Nutritional and Toxicological Evaluation of *Hibiscus sabdariffa* Oil and *Cleome viscosa* Oil

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ABSTRACT

Mesta seed oil (*Hibiscus sabdariffa*), like cottonseed oil, contains cyclopropenoid fatty acids (2.9%) and epoxy fatty acids (2.6%) in addition to normal fatty acids found in vegetable oils. *Cleome viscosa* (Capparidaceae) seed oil is rich in linoleic acid (70%) and free from any abnormal chemical constituents. Nutritional and toxicological evaluations of these two oils were done by multigeneration breeding studies by feeding the respective oils and groundnut oil as control at 10% level in a 20% protein diet with adequate vitamins and minerals. These studies revealed that rats fed mesta oil had inferior growth and reproductive performance and also had altered liver metabolism. Rats fed *C. viscosa* oil did not show any abnormal growth or reproductive performance or altered liver lipid levels. Thus, these studies indicate that raw or refined mesta oil may not be suitable for human consumption whereas *C. viscosa* oil can be used safely by humans.

INTRODUCTION

Oils and fats obtained from unconventional sources are used for human consumption in limited areas where they are grown and are known as minor oils. Localized shortages of traditional edible oils (1) have focused attention on these oils in recent times (2). The available minor oilseed potential of India (3) includes castor (*Ricinus Communis*), linseed (*Linum usitatisum*), neem (*Azadirachta indica*), kokum (*Garcinica indica*), Kusum (*Scheleichera trijuga*), mesta (*Hibiscus sabdariffa, H. cannabinus*), mango (*Mangiferra indica*), Dhupa (*Vateria indica*), Sal (*Shorea robusta*), mowrah (*Bassia lalifolia*) and phulwarah (*Madhuca butyraceae*). These oils, however, need to be evaluated for their chemical, nutritional and toxicological properties before they can be recommended as safe for human consumption. Such studies would also help to indicate the chemical modifications necessary to convert these oils into edible grades or into nonedible grades for use in cosmetic, pharmaceutical, soap and textile industries.

H. sabdariffa and *H. cannabinus* (4), commercially known as mesta (ambadi or ambari in India), are grown primarily as fiber crops. The leaves are edible and the seed contains about 20% oil which is also consumed by certain rural populations. The annual production of mesta seed oil in India is estimated to be 13,000 tons (5). The seed oils of the plants belonging to the Malvaceae family contain some unusual fatty acids known as cyclopropenoid fatty acids (CPFA) and epoxy fatty acids (6). Mesta oil, as well as cottonseed oil, are also known to contain these abnormal fatty acids (4). The CPFA, generally sterculic (C_{19}) and malvalic (C_{18}) acids are known to be toxic (7,8) and cocarcinogenic (9). It was, therefore, considered necessary to test the safety of these oils for human consumption. This paper reports the toxicological, nutritional and chemical evaluation of mesta seed oil which contains 2.9% CPFA and 2.6% epoxy fatty acid.

Cleome viscosa (Capparidacea) grows in the wild all over the barren lands of India and known as wild mustard (10). The seeds, which resemble mustard seeds (11), and the whole plant are consumed by certain populations (12). Preliminary analyses of the oil (13) and the nutritive value of the seed cake (14) have been reported from these laboratories. The seed had 26% oil which is rich in linoleic acid (70%) and apparently has no abnormal chemical constituent. Short-term feeding trials of the oil, using rats, have shown that the oil is nontoxic and has good nutritional quality (13). Now we are reporting the results of long-term toxicological studies in rats by a multigeneration breeding technique.

EXPERIMENTAL PROCEDURES

Mesta oil was obtained in bulk as a pooled sample from the Oil Technological Institute, Ananthapur (A.P.), India. The expeller oil was refined on a laboratory scale according to the method recommended by the AOAC (15). *C. viscosa* seeds were collected from local fields and the oil was obtained by solvent extraction in a pilot plant at the Oil Technological Institute, Ananthapur (A.P.), India.

Physicochemical constants were determined and fatty acid analysis was carried out by conventional methods (gas liquid chromatograph Model 650, Aerograph) using a 15% DEGS column on Chromosorb W (45-60 mesh) and flame ionization detector. Groundnut oil was used as the standard. Separation was done isothermally at 200 C and peak areas were calculated by triangulation. CPFA content was determined by the Halphen test (16) and quantitated by hydrobromic acid titration at 3 C and 55 C as described by Harris (17). The epoxy fatty acid content was determined at 3 C and the infrared (IR) spectrum was taken using Beckman Model 221 IR spectrophotometer in KBr disc. Thin layer chromatography (TLC) was done on silver nitrate-impregnated plates using 30% ether/hexane. Reverse-phase TLC was done on silicone-impregnated plates using acetonitrile/acetic acid/water (70:10:20, v/v), spraying with 20% perchloric acid and charring at 20 C. *Sterculia foetida* and *Vernonia anthelamentica* oils were used as standards.

Toxicological and Nutritional Evaluation

Sixty weanling rats (F_0) of Wistar strain were divided into equal groups of 15 males and 15 females each and were fed ad libitum a diet containing either 10% mesta oil or 10% groundnut oil, 20% protein and adequate vitamins and minerals. Similar protocol was followed for *C. viscosa* oil. Body weights and food intake of the animals were recorded at weekly intervals and growth performance and feed efficiency ratios (FER), which represent the weight gain for unit intake of food, were calculated. Oil absorption studies were done by estimating the oil intake and oil excreted through urine and feces. F_0 rats gave rise to the pups F_{1a} and F_{1b}; F_{1b} rats were used for propagation. F_{1b}, in turn, gave rise to pups F_{2a} and F_{2b} and, similarly, F_{2b} rats gave rise to F_{3a} and F_{3b} pups (Fig. 1).

At the end of 15 weeks, when optimal reproductive performance is expected in rats, 10 males and 10 females were allowed to mate. At 2 to 3 weeks, pregnancy was confirmed by abdominal examination and also by gain in weight. Females were separated and caged individually until delivery, when F_{1a} generation pups were obtained. The rest of

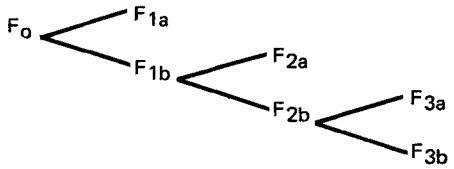

FIG. 1. Propagation of rats fed experimental diets.

the animals were continued on the diet up to 22 weeks and then sacrificed. Liver, kidney, spleen, heart, testis or ovary were weighed and processed by conventional methods of histopathological examination. Toxicological evaluation was done by multigeneration breeding studies by which the cumulative effect of the test substance can be detected (18,19). Reproductive parameters studied were: (a) percentage of conception = no. of females conceived/no. of females mated × 100; (b) average litter size; (c) average birth weight; (d) average weanling weight; and (e) percentage preweanling, where mortality = no. of pups that died from birth to weanling/no. of pups born × 100.

The young ones (F_{1a}) constituting first generation pups were separated and sacrificed on day 21. The organs were weighed and subjected to histopathological examination. The same parents (F_O) were introduced once again for mating one week after the pups (F_{1a}) were weaned. Mating, generation, lactation and weaning were followed as already described when F_{1b} pups were obtained. The parents (F_O) were then sacrificed, organs were weighed and histopathologies of various tissues were studied as done earlier. F_{1b} pups were used for breeding the second generation. They were fed the groundnut oil, mesta oil or *Cleome* oil diets for 15 weeks and mated as described earlier to get F_{2a} and F_{2b}; F_{2b}, in turn, served as parents of F_{3a} and F_{3b}, which are third-generation pups. After the F_{3b} pups were weaned, blood and livers of the parents (F_{2b}) were collected and sacrificed. Total lipids, cholesterol (20) and triglycerides (21) were estimated. At sacrifice, the various organs were weighed and values were expressed as percentage body weight of the animal. These tissues were subjected to histopathological examination. Statistical analysis of the data was done by Students t-test.

RESULTS AND DISCUSSION

Mesta Oil

Chemical analysis. The physicochemical parameters of mesta seed oil used in this study and the fatty acid composition obtained by gas liquid chromatography (GLC) are presented in Table I. Ahmad et al. (22) reported 35.2% pamitic and 14.6% linoleic acid in mesta oil whereas Mohiuddin and Zaidi (23) reported complete absence of palmitic acid and 44.4% linoleic acid. Cornelius et al. (24) and Subbaram et al. (25), on the other hand, reported 18.6 and 19.0% palmitic acid and 38.4 and 46.0% linoleic acid, respectively. The values obtained in the present study (20.6% palmitic acid and 35.3% linoleic acid) resemble very closely the latter values. The differences noticed in these various studies may be attributable to varietal differences and differences in agronomic conditions under which the seeds are grown. The refined mesta oil used in this study contained 5.5% of HBr reacting substances. The oxirane ring oxygen accounted for 2.6% and the remaining 2.9% for CPFA. IR υ max 1,008 cm^{-1} for cyclopropene moiety was obtained using a KBr disc.

Nutritional evaluation. The growth rate and the FER in both the control and experimental groups of animals were calculated up to 22 weeks. Results are presented in Table II. Male rats of the experimental group gained significantly less weight than controls in the F_O generation. In the F_1 and F_2 generations, both males and females showed significantly less gain in weight, although FER of the groups over three generations were similar. Mesta oil was absorbed to 92% and groundnut oil to 94%.

Toxicological evaluation. Results of the reproductive performance of the rats are presented in Table III. Data on the first mating of the first generation rats were not recorded due to unsatisfactory results, as the optimal mating time was delayed beyond 22 weeks, instead of 15 weeks, due to unforeseen reasons. The subsequent matings were done at the optimal time. The percentage preweanling mortality of pups F_{3b} of the group of rats fed mesta oil was significantly high compared to the corresponding controls (100% with mesta oil and 64.6% in the control group). This may be due to severe summer heat, as similar deaths were noticed in the

TABLE I

Physicochemical Constants of Mesta Oil and *Cleome* Oil

	Mesta	Cleome[a]
A: Oil constants		
Unsaponifiable fraction	1.0%	2.0%
Saponification value	188	199
Acid value	0.1%	12.6%
Iodine value	101.0	138.2
Refractive index	1.47	1.48
Thioglucosinolates	–	0.126 mg %
Phytosterols	–	1.37 mg %
B: Unusual acids		
Epoxy acid	2.6%	–
Cyclopropenoid fatty acids	2.9%	–
(IR υ max KBr)	1,008 cm^{-1} (cyclopropene moiety)	
C: Fatty acids (GLC, DEGS column and FID, %)		
Myristic (14:0)	1.4	–
Palmitic (16:0)	20.6	17.2
Palmitoleic (16:1)	0.7	–
Stearic (18:0)	3.7	3.6
Oleic (18:1)	32.6	11.9
Linoleic (18:2)	35.3	67.2

[a]Average of 4 samples.

TABLE II

Growth Rate of Rats Fed GNO/CVO/MO over Three Generations[a]

Source of oil	F_0 Avg gain in body wt in g (at 154 days) M	F_0 Avg gain in body wt in g (at 154 days) F	F_0 FER	F_{1b} Avg gain in body wt in g (at 100 days) M	F_{1b} Avg gain in body wt in g (at 100 days) F	F_{1b} FER	F_{2b} Avg gain in body wt in g (at 100 days) M	F_{2b} Avg gain in body wt in g (at 100 days) F	F_{2b} FER
Mesta oil (MO)	216.6 ± 14.24[b] (12)	103.2 ± 11.3 (12)	19.0 ± 4.20	120.2 ± 10.52 (7)	87.3 ± 6.71 (6)	23.7 ± 6.82	128.6 ± 11.66 (12)	92.8 ± 8.42 (12)	18.2 ± 4.64
Groundnut oil (GNO)	256.9 ± 16.16* (12)	126.0 ± 11.84 (12)	16.0 ± 3.82	160.5 ± 12.4* (10)	120.0 ± 11.60* (10)	22.5 ± 5.96	168.8 ± 12.64* (12)	126.4 ± 12.84** (12)	19.4 ± 4.12
Cleome viscosa oil (CVO)	228.66 ± 16.26 (12)	120.16 ± 8.41 (12)	17.8 ± 3.89	161.6 ± 11.8 (11)	110.6 ± 8.4 (11)	15.22 ± 5.22	166.8 ± 12.2 (12)	119.4 ± 12.0 (12)	19.21 ± 1.98
Groundnut oil (GNO)	229.16 ± 11.97 (12)	132.83 ± 14.24 (12)	18.8 ± 5.7	160.5 ± 12.4 (12)	120.6 ± 9.6 (12)	15.44 ± 4.21	168.4 ± 11.6 (12)	126.9 ± 6.8 (12)	18.86 ± 3.12

[a]M = males; F = females; FER = feed efficiency ratio = body weight gain/food intake weight X 100. Values are mean ± SEM. Figures in parentheses indicate number of animals.
[b]Levels of significance with respect to GNO group: *$p<0.01$; **$p<0.001$.

TABLE III

Reproductive Performance of Rats (F_{1b} and F_{2b}) Fed Mesta Oil, *Cleome viscosa* Oil and Groundnut Oil

Generations	Oil source	No. of animals mated	Conception (%)	Avg litter size	Avg birth wt (g)	Avg weanling wt (g) at 21 days	Preweanling mortality (%)	No. of days taken for delivery from the date of mating
F_{1b} adults								
1st mating (F_{2a} pups)	Mesta	6M + 6F[a]	66.2	5.5 ± 1.5[b]	5.1 ± 1.05	19.0 ± 0.98	18.0	26.2 ± 0.34
	Cleome	6M + 6F	100.0	8.59 ± 1.2	5.7 ± 0.6	20.4 ± 1.2	9.3	24.7 ± 0.23
	Groundnut	6M + 6F	100.0	8.6 ± 0.69	4.7 ± 1.3	18.7 ± 1.46	13.3	28.7 ± 0.4
2nd mating (F_{2b} pups)	Mesta	6M + 6F	100.0	7.3 ± 1.08	5.8 ± 0.25	19.8 ± 12.78	40.0**	24.5 ± 0.21
	Cleome	6M + 6F	100.0	7.8 ± 0.97	5.7 ± 0.18	21.2 ± 11.14	21.6	27.0 ± 0.4
	Groundnut	6M + 6F	85.2	8.0 ± 0.67	5.0 ± 0.17	31.6 ± 10.5	9.3	24.8 ± 0.33
F_{2b} adults								
1st mating (F_{3a} pups)	Mesta	12M + 12F	100.0	4.5 ± 5.9	4.7 ± 0.2	21.6 ± 1.3	62.9*	26.8 ± 0.60
	Cleome	12M + 12F	100.0	7.1 ± 0.63	4.9 ± 0.6	21.2 ± 7.1	21.6	25.5 ± 0.8
	Groundnut	12M + 12F	100.0	5.7 ± 4.0	5.0 ± 0.7	19.3 ± 0.8	19.2	25.8 ± 0.65
2nd mating (F_{3b} pups)	Mesta	12M + 12F	100.0	6.6 ± 6.2	4.6 ± 0.11	—	100*	28.8 ± 0.54
	Cleome	12M + 12F	100.0	7.7 ± 0.66	4.6 ± 1.2	16.9 ± 1.0	64.6	30.0 ± 0.73
	Groundnut	12M + 12F	100.0	6.9 ± 0.8	4.9 ± 0.72	16.9 ± 2.4	70.4	25.2 ± 0.93

[a]M = males, F = females.
[b]Values are mean ± SEM. Level of significance with respect to groundnut oil group: *$p<0.01$; **$p<0.001$.

TABLE IV

Liver Lipids of Adult Rats of F_{2b} at Sacrifice[a]

Oil source	Liver wt as % body wt	Total lipids (g/100 g of liver)	Total cholesterol (mg/100 g lipids)	Triglycerides (mg/g liver)
Mesta oil (8)	3.9 ± 3.5	13.3 ± 1.2	210.0 ± 4.8	42.3 ± 2.6
Cleome oil (8)	3.3 ± 2.7	3.5 ± 0.21	114.0 ± 3.2	24.3 ± 1.2
Groundnut oil (8)	3.5 ± 4.7	3.8 ± 0.46	200.0 ± 3.2	23.8 ± 1.8

[a]Values are mean ± SEM. Numbers in parentheses indicate the number of animals.

stock colony pups (26). No cogenital abnormalities were noticed in the pups of any generation.

Concentrations of total lipid cholesterol and triglycerides in livers of both groups of F_{2b} adult rats were determined and are presented in Table IV. There was a four-fold increase in total lipids in the livers of the animals fed mesta oil over the control group of rats and the triglycerides were almost double. A similar result was reported by Lee et al. (27) in rainbow trout. There were, however, no differences in the cholesterol values in either of the two groups. Increased liver weight, total lipids and triglycerides in the mesta oil group of rats indicated altered lipid metabolism. Serum cholesterol, total lipids and triglycerides were not significantly different in the two groups.

Organ weights of the rats of both the groups at each time of sacrifice, expressed as percentage of body weight, are presented in Table V. Weights of male rats fed mesta oil were significantly higher than the corresponding values of the control group.

Histopathological examination did not reveal any significant abnormalities in the rats fed mesta oil.

There are many reports about the adverse effects of feeding pure CPFA to rats and other animal species (5). Morphological changes in the liver due to lipid accumulation, decreased liver protein levels and reduction in the activity of soluble dehydrogenases were described by Taylor et al. (28). Other abnormalities were inhibition of fatty acyl desaturases, reduction in codeine demethylase activity and aortic atherosclerosis in rabbits fed CPFA (29,30). CPFA acted as a cocarcinogen in rainbow trout fed aflatoxin (8). Pink discoloration in hen's egg and miscibility of the yolk in the egg have also been reported (31). CPFA inhibit the desaturases and, hence, the stearic → oleic → linoleic → arachidonic pathway is inhibited (32).

Thus, the rats fed 10% mesta oil exhibited inferior growth performance, inferior reproductive performance and altered lipid metabolism in the livers of rats over three generations. These probably are due to the CPFA and epoxy fatty acids present in mesta oil. Similar results have been reported (33) after feeding 3% *S. foetida* oil for six months. *S. foetida* oil contains 90% of CPFA, and decreased mating behavior, low fertility and low fetal and newborn viability were observed with the use of this fat in the diet. Similar observations have been made in rats fed diets containing either 1 or 2% pure CPFA. These changes were interpreted as due to high perinatal and prenatal mortality and not due to any teratogenic effects. However, the implication of increased organ weights of the male rats fed mesta oil in the present study is not well understood. Nixon and coworkers (7) reported higher liver weights in rats fed pure CPFA. There is no mention of sex differences in their studies.

Results of the present study indicate that it may not be desirable to use mesta oil as a major dietary source of fat for humans. However, manufacture of hydrogenated vegetable oils involves deodorization and hydrogenation, bringing about the conversion of CPFA to cyclopropane moieties

TABLE V

Organ Weights of Adult Rats Sacrificed during Three Generations (Expressed as % Body Weight)

Organs	F_0				F_{1b}				F_{2b}			
	Mesta oil		Groundnut oil		Mesta oil		Groundnut oil		Mesta oil		Groundnut oil	
	M[a] (12)	F (12)	M (12)	F (12)	M (7)	F (6)	M (10)	F (10)	M (12)	F (12)	M (12)	F (12)
Liver	3.84 ± 0.23*	4.26 ± 0.132	3.0 ± 0.04	3.85 ± 0.52	4.24 ± 0.66*	4.38 ± 0.24*	3.40 ± 0.83	3.69 ± 0.27	3.58 ± 0.22	3.86 ± 0.52*	3.51 ± 0.511	3.15 ± 0.20
Kidney	0.74 ± 0.071	0.77 ± 0.045	0.47 ± 0.15	0.76 ± 0.06	1.02 ± 0.17*	1.06 ± 0.31	0.68 ± 0.61	0.77 ± 0.085	0.59 ± 0.04	0.68 ± 0.09**	0.58 ± 0.04	0.51 ± 0.04
Spleen	0.23 ± 0.01**	0.22 ± 0.01	0.06	0.18 ± 0.26	0.29 ± 0.06	0.31 ± 0.01*	0.24 ± 0.07	0.23 ± 0.04	0.20 ± 0.04	0.25 ± 0.03**	0.19 ± 0.03	0.19 ± 0.02
Heart	0.35 ± 0.03**	0.34 ± 0.12	0.2 ± 0.01	0.31 ± 0.13	0.37 ± 0.08	0.43 ± 0.08	0.33 ± 0.09	0.41 ± 0.01	0.29 ± 0.04	0.30 ± 0.03	0.29 ± 0.05	0.33 ± 0.02
Testis/ovary	0.97 ± 0.07	0.76 ± 0.07	0.93 ± 0.13	0.67 ± 0.07	1.13 ± 0.01**	0.20	0.07	0.20 ± 0.15	0.91 ± 0.04	0.26 ± 0.04	0.90 ± 0.10	0.19 ± 0.08

[a]M = males; F = females. Values are mean ± SEM. Levels of significance with respect to groundnut oil group: *$p<0.05$; **$p<0.01$. Numbers in parentheses indicate the number of animals.

which are less chemically reactive. Inactivated CPFA containing mesta oil toxicity may have to be carefully studied. Destruction of CPFA in cottonseed oil by various methods has been reported (34). The adverse biological effects of CPFA were also shown to be minimized or totally absent in rats fed inactivated CPFA (35). Whether mesta oil could be introduced in the manufacture of hydrogenated fats in a small proportion along with other oils needs to be examined.

Cleome viscosa Oil

Physicochemical characteristics and the fatty acid profile of the *C. viscosa* oil as determined by GLC are presented in Table I. Refining of the oil according to the AOAC method (15) diminished the visible color, and the acid value was also reduced to 0.5-1%. FER and growth performance of the experimental animals are presented in Table II. Table III represents the reproductive performance of the rats over three generations.

The experimental group of animals did not show any significant difference in organ weights when compared with the control group of animals receiving groundnut oil. Histopathological examination of the various organs did not show any abnormalities in either group over three generations. Table IV gives the data on the total lipids, triglycerides and total cholesterol of the livers of the adult rats of F_{2b} at the time of their sacrifice.

Physicochemical constants of the oil and the fatty acid profile (Table I) suggested that the chemical composition of *C. viscosa* oil is similar to sunflower and safflower oils. No abnormal chemical constituents were detected in the unsaponifiable matter of the oil. However, a white solid material separated out from the petroleum ether extract of the oil. A similar compound has been isolated and chemically investigated by Ray et al. (36) and designated as cleomiscosin A, a coumarino-lignoid compound of *C. viscosa* seeds. However, its physiological activity is yet to be evaluated. The oil has a deep green color upon visual comparison with other oils. This visible color of the oil may be due to high tocopherol content. Laboratory-scale refining reduced the color to a lighter shade. The high unsaponifiable matter, the high acid value and dark visible color are some of the undesirable factors which can be easily removed by bleaching, deodorization and refining.

A small-scale organolepic trial done with human volunteers using foods fried in this oil suggested that the oil is acceptable. Nutritional quality of the oil as judged by FER and growth studies over three generations was satisfactory and comparable to that of groundnut oil. Thus, the chemical and nutritional qualities of the oil appear to be satisfactory.

Toxicological evaluation by multigeneration breeding studies revealed that there were no congenital abnormalities seen in the pups. Reproductive performance of both groups over three generations and in both matings were comparable. Since high preweanling mortality was observed in the stock colony pups, also due to severe heat (26), this effect may not be attributable to the quality of the oil.

Liver weights of the rats fed 10% CVO and 10% GNO were comparable over three generations. Thus, the toxicological evaluation indicates that the *C. viscosa* oil is apparently safe for human consumption. The use of the oil for edible purposes is therefore recommended.

The oilseed, at present, is not grown systematically and it is suggested that the seed be domesticated and grown under proper agronomic conditions.

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