Safety Evaluation of Ambrette (*Abelmoschus moschatus* Linn) Seed Oil

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ABSTRACT: The seeds of ambrette (*Abelmoschus moschatus* Linn), after selective extraction of fragrance from the seed coat, are flaked and extracted with hexane to obtain a fatty oil. The FA composition and iodine value of the oil indicate it possesses saturated, monounsaturated, and polyunsaturated FA in ratios close to the recommendations of the United Nations World Health Organization; these characteristics make it suitable as an edible oil. Acute oral toxicity and safety evaluation in a 13-wk feeding trial on albino rats showed the oil is comparable to groundnut oil in all the parameters and is suitable for edible use.

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KEY WORDS: *Abelmoschus moschatus* Linn, abrette seed fatty oil, acute oral toxicity, safety evaluation study.

Hibiscus abelmoschus L. syn. *Abelmoschus moschatus* Linn of family Malvaceae is native to India (1). It is cultivated in the tropical regions of Asia, Africa, and South America for its seeds, which have a characteristic musky odor. The seeds contain about 16% fatty oil. At present, the seeds are used mostly for the isolation of fragrance components, and the fatty oil is wasted. That most of the seeds are processed or exported for essential oil is probably why this oil has not drawn the attention for consideration as edible oil. Nee *et al.* (2) showed that the essential oil of these seeds is localized in the outer layers of the seed coat, whereas the fatty oil is concentrated in the embryo and the endosperm.

We reported the selective extraction of fragrant and fatty oils from ambrette seeds (3,4). Contrary to earlier reports, ambrette fatty oil contains less than 1% epoxy FA and 1.5% cyclopropanoic acid. Although the oil gives positive Halphen test, methyl dihydrosterculate was the only unusual FAME detected in the GLC analysis of ambrette oil FAME.

In view of the widening gap between supply and demand of edible and industrial fatty oils in India, we examined ambrette as a nontraditional oil seed. Under rain-fed conditions, ambrette seed fetches a much higher price, making it an economic crop. Our selective process extracts essential oil in much higher yields and makes the extracted seeds available for fatty oil extraction at an affordable price.

The FA composition and iodine value of the fatty oil indicate ambrette oil has saturated FA (SFA), monounsaturated FA (MUFA), and PUFA in ratios close to the recommendations of the United Nations World Health Organization (WHO) (5,6), making it a suitable candidate for consideration as an edible oil. Nutritional and toxicological aspects of this oil have not been reported in the literature previously. The presence of small amounts of epoxy- and cyclopropanoid-containing FA in the oil makes it obligatory to undertake safety evaluation of this oil if it is to be recommended for edible use. Cyclopropene FA are known to inhibit biodesaturation of stearic acid to oleic acid (7). On the other hand, consumption of epoxy FA is known to increase coronary heart disease (6). We report here on the acute toxicity and a 13-wk feeding trial of amrette in rats.

EXPERIMENTAL PROCEDURES

The ambrette seed oil used for studies is obtained by solvent extraction of ambrette seed flakes with hexane, as described earlier (4). Ambrette seeds are first extracted without grinding with methanol with stirring to remove the fragrance contained in the outer seed layer. The seeds are then dried to remove the adhering methanol and flaked in a roller-type flaking machine without any treatment. The flakes are then extracted twice with hexane by simple percolation in the cold to obtain 14–15% oil (95%). Refined groundnut oil manufactured by Hindustan Lever Ltd. (Mumbai, India) is used as control. Animal experiments are carried out in the Biochemistry Department of Hamdard University (HU) (New Delhi, India). Wistar albino rats that are bred and maintained in the HU animal house are used for studies; animal studies were approved by the Ethical Committee, Government of India. Safety and toxicological evaluations of the oil are carried out on rats as per literature procedures (8,9) by performing acute oral toxicity limit tests to assess its acute toxicity potential and a 13-week feeding study to assess its safety for use as edible oil.

Physicochemical characteristics. The physicochemical characteristics of the oils were determined as per literature methods (10). Groundnut oil FAME were prepared by refluxing the oil with methanol and sulfuric acid following the literature procedure (11). Epoxy and cyclopropene groups cleave in acid medium and hence the FAME of ambrette oil were prepared by saponification of the oil, followed by esterification of the isolated FA with diazomethane. GC of FAME was carried out on a Shimadzu GC-17A gas chromatograph fitted with a 30 m × 0.25 mm capillary column coated with a 0.25-μm thick film of SUPELCOWAX-10 (Supelco, Bellefonte, PA), an FID detector, and

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TABLE 1
Physicochemical Characteristics and FA Composition of SEAO and GNO[a]

	SEAO	GNO
Iodine value	93	97
FFA (%)	1.1	0.2
Unsaponifiable matter (%)	1.5	0.5
FA		
16:0 Palmitic (%)	26.4	12.8
16:1 Palmitoleic (%)		1.4
18:0 Stearic (%)	Trace	2.1
18:0 (epoxy) (%)	0.8	ND
19:0 (Dihydrosterculic) (%)	1.6	ND
18:1 Oleic (%)	32.8	48.8
18:2 Linoleic (%)	37.4	29.5
20:0 Archidic (%)	ND	4.0
22:0 Behenic (%)	Trace	1.3
22:1 Erucic (%)	0.9	Trace
Total SFA (%)	26.4	20.2
Total MUFA (%)	33.7	50.2
Total PUFA (%)	37.4	29.5

[a]SEAO, solvent-extracted ambrette seed oil; GNO, groundnut oil; MUFA, monounsaturated FA; SFA, saturated FA; ND, not detected; Trace, detected in GC/MS but not on GC (<0.1%).

a CR-6A data processor (Shimadzu). The GC analysis was carried out following a temperature program: initial temperature of 160°C; temperature ramped at 2°C/min to 230°C, then at 4°C/min to 250°C, hold for 5 min (total run time 45 min). The samples were then analyzed following the same program on a Shimadzu QP 5000 gas chromatograph/mass spectrometer, and the peaks were identified by comparison of their mass spectra with spectra available in the NIST library and by retention order. Quantification of peaks in the gas chromatogram was done by a peak normalization method assuming equal detector response for all the components. FA compositions of ambrette seed oil and the groundnut oil used are given in Table 1.

Acute oral toxicity limit test. Two-month-old albino rats weighing about 180 g each were distributed in four groups of six each (three male and three female) and starved overnight. The animals were administered solvent-extracted ambrette seed oil (SEAO) orally at 15 and 30 mL per kg body weight. Any adverse reaction or mortality was recorded, and the surviving animals were observed for 2 wk on normal diet (i.e., the cereal diet used for breeding and maintenance of the animals). The weight gain of the animals was noted, and at the end of 2 wk the animals were sacrificed and the organ weights of liver, spleen, and kidneys were recorded. Observations of weight gain and of organs are given in Table 2.

Feeding study of 13 wk in rats. Albino rats (10 male and 10 female), 25–35 d old, were distributed in two groups of 10 each (5 male and 5 female) and were housed individually in wire net cages and allowed feed and water *ad libitum*. One group was kept on the control diet containing 10% groundnut oil (GNO) as the source of dietary fat. The other group received a diet containing 10% SEAO. The composition of the diets is given in Table 3. The animals were fed the experimental diets for 13 wk. Feed intakes and body weights were recorded daily (Fig. 1). The average weight gains, food intakes, and food efficiency ratios are presented in Table 4; and relative weights of liver, kidney, heart, spleen, lung, and testes at the end of the 13-wk period are given in Table 5.

Fat digestibility. The animals were placed in individual metabolic cages on the 30th, 55th, and 80th day and feces were collected. Fat excreted in the feces was estimated as per the literature procedure (12). Feces from the control group of rats on the same diets without added oil but with the same mass of added sugar on the day preceding the collection of feces were also analyzed to determine metabolic fat. The difference between the total fat excreted and the metabolic fat gives the amount of undigested fat, from which percentage digestibility is calculated for the test oil and control GNO. These data are presented in Table 6.

Hematology and histopathology. The animals were sacrificed at the end of 13 wk and blood was collected. Hematological and biochemical analyses were carried out as per the standard procedures. Hemoglobin and blood cell counts were carried out by the Hunter–Bumford method (13). Blood glucose was estimated by Somogyi's method (14); serum protein by biuret test (15); serum cholesterol according to Abel and Brodie (16); serum TG colorimetrically (17); transaminases by Reitman and Frankel's method (18); and alkaline phosphatase by Kind and King's procedure (19). These data were analyzed statistically using Student's *t*-test and presented in Table 7. Liver, kidney, spleen, heart, lungs, and testes were weighed, and the histology of these organs was carried out.

TABLE 2
Results of Acute Oral Toxicity Limit Test[a]

Oil tested	Initial body weight (g)	Final body weight (g)	Body wt gain (g)	Wt. of organ (g per 100 g body weight)		
				Liver	Kidney	Spleen
Male rats						
GNO, 15 mL	180 ± 4	198 ± 6	18 ± 5	3.1 ± 0.2	0.62 ± 0.05	0.21 ± 0.02
GNO, 30 mL	182 ± 4	196 ± 4	16 ± 4	3.3 ± 0.2	0.58 ± 0.04	0.23 ± 0.03
SEAO, 15 mL	176 ± 6	196 ± 5	20 ± 3	3.1 ± 0.1	0.52 ± 0.06	0.24 ± 0.03
SEAO, 30 mL	176 ± 4	194 ± 4	18 ± 2	3.25 ± 0.1	0.60 ± 0.05	0.20 ± 0.05
Female rats						
GNO, 15 mL	176 ± 3	189 ± 4	13 ± 4	3.0 ± 0.2	0.65 ± 0.05	0.20 ± 0.03
GNO, 30 mL	176 ± 5	194 ± 4	15 ± 4	3.1 ± 0.3	0.55 ± 0.04	0.23 ± 0.04
SEAO, 15 mL	176 ± 4	188 ± 4	12 ± 4	2.9 ± 0.1	0.56 ± 0.05	0.21 ± 0.03
SEAO, 30 mL	180 ± 3	193 ± 4	13 ± 4	3.2 ± 0.2	0.60 ± 0.08	0.22 ± 0.06

[a]Values are given as mean ± SD; $n = 3$. For abbreviations see Table 1.

TABLE 3
Composition of the Diets[a] (g/100 g)

	GNO	SEAO
Casein	15	15
Salt mixture	4	4
Groundnut oil	10	—
SEAO	—	10
Cellulose	6	6
Starch	65	65
Vitamins	[b]	[b]

[a]For abbreviations see Table 1.
[b]100 g diet contained thiamine HCl 0.5 mg, riboflavin 6 mg, pyridoxine HCl 0.3 mg, pantothenic acid 2.7 mg, nicotinic acid 54 mg, choline chloride 368 mg, biotin 20 mg, vitamin B_{12} 3 mg, inositol 22 mg, folic acid 1.5 mg, *p*-amino benzoic acid 10 mg, cysteine 15 mg, ascorbic acid 0.5 mg added in starch.

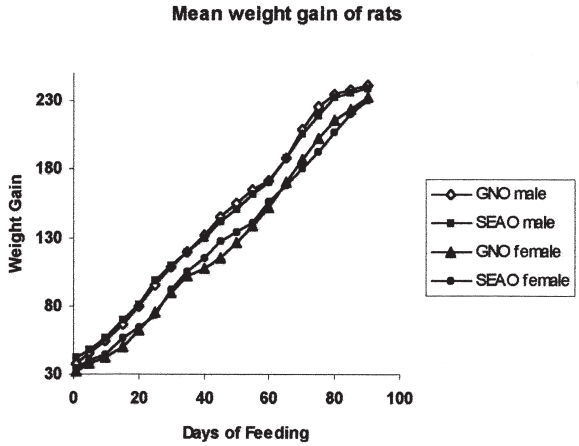

FIG. 1. Mean weight gain of rats fed with diet containing 10% groundnut oil (GNO)/solvent-extracted ambrette seed oil (SEAO).

RESULTS AND DISCUSSION

The physicochemical characteristics given in Table 1 show that SEAO somewhat resembles GNO. The ratios of SFA, MUFA, and PUFA are close to the ratios recommended by WHO for prevention of chronic diseases. Unlike earlier reports (20), epoxy and cyclopropane acids are present to a small extent; these are absent in GNO. Administration of 15 and 30 mL of GNO or SEAO/kg body weight in a single dose did not produce any mortality. The organs did not show any abnormalities, their weights being comparable; nor did they show any microscopic changes. In the 13-wk feeding study, the male and female animals did not show any significant difference in body weight gain (Fig. 1), and there was no mortality in either of the groups. The food efficiency ratio also compared well between the two groups, although a slight excess in food consumption in the case of SEAO was observed (Table 4). There was no significant difference in the organ weights between the two groups (Table 5). The quantity of feces excreted by the group and the residual fat content of the animals fed SEAO were comparable with those receiving the diet containing GNO. Absorption, or digestibility, of fat also compared very well with GNO and was about 96% (Table 6). Hematological analyses did not show any remarkable differences in the hemoglobin or cell counts between the two groups of animals. Biochemical analyses of the serum also did not show any marked difference in the blood glucose, serum cholesterol, TG, protein, urea, and alkaline phosphatase and serum transaminases. Microscopic examination (data not shown) of liver, kidney, spleen, heart, testes, ovary, and lung of all the animals showed no abnormal histopathological lesions, indicating no deleterious effects of SEAO.

The study has demonstrated that solvent-extracted ambrette seed oil is safe to use and could be considered for edible purposes. However, refining, bleaching, and deodorizing would make it more acceptable.

TABLE 4
Weight Gain, Food Intake, and Food Efficiency Ratio[a]

	Male		Female	
	GNO	SEAO	GNO	SEAO
Initial body weight (g)	38 ± 5	42 ± 5	32 ± 5	34 ± 5
Final body weight (g)	241 ± 8	239 ± 11	232 ± 17	230 ± 9
Body weight gain (g)	203 ± 18	197 ± 29	190 ± 25	196 ± 35
Food intake[b] (g)	1105 ± 25	1140 ± 45	1020 ± 32	1105 ± 28
Food efficiency ratio[c]	0.183	0.173	0.186	0.177

[a]Values are given as mean ± SD; $n = 5$. For abbreviations see Table 1.
[b]Total food intake per animal over the entire 13-wk period.
[c]Calculated as (weight gain/food intake); mean of 5 animals.

TABLE 5
Mean Relative Organ Weights Expressed as g/100 g Body Weight

	Liver[a]	Kidney[a]	Heart[a]	Spleen[a]	Lungs[a]	Testes[b]
GNO	3.3 ± 0.5	0.53 ± 0.05	0.32 ± 0.04	0.20 ± 0.06	1.01 ± 0.12	0.94 ± 0.14
SEAO	3.5 ± 0.4	0.60 ± 0.07	0.29 ± 0.03	0.23 ± 0.05	1.22 ± 0.18	1.00 ± 0.12

[a]Mean ± SD of 20 animals: 10 male and 10 female.
[b]Mean ± SD of 5 male animals in each group. For abbreviations see Table 1.

TABLE 6
Analysis of Fat in the Feces and Fat Digestibility

Feed containing 10% test oil/fat (g)	Male rats				Female rats			
	Fat intake (g)	Wt. of excreta (g)	Fat content (mg)	% Digestion[a]	Fat intake (g)	Wt. of excreta (g)	Fat content (mg)	% Digestion[a]
Fat-free feed								
30th d	Nil	3.7	42		Nil	3.2	37	
55th d		4.2	49			4.0	43	
80th d		4.8	53			4.6	50	
GNO								
30th d	2.25	5.5	143	95.5	2.18	3.8	118	96.3
55th d	2.78	6.0	162	96.0	2.47	4.7	151	95.6
80th d	3.48	6.2	192	95.9	2.80	4.9	176	95.5
SEAO								
30th d	2.30	4.8	120	96.7	2.21	3.5	98	97.2
55th d	2.79	5.4	146	96.6	2.50	4.6	129	96.6
80th d	3.21	6.0	180	96.1	2.70	5.1	158	96.1

[a]Calculated as % digestion = fat intake – (fat excreted – metabolic fat)/fat intake × 100. For abbreviations see Table 1.

TABLE 7
Hematological and Biochemical Analysis[a]

Test	GNO	SEAO
HGB (g/dL)	14.2 ± 1.2	13.7 ± 0.83
RBC (mL/mm^3)	6.98 ± 0.2	6.84 ± 0.23
WBC (mL/mm^3)	7260 ± 380	8200 ± 460
Blood glucose (mg/dL)	96.0 ± 8.0	98.0 ± 7.0
Serum protein (g/dL)	7.7 ± 0.4	7.2 ± 0.5
Serum cholesterol (mg/dL)	93.0 ± 7.0	87.0 ± 10.0
Serum TG (mg/dL)	172.0 ± 26.0	180.0 ± 32.0
Serum urea (mg/dL)	40 ± 8.0	45.0 ± 6.0
Serum alkaline phosphatase (KA units[b]/dL)	6.3 ± 0.9	8.0 ± 0.6
SGOT (IU/L)	30.0 ± 3.0	31.0 ± 5.0
SGPT (IU/L)	12.0 ± 2.0	14.0 ± 2.0
HDL cholesterol (mg/dL)	30.0 ± 2.3	25 ± 2.0
VLDL	5.0 ± 0.6	5.8 ± 0.8

[a]Mean ± SD of 10 animals. HGB, hemoglobin; RBC, red blood cells; WBC, white blood cells; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; for other abbreviations see Table 1.
[b]1 KA unit/dL = 7.1 IU/L.

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