vitamins and may potentially be included in animal and human diets. Recently, the effect of virgin fatty oil of Algerian *Pistacia lentiscus* seed on experimental burn wound's healing in rabbits was studied (Djerrou et al., 2010).

Opuntia ficus indica (L.), known as prickly pear, a member of the Cactaceae family, is an arid and semi-arid countries plant (Getachew, 2005; Virginie, 2003). This cactus reaches up to 2 m in height and has a CAM physiology with very high water-use efficiency. It is planted for fruit consumption and also as an ornamental, for wind protection fencing, land reclamation and rehabilitation, and erosion control. Flowers of this plant are pollinated by insects. Seeds are dispersed by birds, feral pigs and lizards that feed upon fruits. Seeds from fruits and scats germinate after long rainfall and warm temperatures (21°C) (Montserrat Vilà, 2009). O. ficus indica produces edible and nutritive fruits whose composition depends on the variety, the maturation state and the environmental conditions (Montserrat Vilà, 2009). In traditional medicine, prickly pear fruits have been used for their hypoglycemic and hypolipidemic actions. Some authors have attributed these beneficial effects to high contents of fibers in these fruits (Virginie, 2003). Opuntia ficus-indica (L.) is used in other regions as laxative, anti-inflammatory, or against diarrhoea (Bullitta, 2007). It is also antacid, astringent, antidermatosic, emollient, antiseptic, vulnerary, analgesic, expectorant, antiviral, sedative, antioxidant (Lamjed, 2011; Bullitta, 2007). The current study was undertaken to highlight certain information, including physico-chemical aspects and safety of *P.lentiscus and O.f.indica* seed oils.

Material and methods

Acquisition of plant material

The *Opuntia ficus indica L.* plant seeds were harvested (August 2009) in Constantine; Algeria, however *Pistacia lentiscus L.* plant seeds were harvested (November 2008) in Skikda; Algeria . A voucher (*P. lentiscus* : PL-1108AB ; *Opuntia.f. Indica* : OFI-0909AB) specimen of both plants has been deposited at the Pharmacognosy and Botany Laboratory, Mentouri Constantine University, Algeria.

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Extraction

The *Opuntia ficus indica L.* and *Pistacia lentiscus L.* seeds were powdered mechanically. The extracts were obtained by Soxhlet extraction of powdered seeds in (3 x 1.5) liters of hexane thrice in 18 hours. The solvent was removed from the extract under reduced pressure.

Experimental animals
Acute toxicity

Acute toxicity studies were carried out in *Mus musculus* mice from the Pharmacy department , 8–10 weeks old and weighing 20–30 g each, using a single dose, administered orally or intaperitoneally. The animals were distributed in plastic cages with free access to water and food. It consists of a standard mixture of corn, barley, soya, added multivitamin complex and packaged in the form of granulated. The procedures adopted were approved by Faculty of Sciences, Mentouri Constantine University, Algeria

a- *Opuntia ficus indica L.* fixed oil acute toxicity

Seventy mice, divided into 7 groups, were designed for study of acute toxicity via the oral route. Each group of 10 mice (5 males and 5 females) received, respectively, a single oral dose of 10, 20, 30, 40, 50, 60 or 70 ml/kg body weight of *Opuntia ficus indica L.* fixed oil. Seventy other mice divided into 7 groups were used to study toxicity intraperitoneally. Each group received, respectively, a single dose of 0.5, 1, 2, 3, 4, 5 or 6 ml/kg body weight of *Opuntia ficus indica L.* fixed oil.

b- *Pistacia lentiscus L.* fixed oil acute toxicity

Sixty mice, divided into 6 groups, were designed for study of acute toxicity via the oral route. Each group of 10 mice (5 males and 5 females) received, respectively, a single oral dose of 10, 20, 30, 40, 50 or 60 ml/kg body weight of *Pistacia lentiscus L.* fixed oil. Sixty other mice divided into 6 groups were used to study toxicity intraperitoneally. Each group received, respectively, a single dose of 0.5, 1, 2, 3, 4 or 5 ml/kg body weight of *Pistacia lentiscus L.* fixed oil. After administration of the plants oil, the mice were observed for gross effects and mortality on the first day every 60 minutes during the first 08 hours, and every day once at the same time for a period of 14 days. Post- mortem examination was carried out on the dead animals.

Results

Table 1 presents the chemical composition of *Pistacia* seeds. *Pistacia* seeds contained 11.6% moisture. We found that the total oil recovered from seeds accounted for 202.5 g/kg, this value being the mean of three replicated extractions. This amount is lower than those reported in previous works where the seed oil content of several species of *Pistacia lentiscus* fruits varied between 312 and 328 g/kg (Charef et al., 2008). The ash percentage was 3.23%. *Pistacia* seeds also contained significant amounts of crude fiber of 24.7%. The shape of *Pistacia lentiscus* berries is spherical with 3mm average diameter and 3g average of 100 seeds.

Table 1: Chemical Composition of the *Pistacia lentiscus* and *Opuntia ficus indica* Seeds oil

Component	Content (mg/100 g dry matter)	
	Pistacia lentiscus	*Opuntia ficus indica*
Moisture content, %	11.6±0.1	6.2±0.1
oil content, % dry matter	20.25±0.10	10.45±0.10
Ash, % dry matter	3.23±0.02	1.46±0.02

Pistacia seed oil gave a high iodine value (87.377) due to its high content of unsaturated fatty acids. This value is lower than that of other seed oils such as corn oil (103–128), cottonseed oil (99–119), and mustard seed oil (92–125) (Yong and Salimon, 2006?). The high iodine value shows that the seed oil upholds the good qualities of edible oil (Yong and Salimon, 2006) The saponification value, determined as 193.3, is comparable with that of safflower, sunflower, and corn oil (O′Brien, 2004) for which the average range of saponification value is 191–250. The density and refractive index are comparable to values reported by (Ennouri et al., 2005).

The effects of oral and intraperitoneal single doses of plants seed fixed oils on mortality and LD_{50} values in Mus Msculus mice are summarized in Table 3. LD_{50} values were determined using the method of Kabba and Berhens (Adjoungoua et al, 2008) to calculate the LD_{50}.

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LD_{50}: dose giving 50% of deaths.
DL_{100}: dose giving 100% of deaths.
a: average deaths between two successive doses.
b: difference between two successive doses.
n: Average of animals used.

$$DL_{50} = DL_{100} - \frac{\Sigma a \times b}{n}$$

Table 2: Physicochemical characteristics of *Pistacia lentiscus* Seed Oil (Mean values of three replicates ± standard deviation).

Properties	*Pistacia lentiscus* Mean	*Opuntia ficus indica* Mean
Acid value	2.27±0.03	1.82±0.01
Iodine value (g of I2/100 g of oil)	87.38±0.31	93.45±0.22
Saponification value (mg of KOH/g of oil)	193.30±0.05	177.10±0.05
Peroxide value (meq O2/kg of oil)		
Density (20°)	1.12±0.06	2.04±0.05
Refractive index (20°)	0.918±0.02	0.909±0.01
Unsaponifiable matter %	1.465±0.03	1.476±0.01
Physical state at room temperature	0.87±0.02	/
Oil color	Liquid Deep green	Liquid yellow brown

All doses, orally and intraperitoneally administered, caused immediate agitation and behavioral perturbations with temporary writhing, followed by a quiet attitude period and sedation. Generally, diarrhea was observed and the animals died 12 hours after the administration of fixed oil. Autopsied dead animals showed congested lungs and hearts stopped in diastole. The surviving animals quickly recovered their normal activity and growth, after a period ranging from 4 to 8 days, depending on the dose and mode of administration.

Table 3: Mortality percentage and LD50 values in *Opuntia ficus indica and Pistacia lentiscus* -treated mice with oral or intraperineal single doses.

Oral administration	*Opuntia ficus indica*	Dose (ml/kg body wt.)	10	20	30	40	50	60	70
		Mortality (%)	0	10	20	40	70	90	100
		LD_{50} : 43 ± 0,8 ; CI: [40.7- 45.4] ml/kg body wt.							
	Pistacia lentiscus	Dose (ml/kg body wt.)		10	20	30	40	50	60
		Mortality (%)		0	10	30	60	80	100
		LD_{50} : 37 ± 1 ; CI: [34.4 – 39.8] ml/kg body wt.							
Intraperitoneal administration	*Opuntia ficus indica*	Dose (ml/kg body wt.)	0.5	1	2	3	4	5	6
		Mortality (%)	0	10	40	60	80	90	100
		LD_{50} : 2.72 ± 0,1 ; CI: [2.52–2.92] ml/kg body wt.							
	Pistacia lentiscus	Dose (ml/kg body wt.)		0.5	1	2	3	4	5
		Mortality (%)		0	10	50	60	80	100
		LD_{50} : 2.52 ± 0,2 ; CI: [2.22 – 2.81] ml/kg body wt.							

LD_{50} – lethal dose 50; CI – confidence intervals; values are expressed as mean ± SD.
LD_{50} was determined using the method of Kabba and BERHENS (Adjoungoua et al, 2008).

Discussion

In the present study, the botanical data collected are comparable to those described in the literature (Chiej, 1982; Hichey and King, 1988; Baba, 1999). In addition a sample of a branch of leaves of *P. lentiscus* with ripe fruit of the plant have been formally identified as those of *P. lentiscus L.*, *Anacardiaceae* family. Density depends on temperature and chemical composition of the oil. These tell us about the nature of the component fatty acids, including the length of the chain, the presence of unsaturation and functionality on the carbon chain. The density value shown in Table 2, measured for mastic oil is from 0.918±0.02 (n = 3) for *P. lentiscus* and 0.909±0.01 for *O. f. indica*. These values are in the same order as those found with certain fatty oils reported by Karleskind (1992); Olive oil has a density between 0.910 and 0.916, Palm oil density

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ranging from 0.895 to 0.900, Avocado oil, density of between 0.910 and 0.920. But some vegetable oils, like almond or argan, have lower densities, respectively equal to 0.906 and 0.900 (Farines et al. 1984; Maurin et al, 1992; Boudjira, 2002).

The refractive index depends on the density, chemical composition of the oil and temperature. It increases with the unsaturation and the presence of the fatty chains of secondary functions. The refractive index measured for the samples of our oils are from 1.465±0.03 (n = 3) for *P .lentiscus* and *1.476±0.01* for *O .f .indica.* These values are close to those reported by Karleskind (1992) on olive oil, palm oil, and avocado, which are respectively (1.468 to 1.470), (1.453 to 1.458) and (1.465 to 1.474). The acid provides information on the rate of free fatty acids existing in the oil. The values of the acid for the samples of *P .lentiscus* and *O .f .indica* oils are respectively 2.27±0.03 and 1.82±0.01 mg KOH / g (n = 3). Charef postpones acid values of 7.7 ± 0.3 and 24 ± 0.5 mg KOH / g for *P. lentiscus* oil, respectively extracted from the black and red berries (Charef et al ., 2008). The second value, relatively high compared to the value of our sample (consisting mainly of fruit and ripe black) reported by Charef for berries red, not yet ripe, can be explained by the fact that these berries contain rate of free fatty acids higher than those from ripe black berries. The value of the saponification of the sample of mastic oil is much more consistent with values reported for other oils. According to Karleskind (1992), vegetable oils of olive, palm and avocado, respectively have saponification indices from 184 to 196, 190 to 205 and from 177 to 198 (Karleskind A., 1992).

Saponification value, determined as 177.10±0.05 for *O. f. indica* oil , is lower than that of safflower, sunflower, and corn oil, but it is so similar to that reported by Mannoubi et al. (2009). *O .f.indica* oil. *P. lentiscus* and *O. f. indica* oils provide relatively high iodine values (Table 1) due to their high content of unsaturated fatty acids (Table 4). These values are comparable to that of other seed oils such as corn oil (103–128), cotton seed oil (99–119), and mustard seed oil (92–125) (Mannoubi and al. 2009). The peroxide values of *P. lentiscus* and *O. f. indica* seed oils were respectively evaluated as 1.12±0.06 and 2.04±0.05 meq/O^2, showing the relative stability to oxidation of these oils. The high iodine value and oxidative stability show that the seed oils uphold the good qualities of edible oil (Mannoubi et al. 2009). The single-dose toxicity test should be conducted in such a way that signs of acute-toxicity and death are revealed. Animals should be observed at regular intervals and all signs of toxicity and the time of their first occurrence and their severity, duration and progression recorded. The time and mode of any death should be documented.

Conclusion

The current study has shown a low toxicity of *Opuntia ficus indica and Pistacia lentiscus* fixed oil. The high values of oral and intraperitoneal lethal doses of both *O. f. indica* fixed oil (LD50 value = 43 ml/kg body wt., p.o. ; LD50 value = 2.72 ml/kg body wt., i.p.); and *P. lentiscus* fixed oil (LD50 value = 37 ml/kg body wt., p.o. ; LD50 value = 2.52 ml/kg body wt., i.p.) show their low acute toxicity.

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