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Nutritional and short term toxicological evaluation of Perilla seed oil

T. Longvah *, Y.G. Deosthale, P. Uday Kumar

National Institute of Nutrition Indian Council of Medical Research, Food Chemistry Division, Jamai Osniania, Hyderabad-500 007, India

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Abstract

Weanling NIN wistar rats appeared to suffer no toxicological effects when consuming 10% Perilla seed oil in their diet for 18 weeks. Serum cholesterol and triglyceride levels were significantly reduced after 18 weeks and this effect is attributed to the high α-linolenic acid level of the oil (57%)

1. Introduction

Perilla frutescens linn, belonging to the family lamiaceae, is a traditional oilseed grown primarily for its food uses at the household level in northeast India (Council of Scientific and Industrial Research [CSIR] 1966). Perilla seed, a rich source of protein (17%) and fat (51%) is used by the northeast tribals as a delicacy after frying or in combination with cereals or vegetables after cooking. To some extent, the expelled oil is also used as a cooking medium (Longvah & Deosthale, 1991).

Most vegetable oils are very good sources of linoleic acid, but only very few vegetable oils contribute significant amounts of α-linolenic acid (α-LNA) in the diet. Among them, Perilla seed oil has the highest content of n-3 fatty acid (α–LNA), accounting for nearly 57% (Longvah & Deosthale, 1991). Several studies have indicated that α-LNA and/or its higher derivatives may indeed have certain unique and essential functions in physiology (Budowski, 1988; Crawford, 1987; Neuringer, Anderson & Lonnor, 1988; Tinoco, 1982; Zollner, 1986). Studies have also shown beneficial effects of increased n-3 fatty acid consumption, especially in lowering blood lipids (Dyerberg, 1986; Harris, 1989; Herold & Kinsella, 1986; Kinsella, Lokesh & Stone, 1990; Nester, 1990). The consumption of Perilla oil has also been reported to improve learning ability, retinal function, suppression of carcinogenesis, metastasis, thrombosis and allergy (Kinsella, 1991). In the light of increasing reports of the beneficial effects of α-linolenic acid (n-3) in health and disease, Perilla seed oil with an exceptionally high α-LNA content (56%) assumes importance. Yet, Perilla seed oil remains a less familiar oil, with little or no information on its nutritional quality. The tribal population in northeast India have been consuming Perilla seed as well as its oil and no ill effects have been reported due to its consumption. However, it was considered necessary to confirm the safety of Perilla oil consumption before exploiting the possible beneficial effects. Therefore, a study was designed to examine the physico-chemical characteristics, the nutritional aspects of Perilla seed oil and evaluate toxicity if any in a short term animal experiment.

2. Materials and methods

Perilla seeds were purchased from Ukhrul, Manipur an transported by air to Hyderabad. Upon arrival at the laboratory, the samples were winnowed, cleaned of all foreign particles an dried in an oven at 60°C overnight. The seeds were then crushed and the oil extracted with *n*-hexane in a Soxhlet extractor continuously for 36 h. The solvent was removed completely under vacuum and the oil thus obtained was used for the study.

2.1. Chemical analysis

The fatty acid methyl esters of the oils used in the diet were prepared according to the method of Lowenstein, Brunengraber and Wadka (1975) The fatty acid methyl esters were extracted in petroleum ether (40–60°C BP) and analyzed on a Varian 3700 gas chromatograph equipped with flame ionization detector. A 12 ft×1/8 inch

* Corresponding author. Tel.: +91-40-7018909; fax: +91-40-7019074.
E-mail address: icmrinin@ren.nic.in (T. Longvah).

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stainless steel column packed with 10% Silar 10C coated in chromosorb W-AW 80/100 mesh was used. The flow rate of the carrier gas was 20 ml/min. The temperature of the injection and detection ports were kept at 230°C. The column temperature initially at 160°C was increased at the rate of 3°C/min and maintained at 226°C for 12 min. The individual fatty acids were identified by frequent comparisons with authentic standards and calculated on a Varian 4270 integrator. All fatty acid methyl ester standards and heptadecanoic acid were purchased from Nuchek laboratories. Solvents used were of analytical grade and contained 25 mg butylated hydroxy toluene (BHT) per litre. The fatty acid composition was expressed as percentage of the total oil.

The oil was analysed for various physico-chemical properties such as iodine value, saponification value, unsaponifiable matter and acid value by conventional methods (British Pharamacopia, 1963).

2.2. Animals and diets

Weanling NIN Wistar rats (21–23 days) were divided into two groups of 24 animals each (12 males and 12 females) and fed a diet containing 10% Perilla seed oil (PSO) or groundnut oil (GNO). The basal diet contained 73.3% corn starch. 20% casein, 1% vitamin mixture, 4% salt mixture, 0.2% choline chloride and 1.5% cellulose. PSO or GNO was added to the basal diet at the expense of starch. Fatty acid profiles of oils used in the preparation of diets are given in Table 1. The diets were prepared weekly, flushed with nitrogen, sealed and stored at −20°C to prevent peroxidative damage of the Perilla oil diet. Daily food and water were supplied to the animals ad-lib for 18 weeks. Weekly food intake and body weight gain of individual animals were recorded. Towards the end of 18 weeks, animals were transferred to metabolic cages and faeces were collected for 3 days. Diet and faeces were analysed for nitrogen, calcium and phosphorus (Association of the Official Analytical Chemists [AOAC] 1990). Apparent retentions of these nutrients were calculated from the dietary intake and faecal excretion. At the end of 18 weeks, final body weights of individual animal were recorded and blood was drawn from each animal for the estimation of serum cholesterol (Abel & Brodie, 1952) and triglyceride (Foster & Dunn, 1973). After sacrifices, the liver, heart, lungs, kidneys and spleen were carefully dissected and immediately weighed. The weights of these organs were expressed per 100 g of final body weight. Samples of liver as well as heart were taken for analysis of cholesterol and triglyceride.

Table 1
Fatty acid composition of Perilla seed oil (values are percentages of total oil)

Fatty acids	Perilla oil	Groundnut oil
C16:0 Palmitic	8.9	12.6
C18:0 Stearic	3.9	1.8
C20:0 Arachidic	ND[a]	4.2
C22:0 Behenic	ND	1.9
C24.0 Lignoceric	ND	0.3
C16:1 Palmitoleic	ND	1.2
C18:1 Oleic	12.5	47.8
C18:2 Linoleic	17.8	30.2
C18:3 Linolenic	56.9	–
Total saturates	12.8	20.8
Total unsaturates	87.2	79.2
Total polyunsaturates	74.7	30.2

[a] ND: not detected.

2.3. Histopathology

All the animals in the GNO group (12 ♂ + 12 ♀) and PSO group (11 ♂ + 12 ♀) were taken for histopathology studies. Tissue samples of liver, heart, lungs, spleen, kidneys, ovary, testes, pancreas and G.I. tract were fixed, processed, embedded and sectioned by conventional methods. Six micrometer sections stained with Myer's hematoxylin and eosin were examined under the light microscope for any changes in the tissues due to the consumption of PSO.

Statistical analysis of the data was carried out by students' '*t*' test.

3. Results and discussion

3.1. Chemical analysis

The fatty acid composition of the oils used in the present study are presented in Table 1. As reported earlier (Longvah & Deosthale, 1991) α-linolenic acid was the major fatty acid in Perilla oil. Other fatty acid contents were also similar to reported values. The fatty acid profile of groundnut oil was also similar and comparable to earlier reported values (Gopalan, Rama Sastin & Balasubramanian, 1996).

The physico-chemical characteristics of Perilla seed oil in the present study are given in Table 2. The saponification value, acid value, iodine value and unsaponifiable matter of PSO in the present study are close to earlier reported values (CSIR, 1966).

3.2. Nutritional evaluation of Perilla seed oil

One male rat in the PSO group died at 10 weeks due to otitis media infection. Table 3 shows the growth pattern of the PSO/GNO fed group of rats over 18 weeks. No differences were observed in the body weight gain of animals fed groundnut oil/Perilla seed oil. The feed efficiency ratio as well as food intake are similar between

the two groups fed either groundnut oil or Perilla seed oil. Hence, the results indicate that Perilla seed oil compares favourably with groundnut oil in its nutritive quality. The data on retention of fat, nitrogen, calcium and phosphorus are given in Table 4. No significant differences were observed in the retention of nutrients between the two groups indicating that PSO is absorbed to the same extent as GNO in these animals.

Table 5 shows the contents of cholesterol and triglyceride in serum, liver and heart of rats fed the PSO/GNO diet for 18 weeks. The cholesterol and triglyceride levels in serum were significantly reduced in animals fed PSO but not in the liver or heart. Decreases in the plasma cholesterol often accompany hepatic cholesterol accumulation (Garg, Sebokova, Weirzbicki & Thomson, 1988; Gerson, Shorland & Adams, 1961; Nakagama, Shimokawa, Noguchi, Ishihara & Kojima, 1986). However, in this study, feeding of Perilla seed oil resulted in decreased plasma cholesterol without significant accumulation of cholesterol in various tissues of rats. Similar observations have been recorded on a linseed oil diet (rich in α-LNA) by Garg et al. (1988). The high α-linolenic acid (56%) of Perilla oil might inhibit cholesterol synthesis or it may stimulate cholesterol catabolism to bile acids and to neutral sterols excreted in faeces, or may stimulate the excretion of cholesterol and its metabolites as dermal lipids (Ishihara, Ito, Sakai, Watanabe, Kopayasi & Okuyama, 1995).

3.3. Organ weights and histopathological studies

Table 6 shows the organ weights of animals fed PSO/GNO diet for 18 weeks. No apparent differences were observed in the organ weights of the animals fed PSO as compared to GNO fed animals.

Histopathological examination of heart, lungs, liver, spleen, kidney, pancreas and gastrointestinal tract revealed no changes except in liver and lungs (Table 7). The various changes observed in the liver and lungs (except parasitic cysts in liver) are features commonly seen in all colony-bred animals and as such do not have any great significance. Hence it was concluded that PSO did not cause any toxic changes in the tissues examined.

The above results indicate that PSO had comparable nutritional qualities to GNO and the retention of fat or nitrogen or calcium or phosphorus were not impaired due to PSO in the diet. This study confirms that PSO can be consumed safely by the population as practised by

Table 2
Physical composition of Perilla seed oil

	No. of Observations	Present study	Reported values[a]
Fat content of the seed	5	50.0%	30–51%
Saponification value	5	195	189–197
Acid value	4	4.1	1–6
Iodine value	6	193	189–197
Unsaponifiable matter	4	1.0%	06–1.3%

[a] CSIR (1966).

Table 3
Growth performance of rats fed 10% GNO/PSO for 18 weeks[a]

Mean values	Groundnut oil	Perilla oil
Final body weight (g)	272.7 ± 90.7	287.0 ± 88.3
Gain in body weight (g)	218.8 ± 88.7	233.0 ± 86.6
Food intake (g)	1070.3 ± 465.5	1095.0 ± 454.1
Feed efficiency ratio	21.4 ± 5.9	21.7 ± 5.7

[a] Mean ± SD of 24 animals (12 ♂ + 12 ♀) in GNO group, and 23 animals (11 ♂ + 12 ♀) in PSO group.

Table 4
Apparent retention of fat, nitrogen, calcium and phosphorus in rats fed groundnut oil and Perilla seed oil for 18 weeks[a]

Dietary Fat	% Retention			
	Fat	Nitrogen	Calcium	Phosphorus
Groundnut oil	98.4 ± 3.6	60.23 ± 2.2	74.8 ± 3.0	83.3 ± 3.6
Perilla oil	98.4 ± 4.1	66.45 ± 6.6	76.5 ± 3.1	81.8 ± 3.2

[a] Mean ± SD of 24 animals (12 ♂ + 12 ♀) in GNO group, and 23 animals (11 ♂ + 12 ♀) in PSO group.

Table 5
Cholesterol, triglyceride and phospholipid contents of serum, liver and heart of rats fed GNO/PSO at the end of 18 weeks[b]

		Groundnut oil	Perilla oil
Serum	Cholesterol (mg/dl)	98.3 ± 7.5	79.3 ± 6.3[a]
	Triglyceride (mg/dl)	142 ± 8.7	108 ± 6.8[a]
Liver	Cholesterol (mg%)	198 ± 7.4	196 ± 8.5
	Triglyceride (mg%)	81.4 ± 9.3	82.8 ± 8.4
Heart	Cholesterol (mg%)	96.5 ± 6.9	94.5 ± 5.8
	Triglyceride (mg%)	71.0 ± 8.3	72.3 ± 8.8

[a] Significnatly different at $P < 0.001$.

[b] Mean ± SD of 24 animals (12 ♂ + 12 ♀) in GNO group, and 23 animals (11 ♂ + 12 ♀) in PSO group.

Table 6
Organ weights of rats sacrificed at the end of 18 weeks expressed as g per 100 g body weights[a]

Organs	Groundnut oil	Perilla oil
Liver	2.66 ± 0.20	2.79 ± 0.29
Spleen	0.18 ± 0.03	0.21 ± 0.04
Kidney	0.61 ± 0.05	0.65 ± 0.09
Heart	0.29 ± 0.03	0.27 ± 0.04
Lungs	0.54 ± 0.16	0.53 ± 0.17

[a] Values are mean ± SD of 24 animals (12 ♂ + 12 ♀) in GNO, and 23 animals (11 ♂ + 12 ♀) in PSO group.

Table 7
Histopathology findings in various organs[a]

	Control (groundnut oil)		Experimental (Perilla oil)	
	Male	Female	Male	Female
Liver				
Focal necrosis	4	1	6	4
Edema	–	–	1	–
Parasitic cysts		2	–	–
Lungs				
Chronic intestinal pn.				
Grade I	2	2	3	–
II	1	9	4	5
III	1	1	4	5
Focal emphysema	1	–	1	–
Focal Bronchiectasis	–	1	2	–

Heart, spleen, kidney, pancreas & GI tract } No abnormality detected

[a] 24 animals (12 ♂ + 12 ♀) in GNO and, 23 animals (11 ♂ + 12 ♀) in PSO group.

the tribal population in northeast India. PSO, being rich in α-LNA, has also shown potential beneficial effects in decreasing the circulating levels of serum cholesterol and triglycerides. In the light of such beneficial effects of n-3 polyunsaturated fatty acids in health and disease, oils rich in α-linolenic acid, such as Perilla seed oil used in the present study, could be exploited for nutritional advantage by blending with other vegetable oils.

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