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ORIGINAL ARTICLE

Safety evaluation of *Simarouba glauca* seed fat

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Abstract *Simarouba glauca* DC is a tree of the family *Simaroubaceae*, which grows well up to 1,000 m above sea level in all types of well-drained soils (pH 5.5 to 8.0) and in places with 250 to 2,500 mm annual rainfall. The seed oil has been extracted both by mechanical expelling and solvent extraction. The fatty acid composition and iodine value of the oil indicate that it possesses saturated (40.8–42.6%), monounsaturated (52.9–55.0%), and polyunsaturated (2.5–3.4%) fatty acid in ratios close to that of palm oil. These characteristics are suitable for its use as edible oil. Acute oral toxicity and safety evaluation in a 13-week feeding trial on albino rats showed that the oil is comparable to groundnut oil in all the parameters.

Keywords *Simarouba glauca* · *Simaroubaceae* · Fatty acid · Refined simarouba oil · GC/MS · Acute oral toxicity

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Introduction

India is one of the major vegetable oil importing countries. The improvement in living standards, increase in rising population and change in food habits, have enhanced the annual per capita oil consumption to a present value of 12 kg. India produces about 7–8 million tonnes of oil and imports about 5–6 million tonnes. Although the cultivated area under oil seeds crops is largest in the world and second only to cereal crops in the country, low productivity hampers the total oil seeds production in India.

Though oil palm provides 4 tonnes oil/ha/year, its cultivation could not be expanded because of the initial high investment, long gestation period, suitable climate, soil conditions and high water requirement of about 200 l per day per tree (Kallarackal et al. 2004). India has many regions of low rainfall with inadequate ground water sources. As most of the cultivable area is already occupied by traditional crops, only those species which can thrive in less favourable climatic conditions have to be considered for the introduction of new oil-seeds crops.

Simarouba glauca DC, commonly known as aceituno, paradise-tree or bitter wood is a medium sized (height 7–15 m) evergreen tree and produces seeds with high oil content (60%). It grows well up to 1,000 m above sea level in all types of well-drained soils (pH 5.5 to 8.0) and is found to establish in places with 250 to 2,500 mm annual rainfall and temperatures going up to 45 °C (Juyal et al. 1991). It can withstand dry and semi arid conditions, and planted in areas where no other plants of economic value can be grown. Since it can adopt very well in areas of low and scanty rainfall, it is an excellent candidate for promoting as an oilseed crop in rural and tribal areas.

Simarouba glauca was first introduced in India from El Salvador (EC-19701) by Indian Council of Agricultural

Research (ICAR), New Delhi, during 1961. Because of its easy adaptability to various soil and climatic conditions, it has received attention for large-scale plantation both for soil conservation and reforestation programs. It is estimated that in Orissa alone, a potential of about 10–12 thousand tonnes of *Simarouba* seeds exists (Pradhan 1995) and still more area is expected to be covered under the crop. More recently, large scale plantation has been taken up in Karnataka and Andhra Pradesh and other states of India.

An estimated 5–6 tonnes of seeds equivalent to about 1 tonne of oil per hectare can be obtained from *Simarouba glauca* under the present conditions of growing it for soil conservation purposes. However, by following the proper agricultural practices, the yields of seeds have been claimed to double that can produce 2 tonnes of oil per hectare (Joshi and Hiremath 2000).

Simarouba seeds do not have lipase and can be stored for more than a year. The hand broken kernels were kept for different times at 40, 50 and 60 °C in an oven and were analysed for free fatty acid (FFA), which increased marginally from 0.5% to 1.5% even after 12 h at 50 °C. However, when the kernels were left in the open, the FFA increased gradually over a period of 3 months to 6.35% (Rao et al. 2002).

Further, the kernels were found to be attacked by the larvae of the common storage pest *Corcyra cephalonica,* family *Galleridae* particularly during rainy season. However, there is no attack by the pests or fungi and the kernels remained un-damaged, when kept in air tight containers (Rao et al. 2002). We have earlier reported the physicochemical characteristics of *Simarouba* seeds and fat of Orissa origin (Sahoo et al. 2002).

Though, *Simarouba* fat is not so far consumed by local population in India, it was reported marketed in El Salvador and other Central American countries for edible purposes (Severen 1953) under the trade name Manteca vegetal "Nieve". Simarouba fat is a good substitute for hydrogenated oils and unlike the latter, has practically no trans fatty acids and has been found more than 94% digestibility in rats (Squibb et al. 1951).

Simarouba glauca is a promising edible oil seed crop, which can grow in waste lands. Though simarouba seeds contain toxic bitter quassinoids to the extent of 2% (Monseur and Motte 1983), however the oil has no bitterness. Thus quassinoids do not pass into the extracted oil because of their polar nature. However, in view of the reported acute cytotoxicity and also phytotoxicity of simarouba quassinoids (Okano et al. 1990; Patro et al. 2002; Prasad et al. 2005) and present efforts to propagate the simarouba on large scale as an alternative oil seed crop, therefore systematic safety evaluation of the oil for human consumption is warranted. We report now the results of our work on the evaluation of feeding and toxicological properties of *Simarouba glauca* seed oils.

Materials and methods

The simarouba seeds were decorticated manually and the oil from kernels was obtained by expelling the kernels in a modified commercial expeller (Jena et al. 2003); the oil was also obtained by solvent extraction with hexane of the powdered material. The expeller oil was degummed and then alkali refined to obtain refined simarouba oil (RSO) (Rao et al. 2002). The animal experiments were carried out at Biochemistry Department, Hamdard University (HU), New Delhi, India. Refined groundnut oil manufactured by Hindustan Lever Ltd. (Mumbai, India) is used as control. Albino rats bred and maintained in the HU animal house were used for studies. Animal studies were approved by the Ethical Committee, Government of India. Safety and toxicological evaluations of the oil were carried out on rats as per BIS procedure (1984) 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. All the solvents used in the experiment were of reagent grade and distilled in the laboratory before use.

Physicochemical characteristics

All the experiments were carried out in triplicate. The physicochemical characteristics of the oils were determined as per AOAC method (1970). The fatty acid composition of the oils were determined by GC-FID of their methyl esters (FAMEs), which were prepared by refluxing the oil with methanol and sulfuric acid following the literature procedure (Hammond 1993). GC analysis 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 a CR-6A data processor following a temperature program as follows: 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 on a Shimadzu QP 5000 GC/MS following the same program. The MS parameters were as follows: Ionization Voltage (EI) 70 eV, peak width 2 s, mass range 40–500 amu and detector voltage 1.5 V. The peaks were identified by comparison of their mass spectra with spectra available in the NIST library and also elution order. The percentage composition was calculated by a peak normalization method assuming equal detector response for all the compounds.

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 expeller extracted *simarouba* oil (EESO), solvent extracted simarouba oil (SESO), refined simarouba oil (RSO) orally at 15 and 30 mL per kg body weight. The adverse reaction or mortality was recorded, and the surviving animals were observed for 2 weeks 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 weeks, the animals were sacrificed and the organ weights of liver, spleen and kidneys were recorded.

Feeding study of 13 week in rats

Albino rats (20 male and 20 female), 25–35 days old, were distributed in four 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 groups have received a diet containing 10% EESO/SESO/RSO. Besides fat, the common composition of diet is casein (15%), salt mixture (4%), cellulose (6%), starch (64%) and vitamins (1%). The vitamins included thiamine HCl (0.5 mg), riboflavine (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), cysteine (15 mg) and ascorbic acid (0.5 mg). The animals were fed the experimental diets for 13 weeks. The daily food intake and body weights were recorded (Figs. 1A, B). The average weight gain, food intake, and food efficiency ratios were recorded.

Fat digestibility

The animals were placed in individual metabolic cages on the 50th and 80th day and feces were collected. Fat excreted in the feces was estimated as per the literature procedure (Folch et al. 1957). 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 the 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.

Hematology and histopathology

The animals were sacrificed at the end of 13 week and blood was collected. Hematological and biochemical analyses were carried out as per the standard procedures.

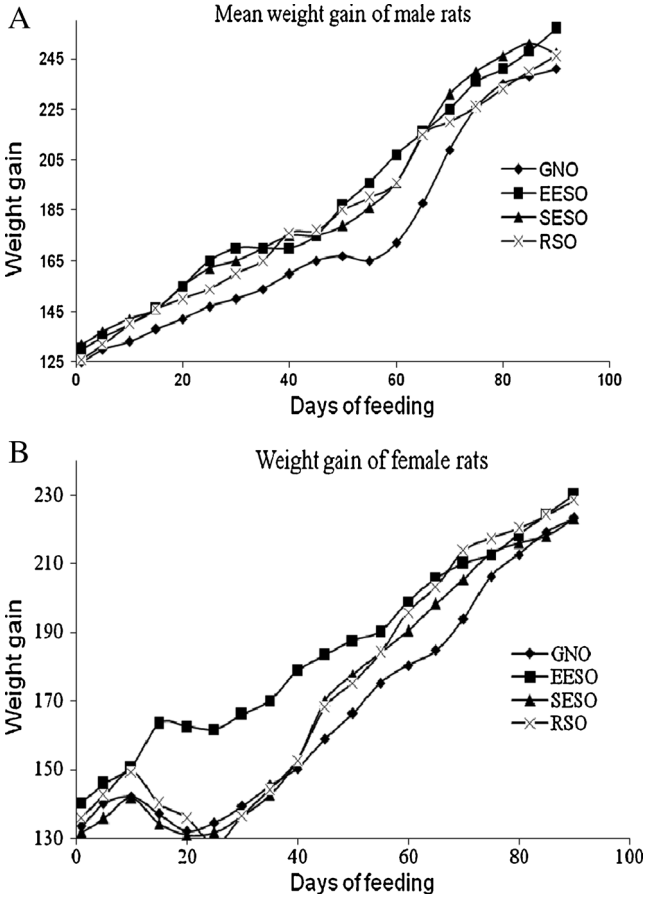

Fig. 1 Growth of (**a**) Male and (**b**) Female rats during feeding (13 week). (Composition of diet is casein (15%), salt mixture (4%), cellulose (6%), starch (64%) and vitamins (1%) along with 10% fatty oil mentioned below). GNO: ground nut oil; EESO: expeller extracted simarouba oil; SESO: solvent extracted simarouba oil; RSO: refined simarouba oil, $n=5$

Hemoglobin and blood cell counts were carried out by the Hunter-Bumford method (Hunter and Bumford 1956). Blood glucose was estimated by Somogyi's method (1952); serum protein by biuret test (King and Wooton 1964); serum cholesterol according to Abel and Brodie (1952); serum TG colorimetrically (Foster and Dunn 1973); transaminases by Reitman and Frankel's method (Reitman and Frankel 1957); and alkaline phosphatase by Kind and King's procedure (Kind and King 1954). The weights of liver, kidney, spleen, heart, lungs, and testes were taken, and the histology of these organs was carried out. The values reported are average of three replications and the results obtained were subjected to analysis of variance (ANOVA) and DUNCAN test using SPSS 10.0 software.

Results and discussion

The physicochemical characteristics of EESO, SESO, RSO and GNO are presented in Table 1(statistically significant at

Table 1 Physicochemical characteristics and Fatty acid composition of simarouba and groundnut oil

Colour, taste	EESO Light yellow-green	SESO Light yellow-green	RSO Colourless to cream yellow	GNO Colourless to cream yellow
Acid value (mg/g)	2.0±0.2[a]	1.6±0.2[b]	1.0±0.1[bc]	0.4±0.1[d]
FFA (%)	1.0±0.1[a]	0.8±0.1[b]	0.5±0.05[bc]	0.2±0.05[c]
Saponication value (mg/g)	192.3±0.5[a]	191.8±0.4[ab]	192.5±0.4[b]	190.6±0.4[c]
Iodine value (g/100 g)	52.8±0.5[a]	52.5±0.4[a]	52.3±0.3[ab]	97.0±0.3[d]
Unsaponifiable matter (%)	0.5±0.05[a]	0.4±0.04[ab]	0.2±0.03[c]	0.5±0.05[d]
Fatty acid (%)				
16:0 Palmitic	12.3±0.4	14.8±0.4	13.5±0.3	12.8±0.4
16:1 Palmitoleic	–	–	–	1.4±0.2
18:0 Stearic	27.3±0.6	26.0±0.5	27.1±0.5	2.1±0.2
18:1 Oleic	54.6±0.9	52.6±0.8	53.3±0.6	48.8±0.5
18:2 Linoleic	2.3±0.4	3.1±0.4	2.9±0.3	29.5±0.8
18:3 Linolenic	0.2±0.1	0.3±0.1	0.2±0.1	–
20:0 Arachidic	1.2±0.3	1.8±0.3	1.7±0.4	4.0±0.4
22:0 Behenic	–	–	–	1.3±0.2
22:1 Erucic	0.4±0.1	0.3±0.1	0.3±0.1	Trace (<0.1)
Total SFA	40.8	42.6	42.3	20.2
Total MUFA	55.0	52.9	53.6	50.2
Total PUFA	2.5	3.4	3.1	29.5

Data are mean values ± standard deviation. n=3, Values in the same rows followed by different letters (a–d) are significant (P<0.05)

EESO expeller extracted simarouba oil; *SESO* solvent extracted simarouba oil; *RSO* refined simarouba oil; *GNO* ground nut oil; *FFA* free fatta acids; *SFA* Saturated fatty acid, *MUFA* mono unsaturated fatty acid, *PUFA* poly unsaturated fatty acid

P<0.05). There were nine fatty acids identified in GC-FID and GC/MS analysis of EESO, SESO and RSO. The major fatty acids were palmitic (12.3–14.8%), stearic (26.0–27.3%), oleic (52.6–54.6%) and linoleic (2.3–3.1%). From the fatty acid composition, it has been observed that, the oil does not contain epoxy or hydroxy or other unusual fatty acids. It consists of about 30% of symmetrical monounsaturated-type triacylglycerides and appears to be a

Table 2 The acute oral toxicity limit test of male and female rats (body and major organs weight gain)

Oil tested and quantity fed	Initial body wt	Final body wt	Body wt gain	Wt of organ g/100 g of body weight		
				Liver	Kidney	Spleen
	g	g	g	g	g	g
Male rats						
GNO, 15 mL	180±4.4	198±6.4	18±5.6	4.7±0.25	0.5±0.05	0.5±0.05
GNO, 30 mL	180±4.5	196±4.3	16±4.8	4.9±0.32	0.5±0.04	0.6±0.07
EESO, 15 mL	180±6.2	196±5.2	16±5.3	4.9±0.16	0.5±0.03	0.6±0.07
EESO, 30 mL	180±4.5	200±4.5	20±4.5	4.9±0.43	0.5±0.04	0.6±0.09
SESO, 15 mL	180±4.6	204±4.8	24±4.6	4.5±0.37	0.4±0.05	0.5±0.07
SESO, 30 mL	180±4.7	211±4.6	31±4.8	4.6±0.38	0.5±0.03	0.8±0.08
RSO, 15 mL	180±4.4	205±4.5	25±4.6	4.4±0.16	0.6±0.05	0.5±0.03
RSO, 30 mL	180±4.8	213±4.6	33±4.4	4.6±0.19	0.5±0.04	0.6±0.06
Female rats						
GNO, 15 mL	176±3.6	189±4.4	13±3.3	4.9±0.24	0.6±0.04	0.5±0.05
GNO, 30 mL	176±5.2	192±5.3	16±4.8	5.0±0.32	0.5±0.03	0.6±0.07
EESO, 15 mL	180±4.6	191±5.2	11±4.5	5.2±0.26	0.6±0.05	0.7±0.06
EESO, 30 mL	176±5.7	192±5.4	16±4.5	5.3±0.28	0.5±0.03	0.6±0.07
SESO, 15 mL	170±3.8	188±4.5	18±4.2	4.8±0.18	0.5±0.03	0.6±0.05
SESO, 30 mL	176±4.7	194±4.5	18±4.4	5.1±0.26	0.6±0.04	0.9±0.07
RSO, 15 mL	174±5.8	190±4.7	16±4.3	4.7±0.19	0.6±0.05	0.6±0.05
RSO, 30 mL	180±4.9	197±5.6	17±5.2	5.0±0.24	0.6±0.04	0.6±0.08

Data are mean values ± standard deviation, n=3

GNO ground nut oil; *EESO* expeller extracted simarouba oil; *SESO* solvent extracted simarouba oil; *RSO* refined simarouba oil

Table 3 The food intake, weight gain and food efficiency ratio of male and female rats

	GNO	EESO	SESO	RSO
Male				
Initial body weight[1]	125±5.2[a]	130±5.3[b]	132±5.4[c]	126±5.3[d]
Final body weight[1]	241±6.3[a]	257±6.4[b]	247±4.5[bc]	246±6.3[d]
Body weight gain[1]	116±6.2[ab]	127±6.4[b]	115±5.2[c]	120±6.4[d]
Food intake[2]	513±8.3[a]	490±7.5[ab]	486±8.3[c]	505±8.2[d]
Food efficiency ratio[3] (weight gain/food intake)	0.226±0.005[a]	0.259±0.008[ab]	0.236±0.006[ab]	0.238±0.005[c]
Female				
Initial body weight[1]	133±4.3[a]	140±5.6[b]	132±4.5[c]	136±6.6[d]
Final body weight[1]	223±6.2[a]	230±6.3[ab]	223±7.4[bc]	228±7.8[d]
Body weight gain[1]	90±5.6[a]	90±5.5[ab]	91±6.5[b]	92±6.8[c]
Food intake[2]	447±8.1[a]	440±7.3[b]	440±8.4[bc]	442±8.6[d]
Food efficiency ratio[3] (weight gain/food intake)	0.201±0.007[a]	0.204±0.005[ab]	0.207±0.006[b]	0.204±0.008[c]

[1] Values are given as mean ± SD, $n=5$

[2] Total food intake per animal over the entire 13 week period

[3] Calculated as weight gain/food intake; mean of 5 animals

Values in the same rows followed by different letters (a–d) are significant ($P<0.05$)

GNO ground nut oil; *EESO* expeller extracted simarouba oil; *SESO* solvent extracted simarouba oil; *RSO* refined simarouba oil

good source of fat for preparation of cocoa butter (CB) extender (Jeyarani and Yella Reddy 2001). It contained saturated fatty acid (SFA) (40.8–42.3%), monounsaturated fatty acid (MUFA) (52.9–55.0%) and polyunsaturated fatty acid (PUFA) (2.5–3.4%). On the other hand, the palm oil contained SFA (50.5%) and monounsaturated fatty acids (49.5%) (Dinc et al. 2011). Thus, the fatty acid composition of simarouba oil somewhat resembles with palm oil.

Administration of 15 and 30 mL of simarouba oils or GNO/kg body weight in a single dose did not produce any mortality. The organs viz. liver, kidney and spleen did not show any abnormalities and microscopic changes, their weights being comparable, which are presented in Table 2. In the 13-week feeding study, the male animals did not show any significant difference in body weight gain (Fig. 1A), while in the case of female rats there was reduction in the body weight during the 3rd and 4th weeks except in the case of feed with EESO (Fig. 1B). The similar trend was also observed in the case of GNO. 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 the efficiency was observed in the case of male rats taking EESO (Table 3) ($P<0.05$). There was no significant difference between food intake and body weight gain in the four groups of male or female rats. But the figure is slightly lower in case of female rats as presented in Table 3. The organ weights such as liver, kidney, heart, spleen, lungs and testes of rats are presented in Table 4 ($P<0.05$). The residual

Table 4 The mean relative organ weights of male and female rats expressed as g/100 g body weight

	Liver	Kidney	Heart	Spleen	Lungs	Testes
Male						
GNO	3.2±0.42[a]	0.300±0.062[a]	0.315±0.073[a]	0.348±0.053[a]	0.539±0.053[a]	0.307±0.036[a]
EESO	3.4±0.53[bc]	0.311±0.046[b]	0.315±0.064[ab]	0.385±0.045[b]	0.863±0.063[b]	0.389±0.038[b]
SESO	3.5±0.45[c]	0.291±0.055[c]	0.318±0.024[b]	0.445±0.055[c]	0.769±0.063[bc]	0.352±0.029[bc]
RSO	3.1±0.43[d]	0.329±0.037[d]	0.333±0.085[c]	0.325±0.034[d]	0.589±0.056[c]	0.361±0.035[d]
Female						
GNO	3.5±0.54[a]	0.318±0.028[a]	0.367±0.042[a]	0.358±0.033[a]	0.741±0.056[a]	
EESO	3.8±0.42[ab]	0.313±0.046[ab]	0.334±0.033[b]	0.360±0.062[b]	0.686±0.076[ab]	
SESO	3.4±0.43[c]	0.318±0.057[bc]	0.336±0.042[c]	0.399±0.054[c]	0.636±0.065[bc]	
RSO	3.3±0.33[d]	0.307±0.038[d]	0.289±0.054[d]	0.328±0.045[d]	0.679±0.048[d]	

Data are mean values ± standard deviation, $n=5$, Values in the same columns followed by different letters (a–f) are significant ($P<0.05$)

GNO ground nut oil; *EESO* expeller extracted simarouba oil; *SESO* solvent extracted simarouba oil; *RSO* refined simarouba oil

Table 5 The digestion of Simarouba fat by male and female rats after 50th and 80th days

Feed containing 10% test oil/fat	Male rats				Female rats			
	Fat intake (g)	Wt of excreta (g)	Fat content (mg)	Digestion (%)	Fat intake (g)	Wt of excreta (g)	Fat content (mg)	Digestion (%)
Fat free feed								
50th day	Nil	3.7±0.42	1.5±0.22		Nil	3.3±0.22	1.2±0.22	
80th day		4.8±0.53	1.2±0.32			4.6±0.38	1.1±0.23	
GNO								
50th day	2.9±0.34^{a}	5.5±0.52^{b}	3.1±0.41^{c}	96.3±0.29^{d}	2.5±0.22^{a}	3.8±0.33^{b}	2.8±0.24^{c}	97.3±0.27^{d}
80th day	3.6±0.42^{a}	6.2±0.63^{ab}	2.3±0.33^{c}	97.3±0.24^{d}	2.8±0.31^{a}	4.7±0.38^{ab}	2.2±0.28^{bc}	98.2±0.24^{d}
EESO								
50th day	2.5±0.31^{a}	4.8±0.33^{b}	1.7±0.32^{bc}	98.9±0.25^{d}	2.4±0.32^{a}	3.5±0.23^{ab}	2.1±0.22^{c}	98.7±0.26^{d}
80th day	3.2±0.33^{a}	5.4±0.52^{b}	1.8±0.41^{c}	98.7±0.23^{d}	2.8±0.43^{a}	5.1±0.39^{b}	2.9±0.28^{bc}	96.6±0.23^{d}
SESO								
50th day	2.6±0.34^{a}	4.5±0.34^{b}	2.1±0.42^{bc}	98.5±0.22^{d}	2.5±0.33^{a}	4.3±0.32^{ab}	2.1±0.28^{c}	98.1±0.22^{d}
80th day	3.3±0.45^{a}	5.6±0.52^{b}	2.5±0.34^{c}	97.5±0.24^{d}	2.8±0.42^{a}	5.2±0.37^{bc}	2.4±0.26^{c}	97.4±0.24^{d}
RSO								
50th day	2.6±0.22^{a}	3.9±0.42^{b}	3.7±0.25^{c}	96.5±0.3^{d}	2.5±0.32^{a}	3.8±0.31^{b}	1.8±0.18^{c}	98.8±0.23^{d}
80th day	3.5±0.31^{a}	5.7±0.43^{bc}	2.3±0.41^{c}	97.9±0.23^{d}	2.8±0.28^{a}	5.3±0.46^{b}	3.1±0.21^{bc}	96.1±0.22^{d}

Data are mean values ± standard deviation, n=5, Values in the same rows followed by different letters (a–d) are significant (P<0.05)

GNO ground nut oil; *EESO* expeller extracted simarouba oil; *SESO* solvent extracted simarouba oil; *RSO* refined simarouba oil

fat content of feces of the animals fed with Simarouba oils was comparable with those receiving the diet containing GNO. Absorption or digestibility of fat was above 96% and compared very well with GNO (Table 5) (P<0.05). Haematological analyses did not show any remarkable differences in the haemoglobin or cell counts between all the groups of animals (Table 6) (P<0.05). Biochemical analyses of the serum also did not show any marked difference in the blood glucose, serum cholesterol, triglyceride, protein, urea, alkaline phosphatase and serum transaminases. Microscopic

Table 6 Hematological and biochemical analysis of rats feed with Simarouba fat and Ground nut oil

Test	GNO	EESO	SESO	RSO
HGB (g/dl)	14.2±1.42^{a}	12.7±2.13^{ab}	14.9±1.24^{bc}	15.8±1.83^{d}
WBC (thousands/Cu.mm)	14.3±1.83^{a}	12.3±1.42^{ab}	12.0±2.42^{bc}	11.8±1.54^{d}
HCT (Hematocrit)%	39.8±3.13^{a}	37.4±2.92^{bc}	37.3±5.34^{bc}	36.9±3.73^{d}
Blood glucose (mg/dl)	90.0±10.5^{a}	96.0±9.4^{bc}	106.0±8.6^{bc}	91.0±9.3^{d}
Serum protein (g/dl)	6.7±0.64^{a}	7.2±0.95^{ab}	6.7±0.44^{bc}	6.8±0.75^{d}
Serum cholesterol (mg/dl)	55.0±6.5^{a}	56.0±6.3^{bc}	52.0±5.8^{c}	59.0±6.9^{d}
Serum triglycerides (mg/dl)	65.0±6.3^{a}	69.0±5.2^{bc}	65.0±8.3^{c}	62.0±7.1^{d}
Serum urea (mg/dl)	10.2±1.62^{a}	12.0±1.34^{ab}	11.0±1.15^{b}	10.8±1.53^{d}
Serum alkaline phosphatase IU/L	166.0 ±5.3^{a}	176.0±8.2^{ab}	184.0±6.2^{b}	174±9.1^{d}
SGOT IU/L	47.2±4.62^{a}	45.6±6.24^{bc}	43.9±4.53^{c}	39.4±3.73^{d}
SGPT IU/L	31.0±2.24^{a}	36.0±1.92^{ab}	29.4±3.82^{bc}	33.2±2.92^{d}
HDL cholesterol mg/dl	27.2±4.23^{a}	25.1±3.93^{ab}	26.6±5.24^{bc}	24.3±3.73^{d}
LDL cholesterol mg/dL	35.4±3.92^{a}	35.8±4.13^{ab}	37.2±4.72^{b}	38.4±3.64^{d}

Data are mean values ± standard deviation, n=5, Values in the same rows followed by different letters (a–d) are significant (P<0.05)

GNO ground nut oil; *EESO* expeller extracted simarouba oil; *SESO* solvent extracted simarouba oil; *RSO* refined simarouba oil; *HGB* Haemoglobin; *WBC* White blood cells; *SGOT* Serum glutamic oxaloacetic transaminase; *SGPT* Serum glutamic pyruvic transaminase; *HDL* High density lipoprotein; *LDL* Low density lipoprotein

examination (data not shown) of liver, kidney, spleen, heart, testes, and lungs of the animals showed no abnormal histopathological lesions, indicating no deleterious effects of EESO and SESO.

Conclusion

The chemical composition of the simarouba oil is comparable to that of palm oil. The toxicological study shows that the simarouba oil is comparable to GNO. Thus the simarouba oil is safe to use for edible purposes. However, in view of the observed acute cytotoxicity of quassinoids, it may be necessary to undertake a three generation toxicological evaluation study on rats to eliminate any possible teratogenic effects. The study has demonstrated that expeller extracted, solvent extracted as well as refined Simarouba oils are safe to use and could be considered for edible purposes. However, refining, bleaching, and deodorizing would make it more attractive and acceptable product.

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