Malaysian Journal of Pharmaceutical Sciences
Vol. 8, No. 1, 1–12 (2010)

NUTRITIONAL AND FUNCTIONAL CHARACTERISATIONS OF *PERILLA FRUTESCENS* SEED OIL AND EVALUATION OF ITS EFFECT ON GASTROINTESTINAL MOTILITY

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Perilla frutescens seeds contain fixed oil which is a useful edible oil. It is an alternative source of unsaturated fatty acids (linolenic acid i.e. omega-3-fatty acids), phenolic compounds (rosemarinic acids, luteolin chrysoeriol, quercetin, catcehin, protocatechuic acid and apigenin), natural antioxidants and minerals. The present study was carried out to investigate nutritional and functional characterisations, and gastrointestinal motility of P. frutescens seed oil. The perilla oil was used in 3 doses i.e. 5 mL/kg, 7.5 mL/kg and 10 mL/kg body weight by p.o. together with 0.5% carboxyl methyl cellulose (CMC) as an emulsifying agent. Gastrointestinal motility effect was evaluated by charcoal meal (10% wt/vol.). Laxative activities were assessed for faecal content (%), frequency (times/day), number of faecal pellets, and intestinal propulsion. Petroleum ether extract of perilla seed contained 30%–35% oil. The physical and chemical characteristics were found to be; specific gravity 0.92396 at 31°C, saponification value 180.92, acid value 1.68, peroxide value 1.68, iodine value 135.0 and unsaponifiable matter 1.8%. It may be concluded that perilla seed oil produced laxative effect and increased gastric motility in constipated rats.

Keywords: *Constipation, Laxatives, Gastrointestinal motility, Linolenic acid, Perilla frutescens*

INTRODUCTION

Perilla frutescens (Shiso) is an annual herb of the Mint family (Lamiaceae) native to East Asia. It is an edible medicinal plant and a traditional crop of China, India, Japan, Korea, Thailand, and other Asian countries and USA (Jackson and Shelton 2002; Siriamornpun *et al.* 2006). In India it is found in northern hillsides of Lohagat, Champawat and Tanakpur. The entire plant is very nutritious, containing vitamins and minerals. There are many scientifically proven medicinal uses for Perilla. It has been used as an antiasthmatic (Okamoto *et al.* 2000a, b), antibacterial, antidote (Yamamoto and Ogawa 2002), antipyretic, antiseptic, antispasmodic, antitussive, emollient, expectorant, antioxidant (Tada *et al.* 1996; Dapkevičius *et al.* 1998; Povilaitytėė and Venskutonis 2000), anti-inflammatory (Simopoulos 2002), analgesic and antiallergic (Shin *et al.* 2000; Yamamoto and Ogawa 2002; Ueda, Yamazaki and Yamazaki 2002; Yoko *et al.* 2004; Kamasa *et al.* 2004). Perilla has been reported to have cardioprotective, antithrombotic, antihypertensive (De Lorgeril *et al.* 1999; Ezaki *et al*. 1999; Bemelmans *et al.* 2002), anticancer (Narisawa *et al.* 1994), insecticidal, tonic and heat-protecting properties (Jackson and Shelton 2002).

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It stimulates the immune function (Kwon *et al*. 2002; Talbott and Hughes 2006). The fatty acid has been associated with benefits to treat heart disease, colitis, asthma and support lungs, protecting them from colds and flu. Perilla oil has its own benefits. In animal experiments, perilla oil proved superior to either soyabean or safflower oil in inhibiting mammary, colon and kidney cancers (Okuyama 1992; Onogi *et al*. 1996; Tripathi 2008).

Perilla oil is a polyunsaturated fatty acid (PUFA). Alpha-linolenic acid (ALA) is found in perilla oil as a triglyceride. It is a very rich source of omega-3 polyunsaturated fatty acid which contains 18 carbon atoms and 3 double bonds (ALA 18: 3n). The functional compounds of perilla include luteolin, apigenin, chrysoeriol, rosemarinic acid, caffeic acid, monoterpene alkaloids, ascorbic-acid, beta-carotene, citral, dillapiol, elemicin, limonene, myristicin, protocatechuic acid, perillaldehyde and xanthing oxidase (Ragažinskienė *et al*. 2004; Talbott and Hughes 2006). ALA is metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the precursors of the series–3 prostaglandins, the series–5 leukotrienes and the series–3 thromboxanes. These eicosanoids have anti inflammatory and anti atherogenic properties (Brenner 1993; Okamoto *et al*. 2000b; Yamamoto and Ogawa 2002; Cleland *et al*. 2005).

Perilla seed oil has also been used in paints, varnishes, linoleum, and printing ink. Volatile oils of the plant are also used in aromatherapy and for perfume (Nagatsu *et al*. 1995; Borchers *et al*. 1997; Povilaitytėė and Venskutonis 2000; Jackson and Shelton 2002).

Since no work has been done on the seed oil of *P. frutescens* regarding its laxative activity, it was thought worthwhile to evaluate its laxative and gastrointestinal motility potential because some herbal drugs like castor oil have been proven to exhibit the laxative effect (Ammon, Thomas and Phillips 1974; Tripathi 2008). Therefore, petroleum ether extracts of *P. futescens* were evaluated for laxative activity and gastrointestinal motility in wistar rats.

METHODS

Sample Collection

The seeds of *P. frutescens* were collected from Navdanya office, near the regional traffic office (R.T.O), Rajpur Road, Dehradun (Uttrakhand). Their qualities were analysed by gas chromatography and by phytochemical analysis to determine the different fatty acids present in the oil.

Extraction of seeds

The seeds were washed, dried, crushed and weighed, before extraction in the soxhlet assembly by using petroleum ether as the solvent and maintained at 50°C–60°C during the whole process. The petroleum ether extract was a yellowish transparent liquid with 30%–35% yield.

Fatty oil analysis

Fatty oil analysis of perilla oil was determined according to the Indian Pharmacopoeia (1996).

Determination of saponification value

The saponification value is the milligrams of potassium hydroxide necessary to neutralise the free acids and to saponify the esters present in 1g of substance. Saponification value is a measure of the equivalent weight of the acids present and is therefore useful as an indication of purity. Adulteration with mineral oils would be shown by low saponification value.

Determination of acid value

The acid value is the number, which expresses in milligrams the amount of hydroxide necessary to neutralise the free acids present in 1g of substancee. It is significant for determination of equivalent weight.

Determination of ester value

The ester value is the number of potassium hydroxide required to saponify the ester present in 1 g of substance. The ester value is used to determine the quality of the fixed oil.

Ester value = Saponification value – acid value.

Determination of iodine value

The iodine value is the number, which expresses in grams the quantity of halogen, calculated as iodine, which is absorbed by 100 gm of substance under the described conditions. Iodine value is a measure of unsaturated compounds present in the substance or measure of double bonds. It is also used in the substitution reaction.

Determination of peroxide value

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of substance.

Determination of unsaponifiable matter

The unsaponifiable matter consists of substance present in oil and fats, which are not saponifiable by alkaline hydroxides.

Determination of specific gravity

Specific gravity is defined as weight per milliliter of the solution at a constant temperature. These constants are used as standards for liquids, including fixed oil, synthetic chemicals and solutions.

Specific gravity of oil = Density of oil/density of water

Experimental Design

The study was approved by the Institutional Animal Ethics Committee (IAEC) with registration no. 1145/a/07/CPCEA of GRD Post Graduate Institute of Management and Technology, Dehradun (Uttrakhand), India.

Animals

Swiss albino rats (150–200 g) of either sex were selected for the experiments. Animals were allowed to be acclimatised for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages, maintained under standard laboratory conditions (*i.e.* 12:12 hour light and dark sequence; at an ambient temperature of 25 ± 2^{o}C; 35%–60% humidity), the animals were fed with standard rat pellet diet and water *ad libitum*.

Experimental design

25 albino rats of either sex were used and divided into 5 groups, each group contained 5 rats. The drugs were administered to all groups by p.o route. Constipation was induced by administration of loperamide (5 mg/kg p.o) before administration of control, test or reference drugs. Group I (control) received 0.5% carboxyl methyl cellulose (CMC) in distilled water. Group II (test drug I) received perilla oil (5.0 mL/kg) suspension in 0.5% sodium CMC. Group III (test drug II) received perilla oil (7.5 mL/kg) suspension in 0.5% sodium CMC. Group IV (test drug III) received perilla oil (10 mL/kg) suspension in 0.5% sodium CMC. Group V (reference Drug) received castor oil (10 mL/kg p.o.).

Laxative activity study by loperamide induced constipation:

Procedure

The methods described by Capasso *et al.* (1986), and Shoba and Thomas (2001) were followed for this study. The animals were weighed and numbered. The animals were fasted for 12 h before the experiment but water was provided *ad libitum*. Loperamide (5 mg/kg) was administered everyday by p.o. route to control and treated groups until constipation occurs (Ishikawa *et al.* 2003). Constipation was induced by administration of loperamide which is identified by decreased stool output (Saito *et al*. 2002). Immediately after dosing, the animals were separately placed in individual cages suitable for collection of faeces. After 8 h of drug administration, the faeces were collected, weighed and the

number of faeces counted. Thereafter, food and water were given to all rats and faecal outputs were again weighed and counted after a period of 16 h. The water contents were also determined in faeces after 8 and 16 hr.

Effect on gastrointestinal motility

The methods, described by Abdullahi *et al.* (2001), and Uddin *et al.* (2005) were adopted to study the effect of the perilla oil on the gastrointestinal transit in albino rats. The test animals were starved for 12 h prior to the experiment but were allowed free access to water. The animals were divided into control and test groups containing five rats in each group. The control group received 0.5% CMC in distilled water at a dose of 10 mL/kg body weight orally. The positive control group received castor oil at the dose of 1.0 mL/kg orally, and the test group received perilla seed oil at the doses of 5.0 mL/kg, 7.5 mL/kg and 10 mL/kg body weight orally. After 30 min, rats from each group were fed with 1.0 mL of charcoal meal (3% suspension of deactivated charcoal in 0.5% CMC). After 30 min of the administration of charcoal meal, the animals of each group were sacrificed and the length of the intestine (pyloric sphincter to caecum) as well as the distance traveled by charcoal as a fraction of that length was measured. The charcoal movement in the intestine was expressed in percentage.

$$\text{Intestinal transit \%} = \frac{\text{Distance traveled by charcoal}}{\text{Total length of small intestine}} \times 100$$

Statistical analysis

Statistical analysis was performed; one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test. The value of $p<0.05$ was considered as significant. All the values were expressed as mean ± SEM.

RESULTS

Perilla seed oil is a light yellow clear and transparent oily liquid, without foreign flavour and is slightly soluble in ethanol. The seeds of perilla contain 30%–35% of oil. Using standard procedures in Indian Pharmacopoeia (1996), the analysis of perilla seed oil found that the specific gravity at 31°C was 0.92396, saponification value was 180.92, acid value was 1.68, peroxide value was 3.98, iodine value was 135.0 and unsaponifiable matter was 1.8%.

The seed oil of *P. frutescens* has been analysed in laboratory on albino wistar rats. Perilla seed oil has shown a laxative effect (stimulant purgative) at a dose range of 5.0 mL/kg, 7.5 mL/kg and 10 mL/kg using both parameters; number of faeces and loss of water content in faeces (Tables 1 and 2). The magnitudes of laxative effects were dose dependent i.e. 10 > 7.5 > 5.0 mL/kg respectively. The petroleum extract of *P. frutescens* (seed oil) showed that the increase in faecal output was comparable with the reference drug (castor oil). It also increased gastrointestinal activity in a dose dependent manner up to 16 hr of drug administration, traversed by charcoal meal at 49.47%, 58.65%, and 66.81%

by 5.0, 7.5 and 10 mL/kg dose of test drug (perilla oil), respectively, and 69.29% by the reference drug (castor oil). Oil promoted evacuation of bowel content, increased faecal weight and water contents without producing diarrhoea. CMC did not produce evident changes in bowel movement. Loperamide (5 mg/kg) decreased (stimulant-purgative effect) bowel output dose-dependently. Constipation induced by decrease in fecal weight was produced by loperamide in rats. Castor oil was used as the reference drug (1 mL) in rats via p.o. route. In the gastrointestinal motility test, perilla oil at doses of 5.0, 7.5 and 10 mL/kg body weight increased the intestinal transit of charcoal meal in rats. The effect was less potent compared to that of the reference drug at 10 mL/kg body weight.

DISCUSSION

On the basis of these findings, it may be inferred that *P. frutescens* is a laxative agent that promotes evacuation of bowel contents and increases gastrointestinal motility in wistar rats based on parameters like the number of faeces and loss of water content in faeces. The underlying mechanism of laxatives is the modification of fluid dynamics of mucosal cell causing fluid accumulation in the gut lumen via inhibition of the Na^+K^+-ATPase of villous cells thereby impairing electrolyte and water absorption (Gaginella and Bass 1978). Other oils like castor oil causes activation of adenylate cyclase or mucosal cAMP-mediated active secretion (Gaginella *et al.* 1978; Capasso *et al.* 1994), and stimulations of prostaglandins (Capasso *et al*. 1986) and platelet activating factors (Pinto *et al*. 1992). Most recently, nitric oxide has been claimed to contribute to the diarrhoeal effect (Mascolo *et al*. 1996).

Perilla oil is a rich source of PUFAs and are useful for the treatment of various diseases as well as maintenance of health. PUFAs like linoleic and linolenic acids, which are found in perilla are termed "essential" because they cannot be synthesised by the body and must be supplied in the diet. Dietary linoleic acid can synthesise arachidonic acid in the body by ALA and is changed into EPA and DHA inside the body. Arachidonic acid is considered an essential fatty acid (EFA) because it is an essential component of membranes and a precursor of a group of hormone-like compounds called eicosanoids including series–3 prostaglandins, the series–5 leukotrienes, the series–3 thromboxanes, and prostacyclins, which are important in the regulation of physiological processes like anti inflammatory and anti atherogenic effects (Brenner 1993; James, Gibson and Cleland 2000; Okamoto *et al.* 2000a; Yamamoto and Ogawa 2002; Cleland *et al.* 2005).

Recent research interest is in the possibility of using dietary intake of perilla oil, to help reduce the risk of various diseases like coronary heart disease (Von Schacky and Dyerberg 2001; Calder 2004) and also in cancer treatment. Unsaturated fatty acids (UFAs) are cholesterol lowering when they replace significant levels of saturated fatty acids in the diet. Clinical and epidemiological studies indicate that PUFAs lower low density lipid (LDL) and total cholesterol. Some studies have found that diets high in monounsaturated fatty acids (MUSFAs) compared with PUFAs decrease LDL cholesterol while maintaining high density lipid (HDL) cholesterol levels (Bemelmans 2002). Other work has suggested that the effect of consuming polyunsaturated fat and monounsaturated fat is similar and results in a decrease in both LDL and HDL cholesterol (Mattson and Grundy 1985; Gardner and Kraemer 1995).

Table 1: Changes in mean number of faeces and water contents during loperamide-induced constipation.

Groups (n = 5)	Induction of constipation						Loss of water in gm	
	Number of faeces per day after administration of loperamide (5 mg/kg)							
	Initial	Day 1	Day 2	Day 3	Day 4	Day 5	Initial	Day 5
1	19.2 ± 1.30	15.6 ± 1.14	11.0 ± 1.00[c]	10.0 ± 1.41[c]	8.50 ± 1.14[c]	7.40 ± 1.14[c]	9.8 ± 2.25	5.2 ± 0.442[a]
2	21.6 ± 1.14	17.5 ± 1.22[a]	12.0 ± 1.58[c]	10.4 ± 1.14[c]	8.60 ± 1.14[c]	7.10 ± 1.14[c]	9.8 ± 1.2	4.1 ± 0.27[b]
3	18.4 ± 0.89	16.4 ± 1.14	10.0 ± 1.58[b]	8.00 ± 1.00[c]	7.00 ± 1.58[c]	6.20 ± 1.30[c]	9.2 ± 2.2	3.1 ± 0.418[c]
4	19.2 ± 0.84	16 ± 1.00	12.4 ± 1.14[a]	10.0 ± 1.14[c]	8.00 ± 1.58[c]	6.40 ± 1.52[c]	9.0 ± 1.4	2.5 ± 0.35[c]
5	18.4 ± 1.14	15.8 ± 1.00	12.4 ± 1.22[b]	9.80 ± 1.0[c]	7.20 ± 0.84[c]	6.20 ± 0.71[c]	8.8 ± 1.8	1.8 ± 0.57[c]

Notes: Values are in mean ± S.E.M, n = 5 for each group, [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ when compared to the effects on initial value.

Table 2: Effects of perilla oil on loperamide-induced constipative rats.

Groups (n = 5)	Change in total number of faeces			Change in water contents present in faeces		
	Initial	After 8 hr	After 16 hr	Initial	After 8 hr	After 16 hr
1 Control (0.5% CMC)	7.40 ± 1.1	8.6 ± 1.12	8.80 ± 0.84	2.00 ± 0.12	2.15 ± 0.42	2.0 ± 0.41
2 Test I drug (5.0 mL/kg)	7.10 ± 1.4	8.4 ± 1.00	9.40 ± 0.55	2.50 ± 0.41	4.0 ± 0.034	4.4 0 ± 0.32[a]
3 Test II drug (7.5 mL/kg)	6.20 ± 1.3	9.0 ± 0.89	10.24 ± 0.55	2.15 ± 0.22	4.0 ± 0.68	5.0 ± 0.42[b]
4 Test III drug (10 mL/kg)	6.00 ± 1.5	9.4 ± 1.14	11.95 ± 1.4	2.50 ± 0.26	4.8 ± 0.68[a]	5.6 ± 0.68[c]
5 Standard drug (10 mL/kg)	6.20 ± 0.71	9.12 ± 0.71	11.60 ± 1.00	2.00 ± 0.32	4.0 ± 0.46	5.0 ± 0.46[b]

Notes: Values are in mean ± S.E.M, n = 5 for each group, [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ when compared with control group. Castor oil 10 mg/kg used as reference drug.

Studies in animals have found that omega-3 fatty acids suppress cancer formation, but at this time there is no direct evidence for protective effects in humans. Oleic acid and saturated fatty acids have not been found to have any specific effects on carcinogenesis (Okuyama 1992; Onogi *et al*. 1996; Caughey *et al*. 1996; Grimble *et al*. 2002; Cleland *et al.* 2005). A group of isomers of EFAs like linoleic acid and "conjugated linoleic acid" (CLA), appear to have both anticarcinogenic and antiatherogenic properties (Chin *et al*. 1992; Ip *et al*. 1996; Thompson *et al*. 1997). CLA differs from linoleic acid by the position and geometric configuration of one of its double bonds. Animal studies have indicated that CLA reduces the incidence of tumours induced by carcinogens.

CONCLUSION

Chemical values of perilla seed oil have been successfully determined. Our results have established a pharmacological evidence for the folklore claim of the drugs to be used as a laxative agent. Further studies on possible mechanisms of action and isolation of active principle(s) responsible for such activity are required.

ACKNOWLEDGEMENT

The authors would like to thank GRD (PG) Institute of Management and Technology, Dehradun (Uttrakhand), India for providing technical support and facilities to carry out this work.

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