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Effects of diet supplementation with cactus pear seeds and oil on serum and liver lipid parameters in rats

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Abstract

The purpose of this investigation was to evaluate the effects of diets enriched with cactus pear oil (CPO) or seeds (CPS) on serum and liver lipid parameters compared to those of adult rats submitted to a standard diet. Male rats were divided into three groups, the first group represented control group, fed with standard diet, the second group was fed with control diet supplemented with CPO (2.5%, wt/wt) and the third group fed control diet supplemented with CPS (33%, wt/wt), for nine weeks. Feed intake and body weight of rats were measured every two days. Organ weights were determined at the end of treatment; cholesterol, HDL and triglycerides levels were determined by enzymatic methods. Liver and serum lipid extracts were analysed for their fatty acid composition for the three groups of rats. No differences in pancreas, kidney or liver weights were observed in the CPS diet whereas the CPO diet induced a significant increase in liver and pancreas weights. The tested diets significantly decreased the atherogenic index compared to the control diet, whereas serum cholesterol level was only reduced by the supplementation with CPO diet. No variations in serum lipids were observed among the groups, whereas liver lipids showed slight variations. Accordingly, these results indicated that the supplementation with CPO or CPS could be effective in decreasing the atherogenic risk factors in rats.

Keywords: *Opuntia ficus indica* seeds; Fatty acids profile; Liver; Serum; Cholesterol

1. Introduction

Opuntia ficus indica belongs to the *Cactaceae* family and the order *Centrospermae*, and their fruits are the cactus pear. This plant grows wild in arid and semi-arid regions, where the production of more succulent food plants is severely limited. Many uses of cladodes and cactus pear fruit are reported (Hoffmann, 1980). Cladodes are consumed as fresh vegetables or added to casseroles (Hamdi, 1997; Saenz, 2000). Cladodes have been investigated as a possible treatment for gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostatic hypertrophy (Frati, Jimenez, & Ariza, 1990; Hegwood, 1990; Palevitch, Earon, & Levin, 1993). The fruits are used for the manufacture of food products such as juices (Espinosa et al., 1973) and alcoholic beverage (Bustos, 1981), jam (Sawaya, Khatchodourian, Safi, & Almuhammad, 1983a) and in the production of natural liquid sweetener (Saenz, Mecklenburg, Estevez, & Sepulveda, 1996), the production of cocoa butter equivalents from cactus pear juice fermentation (Hassan, Blanc, Pareilleux, & Goma, 1995) and the production of fungal protein by solid substrate fermentation (Oliveira, Rodrigues, Dos Reis, & Nozaki, 2001). Juice and cladodes have

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been extensively studied. There are, however, no reports about seeds, which are usually discarded.

The seeds are made of two different tissues, the endosperm and the pericarp in the relative proportion of 1:9, respectively. Analysis of the main constituents of prickly pear seeds showed a significant amount of polysaccharides, cellulose and hemicelluloses, and the structure of their glucuronoxylans has been identified by Habibi, Mahrouz, and Vignon (2002). The seeds of *Opuntia ficus indica* (cv. Gigante) contain a storage protein having a molecular mass of 6.5 kDa isolated and characterized by Uchoa, Souza, Zarate, Gomez-Filho, and Campos (1998). The cactus pear seeds proteins are rich in mineral and sulfur amino acids, such as methionine and cysteine, which represent nearly twice the FAO/WHO (FAO/WHO, 1973) recommended requirements for humans. Seeds composition of cactus pear fruits during the maturation period has been studied by Coskuner and Tekin (2003). Their nutritive value has been studied by Sawaya, Khalil, and Al-Mohammad (1983b). The cactus pear seed oil composition and its chemical characteristics were investigated by Sawaya and Khan (1982), and by Salvo, Galati, Lo Curto, and Tripodo (2002).

Dietary fatty acids have a significant effect on plasma cholesterol and levels of lipoproteins, which are linked to the incidence of coronary heart disease (CHD) (Hu, Mason, & Willet, 2001; Yu, Derr, Etherton, & Kris-Etherton, 1995).

Recently, the use of functional food and/or nutraceuticals has increased due to their beneficial effects on human health (Dubick, 1986). Among Mediterranean plants, prickly pear seeds and oil exhibit hypoglycemic and hypocholesterolemic effects (Ennouri, Fetoui, Bourret, Zeghal, & Attia, in press-a, in press-b), probably due to the fatty acid composition of the prickly pear seeds oil (Ennouri, Bourret, Mondolot, & Attia, 2005). Changes in the fatty composition of several tissues such as liver and serum, could be attributed to the type of fat ingested in the diet. Therefore, the aim of the current study was to evaluate the effect of diet supplemented with cactus pear oil (CPO) or cactus pear seeds (CPS) comparatively to a control diet, given to adult rats, on some organ weights and fatty acid profiles in their serum and liver.

2. Materials and methods

2.1. Cactus pear seed and oil preparation

Cactus pear seeds were obtained after juice extraction. The seeds were washed with distilled water several times, air-dried at room temperature and then ground with a crusher (Dietz-motoren GmbH & Co. KG, Germany). The oil of *Opuntia ficus indica* was extracted from the seed powder with hexane in a Soxhlet extractor (Quichfit, England) for 9 h. The organic phase was then removed using a Rotavapor apparatus under reduced pressure and the oil was flushed with a stream of nitrogen and stored at −20 °C in sealed tubes.

2.2. Physicochemical analysis of diets

The diet enriched with cactus pear oil (CPO) was prepared using the control diet treated with 25 g kg^{-1} of oil, and the diet supplemented with cactus pear seeds powder (CPS) was prepared using the control diet substituted at a ratio of 1 to 3. The loss in minerals and in protein caused by this supplementation was compensated by an addition to diets of casein (Merck, Germany) and minerals (Interchem, Tunisia). Nitrogen was determined by a Kjeldahl procedure and crude protein was calculated as $N \times 6.25$ (Balogun & Fetuga, 1986). Crude fibre and ash contents were determined according to the AOAC method (AOAC, 1990). Starch content was determined according to McCready, Guggolz, Silviera, and Owen (1950).

2.3. Test animals

Twenty one adult male rats of Wistar strain, weighing about 123 g each, were purchased from Central Pharmacy (Siphat, Tunisia), and were divided into three groups. Seven rats of each group were housed in one cage (Phywe, Gottingen).

The first group was fed with a control diet, the second group (CPO) was given the control diet enriched with 25 g kg^{-1} of cactus pear seed oil and the third group (CPS) was given the control diet supplemented seed powder at a ratio of 1 to 3 and complemented with casein and minerals, as illustrated in Table 1. Body weights and feed intake were measured every 2 days during 9 weeks. The amount of diet ingested was calculated as the difference between the weight of feed that remained in the bin feed (D_a) and the amount placed therein 2 days previously (D_b). These data were then used to calculate a daily feed intake according to the formula:

$$\text{Feed intake (g)} = \left(\frac{D_b - D_a}{7}\right) \Big/ 2$$

where the number 7 corresponds to the animals' number in each cage.

2.4. Experimental procedure

After 63 days of feeding, to avoid the stress of rats, blood samples were withdrawn without heparin by decap-

Table 1
Chemical composition of fed diets (g/100 g d.m.)[a]

Ingredients	Control	CPO	CPS
Lipids	3.37 ± 0.17^{x}	5.90 ± 0.10^{y}	5.87 ± 0.36^{y}
Protein	15.2 ± 1.35^{x}	14.7 ± 0.52^{x}	12.0 ± 1.12^{x}
Ash	4.63 ± 0.09^{x}	4.50 ± 0.15^{x}	3.53 ± 0.11^{x}
Cellulosic polysaccharides	5.12 ± 1.06^{x}	5.20 ± 0.09^{x}	23.4 ± 1.25^{y}
Starch	11.8 ± 1.86^{x}	11.6 ± 1.50^{x}	8.30 ± 1.32^{x}

Figures having different letters (superscribed) in the same row are significantly different ($P \leqslant 0.05$).

[a] Each value is the mean of three observations ± standard error.

itation. Serum samples were drawn from blood after centrifugation at 2500 × g for 10 min. They were kept at −20 °C prior to analysis of lipid parameters. Cholesterol and HDL in serum were determined using commercial kits from Biolabo (Fismes, France). Serum triglyceride levels were measured enzymatically using a kit from Biomaghreb (Fossati & Prencipe, 1982). Liver, pancreas and kidney were taken and weighed for determination of relative organ weight. Liver was stored at −20 °C prior to analysis of lipid content and fatty acid composition.

2.5. Lipid extraction from serum and liver

Five hundred microliters of serum and 1–2 g of liver were used to extract the total lipids with chloroform:methanol mixture (2:1, v/v) according to the method of Folch, Lees, and Stanley (1957). The content of total lipids in the liver and serum was quantified gravimetrically by evaporating off the solvents in the liver and serum lipid extracts.

2.6. Fatty acid composition profile

Fatty acid composition of liver and serum extracts were analyzed by GC–MS after transesterification. The lipid fractions were transesterified to their fatty acid methyl esters by heating at 80 °C for 2 h with MeOH, containing 2% concentrated H_2SO_4 and 0.005% lipid and analyzed on a Hewlett–Packard model 5890 series II gas chromatography (H.P.Co., Amsterdam, The Netherlands) equipped with a flame ionization detector and a polar capillary column HP Innowax with cross linked PEG, Carbowax 20 M (0.32 mm internal diameter, 30 m length and 0.25 μm film thickness). The operational conditions were: injector temperature 220 °C, detector temperature 275 °C, and column temperature 50 °C for 5 min, then a gradient of 10 °C/min up to 240 °C, carrier gas was nitrogen at a flow of 1.47 ml/min. Three injections per sample were accomplished.

2.7. Statistical methods

Data were analysed statistically by Student's *t*-test. Mean values were obtained by averaging independent measurements. Differences between groups were considered significant at least at $P \leqslant 0.05$.

3. Results and discussion

3.1. Chemical composition of diets

Table 1 shows the chemical composition of fed diets substituted with oil (CPO) or seed powder (CPS) and the control diet. The CPS diet was rich in lipids and fibre compared to the control. The CPO diet was also rich in lipids compared to control.

3.2. Effect on feed intake, weight gain, and some organ weights

3.2.1. Feed intake and body weight of rats

A significantly lower average gain of body weight in the CPS group was obtained during the 9 weeks of treatment despite a greater feed consumption than those of the control and CPO groups. In the CPO group, no significant body weight gain was obtained compared to the control (Table 2).

3.2.2. Organ weights

In a previous study, we have found a hypoglycaemic effect after enrichment of diet with CPO (Ennouri et al., in press-a) and CPS (Ennouri et al., in press-b) in treated rats. So we have chosen some organs having an impact on the glycaemia, such as pancreas, liver and kidney. Relative weights, in the CPS group, of liver, pancreas and kidney were similar to those of control, while, in the CPO group, weights of liver and pancreas were lower than those in the control group (Table 2).

Table 2
Initial body weight, body weight gain, feed intake and relative weights of some organs (g/100 g of body weight) in control, CPO and CPS groups after 9 weeks of treatment[a]

Parameters	Control	CPO	CPS
Initial weight (g)	122.35 ± 1.61^{x}	123.7 ± 0.9^{x}	123.8 ± 0.7^{x}
Average weight gain (g/rat)	143.3 ± 31.2^{x}	141.8 ± 22.6^{x}	93.7 ± 15.5^{y}
Average feed intake (g/day)	20.7 ± 4.6^{x}	18.6 ± 1.5^{y}	25.2 ± 3.7^{z}
Relative weight of organs			
Liver (g/100 g BW)	2.95 ± 0.15^{x}	3.58 ± 0.12^{y}	2.81 ± 0.15^{x}
Pancreas (g/100 g BW)	0.29 ± 0.01^{x}	0.22 ± 0.02^{y}	0.32 ± 0.09^{x}
Kidney (g/100 g BW)	0.401 ± 0.02^{x}	0.376 ± 0.02^{x}	0.401 ± 0.03^{x}

Figures having different letters (superscribed) in the same row are significantly different ($P \leqslant 0.05$).
[a] Each value is the mean of seven rats ± standard error.

Table 3
Lipid parameters in serum of rats in CPO, CPS and control groups

Parameters	Control	CPO	CPS
Cholesterol (g/l)	0.66 ± 0.03^{x}	0.43 ± 0.06^{y}	0.76 ± 0.03^{y}
HDL cholesterol (g/l)	0.36 ± 0.03^{x}	0.35 ± 0.08^{x}	0.48 ± 0.03^{y}
HDL cholesterol/total cholesterol (%)	0.55 ± 0.06^{x}	0.81 ± 0.20^{y}	0.64 ± 0.02^{z}
Triglycerides (g/l)	0.717 ± 0.079^{x}	1.38 ± 0.182^{y}	0.333 ± 0.075^{z}
Athero. index[A]	0.822 ± 0.204^{x}	0.505 ± 0.205^{y}	0.564 ± 0.071^{y}

Each value is the mean of seven rats ± standard error.
Figures having different letters (superscribed) in the same row are significantly different ($P \leqslant 0.05$).
Normal range for cholesterol level, HDL cholesterol and triglycerides in serum of adult rats: $(0.80 \pm 0.07)^{a}$, $(0.40 \pm 0.20)^{b}$ and $(0.70 \pm 0.10)^{a}$, respectively. [a]: Alonso et al. (2001); [b]: Gaiva et al. (2003).
[A] Athero. index (atherogenic index) = (total cholesterol − HDL cholesterol)/HDL cholesterol (Deguchi & Ogata, 1991).

3.3. Effect of diet substitution on plasma cholesterol and triglyceride concentrations

The CPO group had a significant reduction in total cholesterol and the ratio of HDL cholesterol to total cholesterol was significantly higher than that of the control group (Table 3). The last parameter was negatively correlated with the risk of coronary heart disease, as reported by Barter and Rye (1996). An increase, by 93%, of serum triglyceride level was noted in the CPO group. This increase may be explained by the rise of serum lipid level, as reported in our results. In the CPS group, the ratio of HDL cholesterol to total cholesterol increased significantly compared to the control, since HDL cholesterol was increased, while serum triglyceride level decreased by 53% compared to the control group. The atherogenic index was significantly lower in CPO and CPS than in the control group.

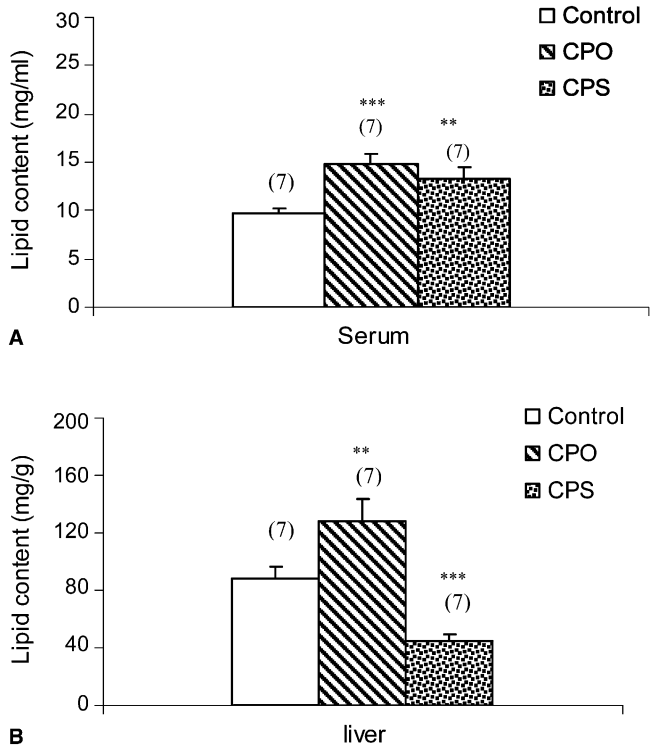

Fig. 1. Effect of diet supplemented with CPO or CPS on serum lipid content (A) and liver lipid content (B) compared to control. Values are given as means ± SEM. Significant differences between the treated and controls groups ***: $P \leqslant 0.001$ and **: $P \leqslant 0.01$. The number of rats studied are shown between brackets.

3.4. Serum and liver lipid contents

The lipid content of serum increased significantly in the CPO and CPS groups compared to control (Fig. 1A) whereas in the CPS group, a significant decrease in liver lipid content was noted (Fig. 1B). The reduction in the level of liver total lipids in the CPS group paralleled that of serum triglycerides. The liver weight was greater in the CPO group than in the control group, probably due to the accumulation of lipids in liver. As the fibre content was the only major variable in the ingredients of the CPO and CPS diets (Table 1), the supplementation of diet with whole cactus pear seeds was better than that with cactus pear oil for lowering the lipid levels of hepatic tissues.

3.5. Serum and liver lipid profiles

In a previous study we have demonstrated that cactus pear seed oil was rich in oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids (16.7% and 70.3%, respectively), which represented 87% of the total fatty acids (Ennouri et al., 2005). The type

Table 4
Serum fatty acid compositions of rats in CPO, CPS and control groups (g/100 g of total fatty acid)[a]

Fatty acid	Control	CPO	CPS
SFA	32.0 ± 5.75^{x}	32.4 ± 0.87^{x}	34.9 ± 0.23^{y}
Myristic acid (C14:0)	0.45 ± 0.00^{x}	0.41 ± 0.00^{y}	0.50 ± 0.04^{z}
Palmitic acid (C16:0)	21.2 ± 0.31^{x}	20.0 ± 0.84^{y}	20.3 ± 1.01^{y}
Stearic acid (C18:0)	9.23 ± 0.34^{x}	11.0 ± 0.01^{y}	12.7 ± 0.68^{z}
MUFA	18.7 ± 0.70^{x}	14.75 ± 1.02^{y}	16.7 ± 2.46^{x}
Palmitoleic acid (C16:1)	1.98 ± 0.53^{x}	0.69 ± 0.50^{y}	1.26 ± 0.03^{y}
Oleic acid (C18:1)	15.8 ± 0.47^{x}	13.5 ± 0.71^{a}	13.1 ± 2.41
PUFA	52.0 ± 1.36^{x}	52.7 ± 2.35^{x}	47.6 ± 2.25^{y}
Linoleic acid (C18:2)	24.0 ± 1.23^{x}	22.7 ± 1.43^{y}	17.2 ± 0.45^{z}
Linolenic acid (C18:3)	0.80 ± 0.04^{x}	0.73 ± 0.01^{y}	0.95 ± 0.18^{z}
Eicosatrienoic acid (C20:3)	0.26 ± 0.01^{x}	0.92 ± 0.36^{y}	0.71 ± 0.17^{y}
Arachidonic acid (C20:4)	23.8 ± 0.86^{x}	25.3 ± 2.36^{x}	25.7 ± 2.99^{x}
Eicosapentaenoic acid (C20:5)	0.16 ± 0.07^{x}	0.16 ± 0.07^{x}	0.33 ± 0.10^{x}
Docosatetraenoic acid (C22:4)	0.71 ± 0.02^{x}	0.82 ± 0.02^{y}	0.77 ± 0.03^{x}
Docosahexaenoic acid (C22:6)	2.13 ± 0.22^{x}	1.11 ± 0.51^{y}	2.08 ± 0.42^{z}
PUFA/SFA	1.61 ± 0.02^{x}	1.62 ± 0.11^{x}	1.36 ± 0.05^{y}

Figures having different letters (superscribed) in the same row are significantly different ($P \leqslant 0.05$).
[a] Each value is the mean of seven rats ± standard error.

Table 5
Liver fatty acid compositions of rats in CPO, CPS and control groups (g/100 g of total fatty acid)[a]

Fatty acid	Control	CPO	CPS
SFA	38.9 ± 2.99^{x}	36.5 ± 0.32^{x}	44.9 ± 3.82^{z}
Myristic acid (C14:0)	0.26 ± 0.06^{x}	0.51 ± 0.05^{y}	0.236 ± 0.04^{x}
Palmitic acid (C16:0)	18.9 ± 0.64^{x}	19.4 ± 0.18^{x}	20.7 ± 1.68^{x}
Stearic acid (C18:0)	18.0 ± 2.64^{x}	15.2 ± 0.01^{y}	22.9 ± 2.09^{y}
MUFA	10.6 ± 2.56^{x}	15.0 ± 0.46^{y}	10.1 ± 0.91^{x}
Palmitoleic acid (C16:1)	0.77 ± 0.14^{x}	0.97 ± 0.34^{x}	0.72 ± 0.06^{x}
Oleic acid (C18:1)	8.62 ± 2.60^{x}	13.6 ± 0.34^{y}	7.74 ± 1.26^{x}
PUFA	50.1 ± 0.46^{x}	48.2 ± 0.47^{y}	46.4 ± 2.43^{y}
Linoleic acid (C18:2)	17.5 ± 1.70^{x}	17.3 ± 0.48^{x}	14.4 ± 1.69^{y}
Linolenic acid (C18:3)	0.48 ± 0.07^{x}	0.25 ± 0.17^{y}	0.28 ± 0.01^{y}
Eicosatrienoic acid (C20:3)	0.34 ± 0.11^{x}	0.58 ± 0.02^{y}	0.42 ± 0.03^{x}
Arachidonic acid (C20:4)	23.4 ± 1.04^{x}	22.5 ± 0.16^{x}	24.0 ± 2.15^{x}
Eicosapentaenoic acid (C20:5)	0.43 ± 0.32^{x}	0.06 ± 0.05^{y}	0.11 ± 0.04^{x}
Docosatetraenoic acid (C22:4)	1.29 ± 0.21^{x}	1.52 ± 0.34^{x}	1.15 ± 1.06^{x}
Docosahexaenoic acid (C22:6)	4.76 ± 0.13^{x}	4.11 ± 0.54^{x}	4.21 ± 0.38^{x}
PUFA/SFA	1.29 ± 0.08^{x}	1.32 ± 0.02^{x}	1.04 ± 0.14^{y}

Figures having different letters (superscribed) in the same row are significantly different ($P \leqslant 0.05$).
[a] Each value is the mean of seven rats ± standard error.

of fat ingested in the diet could provoke changes in the fatty composition of tissues, which is why we have tried to study the fatty acid composition of serum and liver lipid extracts in rats after enrichment of diets with CPO and CPS. The major fatty acids in serum, as well as on liver of all groups, were arachidonic, palmitic, linoleic, oleic, and stearic acids (Tables 4 and 5). Netherless the fatty acid analysis in liver showed that, in the CPO group, the significant increase in oleic acid level resulted in an increase of MUFA. A reduction in linolenic acid in this group provoked a significant decrease in PUFA. But, in the CPS group, the level of stearic acid was increased by 27%, while linolenic acid was decreased by 52%. The levels of docosahexaenoic, docosatetraenoic, arachidonic and linoleic acids, representing 45% of total liver fatty acids were similar in CPO and CPS (Table 5). In the CPS group, a decrease, by 8%, of PUFA in serum and liver was observed compared to the control. Indeed, the ratio of PUFA to SFA decreased significantly in the CPS group (Tables 4 and 5). CPO and CPS diets contained the same amount and quality of oil. It is known that oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids have beneficial health effects, including alleviating cardiovascular complaints, inflammatory conditions, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Hegsted, McCrancy, Myers, & Stare, 1965). Linoleic acid is an essential fatty acid and a precursor of arachidonic acid biosynthesis, the substrate for eicosanoid synthesis. According to Keys, Anderson, and Grande (1957), linoleic acid has hypocholesterolemic effects.

4. Conclusion

The present study has demonstrated, for the first time that the enrichment of diet with CPS had of very pronounced hypolipidemic effect as compared to the CPO diet. It could significantly decrease the levels of triglycerides in serum and total lipids in liver. There were no major variations of the fatty acid composition of liver and serum extracts. More studies are needed of to explain the potential hypocholesterolemic and hypolipidemic effects of *Opuntia ficus indica* seed and oil extracts on hypercholesterolemic and other pathologies on rats.

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