Serum Cholesterol, Triglycerides and Total Lipid Fatty Acids of Rats in Response to Okra *(Hibiscus Esculentus)* Seed Oil[1]

P. Srinivasa Rao*, **P. Udayasekhara Rao** and **B. Sesikeran**
National Institute of Nutrition, Indian Council of Medical Research, Jamai Osmania P.O., Hyderabad-500 007 India

Tender pods of okra are commonly consumed vegetables in India. Okra seed kernel, like soybean, is a rich source of protein and fat. Its fat, with its appreciable linoleic acid content (>42%), prompted us to look into its metabolic utility in comparison with commonly consumed groundnut oil. Serum lipid profiles, with respect to cholesterol, triglycerides and total lipid fatty acids were determined in rats receiving okra seed oil at a level of 10% in the casein based diet which was adequate with respect to vitamins, minerals, etc. The control group received a casein based diet in which groundnut oil was the source of fat. Serum lipid profiles in this group were similarly monitored. The feeding trial was carried out for a period of 90 days.

Results showed that serum cholesterol content of rats receiving okra seed oil was significantly lower compared to those consuming groundnut oil. A decreasing trend in total lipids as well as triglycerides was also evident in animals fed okra seed oil. Serum fatty acid profiles showed a relatively higher proportion of long chain and polyunsaturated fatty acids in this group as compared to the group receiving groundnut oil. These results indicate that okra seed oil consumption has a potential hypocholesterolemic effect.

KEY WORDS: Fatty acids, okra seed oil, serum cholesterol, triglycerides.

Okra *(Hibiscus Esculentus)* is commonly known as Bhendi in India and its tender pods are used as vegetables and its seeds as chutney. It is mostly grown in tropical and subtropical countries like India, Malaysia, East and West Africa and Central America (1). Egyptians use okra flour as a supplement to corn flour to improve dough quality (2).

Chemical composition of whole seed and kernel is close to that of leguminous seeds (3). Not only is it a rich source of protein (whole seed 21%, kernel 38.9%) it has high fat content (17.9% in the whole seed, 36.5% in its kernel). Like legumes, it is a good source of lysine, methionine and tryptophan (3). More interestingly, its fat is a rich source of linoleic acid (Table 1). This prompted us to examine the metabolic utility of its fat and ascertain the beneficial effect, if any exist.

MATERIALS AND METHODS

Weanling rats of Wistar Strain (individually caged) were maintained on casein based diet providing 20% protein (Table 2). They were divided into two groups. Group 1 consisted of 12 male and 12 female rats who received casein based diet with groundnut oil as source of fat, added at a level of 10% in the diet. Group 2, also comprised of 12 male and 12 female rats, received diet with 10% okra seed oil as source of fat (Table 2). After the experimental period of 90 days, blood was collected in heparinized tubes and serum was separated. Liver, spleen, kidneys, lungs, heart and the entire gastrointestinal tract were dissected out of each of these animals and immediately fixed in 10% neutral buffered formalin. They were subsequently sampled out and processed by conventional methods. Paraffin sections of about 6 μm thickness were made on a rotary microtome and stained with Hematoxyline (Meyers) and Eosin and examined under a light microscope.

Total lipids from serum were extracted using the method of Folch *et al.* (6). Different aliquots of serum were used for estimation of cholesterol (7) and triglycerides (8). However, serum lipid extraction was used for determination of total lipid phosphorus (9). Total lipids were subjected to methanolysis and transesterification to yield methyl esters of fatty acids. The pattern of distribution of fatty acid methyl esters was determined using a Varian model 3700 GLC (Varian Associates, Palo Alto, CA) equipped with FID detector, using 10% Silar 10C adsorbed on Chromosorb W as the stationary phase (10).

TABLE 1

Fatty Acid Composition of Groundnut Oil and Okra Seed Oil (Weight Percentages of Total Fatty Acid Methyl Esters)

Carbon no. and unsaturation	Groundnut oil	Okra seed oil
16:0	12.6	23.5
16:1	1.4	tr
18:0	1.7	4.3
18:1	47.4	28.9
18:2	29.9	42.4
20:0	4.2	—
Others	2.8	<0.9

TABLE 2

Composition of Experimental Diets (g/100 g Diet)

Vitamin mixture[a]	1
Salt mixture[b]	4
Groundnut oil/okra seed oil	10
Casein[c]	26
Corn starch	59

[a] Vitamin mixture according to Campbell (4).

[b] Salt mixture according to USP XVII (5).

[c] 100 g of this diet which contained 26 g casein, provided 20% of the protein.

RESULTS AND DISCUSSION

Gain in body weight between the two groups was not

*To whom correspondence to be addressed

[1]Part of this work was presented at 45th Annual Meeting of Oil Technologists Association of India, New Delhi-Feb. 9-10, 1990.

TABLE 3

Body Weights and Organ Weights of Rats Fed Groundnut Oil and Okra Seed Oil[a]

Group[b]	Body weight (g)		Organ weights (g)				
	Initial	Final	Liver	Kidney	Lungs	Heart	Spleen
G M	41 ± 0.3	327 ± 9.0	3.59 ± 0.19	0.69 ± 0.02	0.4 ± 0.01	0.29 ± 0.01	0.18 ± 0.01
O M	41 ± 1.3	317 ± 12.2	3.54 ± 0.16	0.74 ± 0.02	0.46 ± 0.02	0.28 ± 0.01	0.18 ± 0.01
G F	39 ± 1.2	179 ± 8.4	3.35 ± 0.07	0.76 ± 0.03	0.60 ± 0.01	0.35 ± 0.01	0.25 ± 0.01
O F	39 ± 1.2	189 ± 4.5	3.43 ± 0.08	0.72 ± 0.01	0.56 ± 0.02	0.34 ± 0.01	0.24 ± 0.01

[a] All are mean ± S.E. of 12 observations.

[b] Please see Table 4 for details regarding individual groups.

different. Even weights with respect to liver, heart, lung, kidney and spleen in animals of the same sex were comparable irrespective of the nature of the oil fed (Table 3). Further histopathological examination of the organs in these animals indicated that there were essentially no significant differences in the histology of the organs and tissues tested between the two groups of rats which received 10% groundnut oil and 10% okra seed oil, respectively, in their diets at the end of 13 weeks of feeding.

Concentration of total lipid classes *viz.* cholesterol, triglycerides and phospholipids in serum of male and female rats consuming groundnut and okra seed oils is depicted in Table 4. Although total lipids in serum were unaffected due to okra seed oil consumption, a significant decrease in serum level of cholesterol was evident in both male and female rats consuming this oil, as compared to those receiving groundnut oil as the source of fat in the diet. Neither the triglycerides concentration nor the phospholipid concentrations showed any difference under study.

Fatty acid profiles of serum lipids (Table 5), showed a lower value for oleic acid in rats receiving okra seed oil as compared to those receiving groundnut oil. This probably reflected the relative differences in oleic acid concentration of dietary groundnut fat and that of okra seed.

It is an established fact that fats with greater degree of unsaturation, especially those rich in essential fatty acid like linoleic acid, exert beneficial effects by lowering circulating levels of cholesterol. High levels of circulating cholesterol in serum are generally considered risky and the level of serum cholesterol is considered as one of the diagnostic measures for detecting coronary heart disease. It is thus interesting to note that okra seed oil feeding brought about a significant decrease in serum cholesterol level. Further, polyunsaturated fatty acids like arachidonic acid (20:4ω6) and docosatetraenoic acid (22:4ω6) increased significantly in the serum of the okra

TABLE 4

Total Lipids and the Relative Concentration of Lipid Classes in Serum in Male and Female Rats Fed Okra Seed Oil and Groundnut Oil

Group[a]	Total lipids (g/100 mL)	Cholesterol (mg/100 mL)	Triglycerides (mg/100 mL)	Phospholipids (mg/100 mL)
GM	1.42 ± 0.78	112 ± 5	380 ± 45	100 ± 7
OM	1.83 ± 0.79	64 ± 7[b]	310 ± 44	97 ± 14
GF	1.58 ± 0.36	101 ± 4	355 ± 14	96 ± 14
OF	1.68 ± 0.38	66 ± 5[b]	330 ± 21	100 ± 11

[a] GM, groundnut oil fed male rats; GF, groundnut oil fed female rats; OM, okra seed oil fed male rats; OF, okra seed oil fed female rats. All are mean ± S.E. for 10 observations.

[b] $P < 0.001$; GM vs. OM; GF vs. OF.

TABLE 5

Fatty Acid Distribution of Total Serum Lipids in Rats Reared on Different Oil Regimen (Weight Percentages of Total Fatty Acid Methyl Esters)

Group[a]	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:4	22:0	22:1	22:4	Total tetraenes[b]
GM	3.8±1.1	21.8±0.6	1.1±3	12.5±1.1	29.0±2.1	18.1±1.2	1.1±0.2	1.1±0.1	3.0±0.4	tr	0.8±0.1	5.1±0.6	8.1±0.7
OM	1.1±0.7	24.9±1.6	0.5±0.1	16.5±1.6	12.2±0.9	16.4±2.4	0.2±0.1	1.4±0.1	8.5±2.3	0.4±0.1	1.9±0.6	11.7±3.9	20.2±3.5[c]
GF	4.6±1.0	19.5±0.6	tr	18.1±1.5	24.8±1.7	17.9±1.0	1.5±0.1	0.8±0.2	7.4±1.2	tr	0.8±0.1	5.6±0.3	13.0±1.2
OF	7.1±2.2	17.2±1.3	1.8±0.3	23.0±1.5	8.7±1.4	7.4±2.5	0.5±0.1	0.8±0.1	6.3±0.7	1.5±0.2	6.1±0.7	16.9±2.3	23.2±2.1[c]

[a] All are mean ± S.E. of 10 observations. See Table 4 for descriptions of groups.

[b] Tetraenes, total tretraene fatty acid methyl esters ($C20:4_{\omega6}$ + $C22:4_{\omega6}$).

[c] $P < 0.01$. GM vs. OM; GF vs. OF.

tr, Traces.

seed oil fed rats, indicating better utilization of medium chain precursors for biosynthesis of long chain and polyunsaturated fatty acids. While there is a need to explore the active principle present in okra seed oil which may be responsible for lowering cholesterol, these experiments show a definite beneficial potentiality of okra seed oil to influence the lowering of cholesterol in experimental animals.

ACKNOWLEDGMENTS

The authors wish to thank V. Reddy, Director of the Institute, for her keen interest in the work. Technical assistance of P. Padmini and P. Ramulu is gratefully acknowledged.

REFERENCES

1. *Wealth of India. Raw Materials*, edited by B.N. Sastry, Vol. V, New Delhi, Council of Scientific and Industrial Research, 1959, p. 84.
2. Taha-el-Katib, M.M., *Nature 159*:716 (1947).
3. Udayasekhara Rao, P., *Plant Foods Human Nutr. 35*:389 (1985).
4. Campbell, J.A., in *Evaluation of Protein Quality*, Washington, D.C., National Academy of Sciences, National Research Council Publication 1100, 1963, p. 32.
5. *Official Methods of Analysis*, 11th edn., edited by William Horwitz, Washington, D.C., Association of Official Analytical Chemists, 1970, p. 775.
6. Folch, J., M. Lees and G.H. Sloane-Stanley, *J. Biol. Chem. 226*:497 (1957).
7. Zlatkis, A., B. Zak and A.J. Boyle, *J. Lab. Clin. Med. 41*:486 (1952).
8. Lowell, B.F., and T.D. Ralph, *Clin. Chem. 19*:338 (1973).
9. Bartlett, G.R., *J. Biol. Chem. 324*:466 (1959).
10. Stoffel, W., C. Florence and J.E.H. Ahrens, *Anal. Chem. 31*:307 (1959).

[Received August 28, 1990; accepted January 23, 1991]