Nutritional and Toxicological Evaluation of Rubber Seed Oil

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Rubber *(Hevea brasiliensis)* seed oil (RSO) is available in India (Ca. 4500 tons per year) and is used mainly as a drying oil. The oil does not contain any unusual fatty acids, and it is a rich source of essential fatty acids $C_{18:2}$ and $C_{18:3}$ that make up 52% of its total fatty acid composition. Acute toxic potential in rats and the systemic effects and nutritional quality were assessed in a 13 week feeding study in weanling albino rats using a diet containing RSO or groundnut oil (GNO) (as the control) at a 10% level as the sole source of dietary fat. RSO did not manifest any acute toxic potential. Food consumption, growth rate and feed efficiency ratio of rats fed RSO were similar to those fed GNO. The digestibility of this oil was found to be 97%, as compared to 94% for GNO. There were no macroscopic or microscopic lesions in any of the organs which could be ascribed to the RSO incorporation in the diet. Thus the current data show that RSO could be used for edible purposes. However, it will be necessary to process the oil to achieve deodorization and to remove free fatty acids to make it organoleptically acceptable.

KEY WORDS: Fat digestibility, *Hevea brasiliensis*, nutritional and toxicological studies, rubber seed oil.

The shortage of traditional edible oils in India has stimulated the studies of nutritional and toxicological aspects of tree-born oilseeds usually referred to as minor oilseeds. Earlier studies in this laboratory carried out on sal fat in rats showed it to be innocuous and that it could be used for edible purposes (1). We also have reported a comparative evaluation of the toxicological status of some unconventional oils (2).

The rubber tree *(Hevea brasiliensis, Fam. Euphorbiaceae)* is a large tree attaining a height of 60–100 feet indigenous to Brazil that is cultivated in Southeast Asia (3). It is cultivated as a plantation crop mainly in Kerala (India), which accounts for 92% of India's production. The estimated availability of rubber seed in Kerala alone is about 33,600 tons and that of rubberseed oil (RSO) is about 4,200 tons per annum (4). The oil content of the kernel is 45–52%. The oil has a yellowish-brown color with a characteristic odor. The seeds deteriorate very rapidly after falling on the ground due to moisture and an endogenous lipase contributes to a rapid rise in free fatty acids (3). No unusual fatty acids have been reported in this oil. The oil is a rich source of essential fatty acids (5) $C_{18:2}$ and $C_{18:3}$, which amount to 51–63% of the total fatty acids. The oil is used in soap making and also as a substitute for linseed oil in paints and alkyd resins (6). Nutritional and toxicological aspects of the oil have not been reported in the literature so far. In view of this lack of scientific data it was thought worthwhile to evaluate the nutritional quality and toxicological status of RSO in a feeding study in rats. This paper reports findings on the acute toxicity and 13-week feeding studies performed on rats using RSO.

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MATERIALS AND METHODS

Materials. The RSO used in the experiment is a commercial oil of dark, brownish color. Refined groundnut oil (GNO) was used as a control. Haffkine Wistar strain albino rats that were bred and maintained in our animal house facilities were used for the studies.

Methods. The physicochemical characteristics of the oil were determined according to official AOCS methods (7). The fatty acid compositions of RSO and GNO were analyzed on their respective methyl esters using a gas liquid chromatograph (GLC) (Chemito-3800, Toshniwal Bros., Worli, Bombay, India) fitted with flame ionization detector (FID) on a column of 10% diethylene glycol succinate (DEGS) coated on Chromosorb-W. Separations were carried out isothermally at 200°C. Nutritional and toxicological evaluations of the oil were carried out in rats by performing an acute oral toxicity limit test to assess its acute toxicity potential, and a 13-week feeding study to assess its nutritional quality. (each weighing about 80 g) were distributed into two groups of four each (two male and two female) and starved overnight. The animals were administered RSO by oral intubation at 15 and 30 mL/kg body weight. Any adverse sign and mortality were recorded and the surviving animals were observed for two weeks.

Thirteen-week feeding study in rats. Twenty-four rats (12 male and 12 female), 25–30-days-old, were distributed into two groups of 12 (six male and six female) each, and were housed individually in wire net cages and allowed feed and water *ad libitum.* One of the groups received the control diet containing 10% GNO as the sole source of dietary fat. The other group received a diet containing 10% RSO. The composition of the diets is given in Table 1.

The animals were fed on the experimental diets for 13 weeks. Feed intakes and body weights were recorded daily. Fifteen days prior to sacrifice the animals were placed in individual metabolism cages, and the feces were

TABLE 1

Composition of Diets

	Gr. I	Gr. II
Casein	15	15
Salt mixture[a]	4	4
Groundnut oil[b]	10	—
Rubberseed oil[b]	—	10
Cellulose	6	6
Starch + vitamins[c]	65	65

[a] Reference 8.

[b] 325 I.U. of vitamin A acetate, 85 I.U. of vitamin D_2 and 10 mg DL α-tocopheryl acetate were dissolved in oil.

[c] Thiamine HCl 0.5 mg, riboflavin 0.6 mg, pyridoxine HCl 0.3 mg, pantothenic acid 2.7 mg, nicotinic acid 54 mg, choline chloride 368 mg, biotin 20 μg, vitamin B_{12} 3 μg, inositol 22 mg, folic acid 1.5 mg, PABA 10 mg, cystine 15 mg, and ascorbic acid 0.5 mg, were added in starch.

collected for two consecutive days. Fat digestibility was carried out according to the method described by Triscari *et al.* (9) for all the animals. The urine was tested for protein and glucose. At the end of 80 days the animals were placed individually in the animal compartment of an actophotometer (Electrolab, Bombay, India) to monitor their locomotor activity. After allowing 5 min for the animals to acclimatize to the new surroundings, the actophotometer was switched on for 10 min. Any spontaneous movement of the animal cuts off a beam of light from falling on a photocell located inside the compartment. Each such movement is recorded as a cumulative count and displayed on the panel. The number of counts is proportional to the locomotor activity of the animal, indicating whether the animal is hyperactive, normal or hypoactive.

The animals were sacrificed at the end of 13 weeks and blood was collected. Haematological and biochemical analyses were carried out as per the following procedures: Haemoglobin and blood cell counts were carried out by standard methods (10); blood glucose was estimated by Somogyi's method (11); serum protein by the Biuret test (12); serum cholesterol by Sackett's method (13); and serum triglyceride by enzymatic methods (14). The diacetylmonoxime method was used to quantitate serum urea (13); serum alkaline phosphatase as estimated using Kind and King's method (15); and serum transaminases were quantitated by King's method (13). Liver fat was estimated according to Folch's method (16). Liver, kidney, spleen, heart and testes were weighed and histology of these organs along with that of adrenals, thyroid, ovary and lungs was carried out. The quantitative data were analyzed for statistical difference using the Student's *t*-test.

RESULTS

Physicochemical characteristics. The physicochemical characteristics and fatty acid composition of RSO, along with those of GNO, are given in Table 2. These values are in agreement with those reported in the literature (5).

Acute single dose oral administration of RSO at 15 and 30 mL/kg body weight did not produce any adverse signs or mortality. The animals did not display any behavioral changes and there was no mortality in any of the groups during the 13-week feeding study. There was no difference in the locomotor activity as observed in the actophotometer (Table 3). At autopsy the animals showed no abnormalities. The organs appeared normal and showed no macroscopic changes.

Food consumption and weight gain. The male and female animals showed no significant differences in food consumption, body weight gain and FER between the two groups. The growth rate of rats is shown in Figure 1 and Table 4. Urine showed an absence of albumin and glucose. There were no significant differences in the organ weights between the two groups (Table 5).

Fat digestibility. The amount of feces excreted by the two groups did not show any significant difference, but the fat excreted in the feces of rats fed RSO was significantly lower as compared to rats fed GNO. Absorption of fat by the rats receiving GNO was 94.3% [which is similar to other reported values in the literature (17,18)] compared to 96.5% for rats fed RSO (Table 6).

Haematological and biochemical analyses. There were no significant differences in the haemoglobin and cell

TABLE 2

Physicochemical Characteristics of the Oils

	GNO	RSO
Iodine value	98	133
Free fatty acid %	0.14	13
Unsaponifiable matter %	0.43	2.1
Fatty acid % (GLC analyses)		
C_{14}	—	0.2
C_{16}	14.3	10.1
$C_{16:1}$	—	0.3
C_{18}	3.8	8.8
$C_{18:1}$	44.6	24.6
$C_{18:2}$	33.0	38.9
$C_{18:3}$	—	17.1
C_{20}	1.6	—
C_{22}	2.7	—

TABLE 3

Spontaneous Motor Activity Exhibited by Rats in the Actophotometer

Group	Mean[a] count for 10 min in actophotometer
GNO	173 ± 14
RSO	169 ± 14

[a]Mean of 12 animals ± S.E.

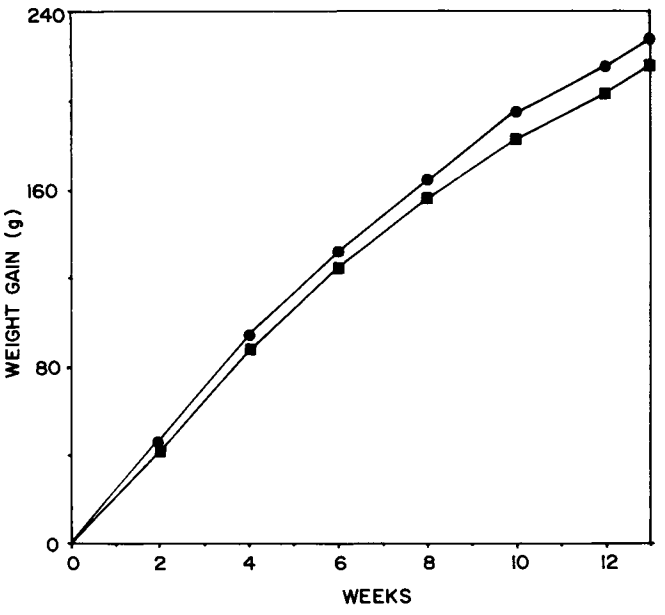

FIG. 1. Mean body weight gain of rats fed groundnut oil (■) and rubberseed oil (●) diets.

TABLE 4

Weight Gain, Food Intake and Food Efficiency of Rats[a]

	Male		Female	
	GNO	RSO	GNO	RSO
Initial body weight (g)	39 ± 2	39 ± 2	35 ± 2	35 ± 2
Final body weight (g)	308 ± 11	334 ± 25	204 ± 14	203 ± 7
Body weight gain (g)	269 ± 10	295 ± 24	169 ± 13	168 ± 7
Food intake (g)	1180 ± 29	1210 ± 54	978 ± 29	967 ± 11
Food efficiency ratio (weight gain/food intake)	0.228 ± 0.004	0.243 ± 0.012	0.173 ± 0.012	0.173 ± 0.004

Mean of six animals ± S.E.

TABLE 5

Mean Relative Organ Weights

	Liver[a]	Kidney[a]	Heart[a]	Spleen[a]	Testes[b]
GNO	3.03 ± 0.10	0.56 ± 0.03	0.28 ± 0.01	0.18 ± 0.01	0.89 ± 0.06
RSO	3.00 ± 0.11	0.56 ± 0.02	0.27 ± 0.01	0.17 ± 0.01	0.90 ± 0.08

[a]Mean of 12 animals ± S.E.

[b]Mean of six animals ± S.E.

TABLE 6

Analyses of Fat in the Feces[a]

	GNO	RSO
Dried feces excreted during 24 hr (g)	1.296 ± 0.046	1.054 ± 0.109
Fat intake during 24 hr (g)	1.167 ± 0.063	1.201 ± 0.082
Fat excreted in the feces (g)	0.067 ± 0.005	0.042 ± 0.004[b]
% Fat absorption in rats	94.26 ± 0.35	96.50 ± 0.327[c]

[a]Mean of six male + six female animals.

[b]Significantly different from GNO ($P < 0.01$).

[c]Significantly different from GNO ($P < 0.001$).

TABLE 7

Haematological and Biochemical Analyses[a]

	GNO	RSO
Haemoglobin (g/dl)	14.07 ± 0.4	14.98 ± 0.25
RBC (millions/Cu mm)	5.21 ± 0.20	5.74 ± 0.17
WBC (Cu mm)	7016 ± 211	6687 ± 197
Blood glucose (mg/dl)	99 ± 5	95 ± 4
Serum protein (g/dl)	7.8 ± 0.17	7.63 ± 0.14
Serum cholesterol (mg/dl)	96 ± 6	95 ± 5
Serum triglyceride (mg/dl)	163 ± 26	178 ± 22
Serum urea (mg/dl)	41 ± 3	46 ± 3
Serum alkaline phosphatase (KA Units/dl)	6 ± 1	5 ± 1
Serum glutamic oxalacetic transaminase (Units/L)	30 ± 2	31 ± 2
Serum glutamic pyruvic transaminase (Units/L)	12 ± 4	8 ± 4
Liver fat %	3.75 ± 0.4	3.97 ± 0.1

[a]Mean of 12 animals ± S.E.

counts between the two groups of animals. There were no significant differences in the serum analyses of protein, cholesterol, triglycerides, urea, alkaline phosphatase serum transaminases and in the liver fat (Table 7).

Histopathological observations. Microscopic examination of liver, kidney, spleen, heart, adrenal, thyroid, testis, ovary and lung of all the animals which received diets containing 10% GNO or RSO was carried out. Except for the slight presence of haemosiderin pigments in the spleen of 5 out of 12 animals fed the RSO diet, none of the animals from the two groups showed any abnormal histopathological lesions.

DISCUSSION

On the basis of the fatty acid composition and physicochemical characteristics it is reasonable to think that RSO could be used for edible purposes. RSO is comparable to rice bran oil (RBO) in terms of its free fatty acid (FFA) content, and although the linolenic acid content (ca. 17%) in RSO is high, it is much less than that present in linseed oil (26–58%). We have observed (2) that both raw linseed oil containing 51% of linolenic acid as well as raw RBO with 48% FFA did not induce any adverse biological response in a short term (six week) feeding study in rats. Rukmini *et al.* (19) also reported the innocuous nature of RBO containing 7% FFA. Rahmani-Jourdheuil and Entressangles (20) fed LSO at 10 or 20% levels to rats for 17 weeks and found a slight increase in liver weights without any increase in serum transaminases levels. Current Indian government legislation classifies LSO as an edible oil (21).

Free fatty acids present in RBO or the high linolenic acid present in linseed oil do not cause any adverse biological response and RSO, which also contains these acids, would likewise be innocuous when ingested. The chances of consuming cyanogenic glucoside present in the seed (6) is remote, as it is unlikely to get extracted into the oil from the seed.

The results of the current study are in agreement with the above hypothesis. No acute toxic potential was observed with RSO. In the 13-week feeding study there was no deleterious effect on the food consumption, growth rate or feed conversion. The RSO is found to be well absorbed by the animals. The locomotor activity of the animals was not altered. RSO ingestion did not affect the haematological or serum biochemical profile of the animals. There were no pathological-macroscopic or microscopic lesions in any of the animals, indicating the innocuous nature of the oil. It is also unlikely to have any adverse effect on the reproductive performance as the testes and ovaries of the animals showed normal histological pattern. It is also reported that rubber seeds are eaten by the people living near plantations after soaking the seeds for 24 hr in water with repeated changes and boiling (22). In conclusion, the current study has shown that, from the nutritional and toxicological aspects, RSO could be considered for edible use. However, processing to deodorize and to reduce FFA would be necessary to make it organoleptically acceptable.

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