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Effect of colocasia leaves (*Colocasia antiquorum*) on serum and tissue lipids in cholesterol-fed rats

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Abstract. Dried colocasia powder (10% and 20%) was fed to hypercholesterolemic rats for a period of one month to explore the effect on serum lipids and tissue lipids. The results indicated that there was a significant increase in total lipids, total cholesterol, and triglycerides levels in the cholesterol fed rats. Liver cholesterol showed a slight reduction which was non-significant, while an increase in liver triglyceride was observed in rats fed with 10% and 20% colocasia leaves diet with or without cholesterol. All these observations indicate an aggravating effect of colocasia leaves on serum and tissue lipids in cholesterol-fed rats.

Introduction

During the past few years considerable interest has been developed regarding the role of dietary fibre from different sources in reducing the serum cholesterol in hypercholesterolemic conditions. Soluble fibres like pectin and guar gum have given a significant decrease in serum cholesterol level [1–4] whereas particulate fibres such as wheat bran have not given any significant decrease in total cholesterol or lipoprotein cholesterol [5–10]. Normally fruits and vegetables contain more soluble fibres which imparts the beneficial effects [11–13]. Thus from the above studies its seems that plant fibres play a regulatory role in the metabolism of cholesterol by sequestration of bile acids and hence in lowering the concentration of cholesterol in hypercholesterolemic conditions. Since the physiological effects of dietary fibre appear to depend heavily on the source and composition of fibre, an attempt was made to study the effect of colocasia leaves (*Colocasia antiquorum*) on serum and tissue lipids in cholesterol fed rats, as colocasia is cultivated throughout India and is widely available and consumed by the Indian population as a green leafy vegetable and snack. Further, it is found to be rich in iron, fibre, protein and calcium.

Materials and methods

All chemicals used were of analytical reagent grade or the best commercially available grade. Triolein and diethyldithiocarbamate were purchased from Sigma Chemical Co., St. Louis, U.S.A. Phosphotungstic acid and magnesium chloride hexahydrate were obtained from Loba Fine Chemicals, Bombay, India. All other reagents were from E. Merck, S.D. Fine and B.D.H. firms.

Colocasia leaves were brought from the local market. The leaves were washed thoroughly with distilled water and dried under the sun for 3–4 days until it became moisture free. The dried leaves were crushed by hand and then ground in Baby Prince flour mill to a very fine powder, was sieved through the sieve of mesh size 1 mm^2. Then the sieved powder was incorporated into the diet. Dried colocasia leaves contained on analysis 41.03 g carbohydrates, 20.79 g protein, 9.28 g fat and 11.25 g of crude fibre.

Experimental design

Forty eight male albino rats of Charles Foster strain weighing between 100–130 g were divided into 6 groups of 8 rats each on the basis of restricted random sampling procedure so that initial mean weights of rats in each group was comparable. They were maintained on different dietary regimen for 30 days as given in Table 1.

The rats were caged separately and were given food and water *ad libitum*, sacrificed on the 30th day after an overnight fast and their tissues viz. blood, liver and kidney were collected. Serum was separated from the blood and was used for various biochemical estimations as described elsewhere. The tissues were washed well in cold saline to remove any blood, blotted on a filter paper and weighed. The tissues were further processed as follows:

One gram of tissue was suspended in 20 ml of Folch solvent (16) (2:1, vol/vol; $CHCl_3$ and CH_3OH) for 4–6 h, ground in a pestle and mortar containing 2–3 g of Na_2SO_4. The powder thus obtained was poured into $CHCl_3$:CH_3OH mix (used for suspending the organ) and allowed it to stay in the solvent mixture overnight. The clear supernatant was transferred to a separating funnel and was mixed with an equal volume of normal saline and the mixture was allowed to stand for 4–6 h. The lower layer was collected and filtered through a Whatman No. 1 filter paper (to remove any suspended particles) rinsing the filter paper 2–3 times with Folch solvent and the filtrate and washings were pooled. The extract was evaporated at 45–50 °C, dissolved in 1 ml of chloroform and stored at 5 °C in a tightly screw-capped bottle containing a crystal of butylated hydroxytoluene as a preservative, until further analysis.

Table 1. Composition of experimental diets (g/100 g)

Ingredients	Basal diet	Basal + 10% colocasia*	Basal + 20% colocasia	Basal + cholesterol (HID)	HID + 10% colocasia	HID + 20% colocasia
Casein	10.8	8.7	6.6	10.8	8.7	6.6
Vitamin mix [14]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix [15]	4.0	4.0	4.0	4.0	4.0	4.0
Groundnut oil	5.0	4.0	3.2	5.0	3.0	3.2
Cholesterol	–	–	–	2.0	2.0	2.0
Bile salt	–	–	–	0.5	0.5	0.5
Colocasia	–	10.0	20.0	–	10.0	20.0
Corn starch	78.2	71.3	64.2	76.7	68.8	61.7
	100.0	100.0	100.0	100.0	100.0	100.0
Calories (Kcal/100 g)	401.0	389.0	377.0	395.0	379.0	368.0
Protein (g/100 g)	10.0	10.0	10.0	10.0	10.0	10.0

* 10 g of colocasia powder provides 2.07 g protein, 1.0 g fat and 4.10 g of carbohydrates.
HID – Hypercholesterolemia Induced Diet.

Assay methods

The following biochemical determinations were done in the serum and tissues:

Total lipids as per the method of Christopher *et al.* [17];
Triglyceride by the method of Foster and Dunn [18];
Phospholipids by the method of Marinetti *et al.* [19];
Cholesterol by the method of Carr and Drekter [20].

In serum, LDL cholesterol and VLDL cholesterol were precipitated by the addition of phosphotungstic acid and magnesium chloride [21]. The supernatant obtained at this stage was used for the determination of HDL cholesterol as per the procedure of Carr and Drekter [20]. LDL and VLDL cholesterol was calculated by subtracting HDL cholesterol value from total cholesterol.

Statistical analysis

All data were subjected to a one-way analysis of variance. When treatment effects were found to be statistically significant, pair-wise comparisons among treatment were made using Tukey's studentized range test at a procedure-wise error rate of 0.05.

Results

Feeding colocasia leaves along with or without cholesterol had no effect on the growth rate of the rats (Table 2). However, an increase in the weight of the liver and kidney of the rats fed cholesterol along with or without colocasia was noticed which was statistically significantly when compared to their controls.

Cholesterol feeding along with or without colocasia leaves showed an increase in the total lipid level in the serum which was statistically significant ($p < 0.001$). The increase in the total lipid content in the cholesterol fed rats was 3 folds whereas no change in total lipid level was noticed in rats fed either 10% or 20% colocasia leaves along with the basal diet (Table 3). The serum phospholipid levels remained nearly unaltered except for a small rise in the cholesterol fed rats which was insignificant (Table 3). Amongst the various groups studied, only the groups fed 10% and 20% colocasia leaves exhibited significant increase in serum triglyceride levels as compared to the controls (Table 3). However there was no significant effect on the triglyceride content of rats maintained on diets supplemented with cholesterol.

Table 2. Effect of colocasia leaves on weight gain and organ weights in rats[1] (Mean ± SD)

Groups	Weight gain (g)	Liver (g/100 g body weight)	Kidney
I. Control	46.2[a] ±15.00	3.0[a] ±0.31	0.7 ±0.09
II. Cholesterol control	48.2[a] ±14.83	4.8[b] ±0.42	0.8 ±0.06
III. Control + 10% colocasia	58.6[a] ±23.00	3.1[a] ±0.10	0.7 ±0.06
IV. Control + 20% colocasia	60.2[a] ±26.51	3.2[a] ±0.10	0.7 ±0.07
V. Cholesterol + 10% colocasia	50.0[a] ±16.31	5.0[b] ±0.10	0.8 ±0.03
VI. Cholester + 20% colocasia	47.4[a] ±15.52	5.0[b] ±0.10	0.8 ±0.05

[1] Means sharing a common superscript letter (within a single column) are not significantly different.

Table 3. Effect of colocasia leaves on serum total lipids, phospholipids, triglycerides, cholesterol and lipoprotein cholesterol levels in rats[1] (Mean ± SD (mg/dL))

	Groups	Serum total lipids	Serum phosopholipids	Serum triglycerides	Serum cholesterol	Serum HDL cholesterol	Serum LDL + VLDL cholesterol
I.	Control	355^{a}	79^{a}	71^{ab}	96^{a}	56^{b}	39^{a}
		±74	±20	±17	±15	±16	±8
II.	Cholesterol	975^{b}	93^{a}	66^{a}	399^{b}	22^{a}	325^{b}
	control	±253	±22	±21	±98	±13	±61
III.	Control + 10%	270^{a}	79^{a}	100^{ab}	79^{a}	44^{b}	32^{a}
	colocasia	±92	±16	±26	±15	±6	±15
IV.	Control + 20%	299^{a}	89^{a}	107^{b}	74^{a}	42^{b}	27^{a}
	colocasia	±48	±17	±34	±9	±6	±5
V.	Cholesterol + 10%	960^{b}	102^{a}	74^{ab}	523^{c}	22^{a}	431^{c}
	colocasia	±203	±48	±32	±135	±7	±97
VI.	Cholesterol + 20%	981^{b}	99^{a}	94^{ab}	530^{c}	23^{a}	420^{c}
	colocasia	±190	±24	±33	±61	±9	±39

[1] Means sharing a common superscript (in the same column) are not significantly different.

On feeding HID to rats, it was noticed that serum cholesterol levels increased by approximately 4 times. However, when colocasia leaves were fed along with HID, the increase in total cholesterol was approximately 1–3 times higher than the cholesterol fed (HID) group. But as compared to the control group slight statistical decreases were observed in the serum cholesterol levels of rats fed 10% and 20% colocasia leaves along with the basal diet (Table 3). In case of lipo-protein fractions, the data revealed that there was a significant two-fold decrease in serum HDL cholesterol levels in rats fed HID as compared to the controls. But, feeding colocasia leaves along with cholesterol in the diet showed no change in HDL-cholesterol levels when compared to cholesterol fed group (Table 3). LDL and VLDL cholesterol levels were significantly increased in cholesterol fed rats with or without colocasia leaves. This increase was 1–3 times higher when compared with only cholesterol fed rats. However, no change in the LDL-cholesterol + VLDL-cholesterol was observed in the group fed with colocasia leaves along with the basal diet (Table 3).

The total lipid content in both liver and kidney was increased to about 3 times in groups fed HID with or without colocasia as compared to the controls. The total lipid content in the tissues was not greatly affected when the rats were fed colocasia leaves with the basal diet (Table 4). The observation on the total lipid content of both liver and kidney was found to go hand in hand with the rise observed in the serum level. No significant differences was observed in the liver and kidney phospholipid content, except in the liver, phospholipid content of cholesterol fed rats was found to be statistically higher ($p < 0.05$) when compared to the basal diet fed rats (Table 4). The change observed in the serum was not magnified much. The triglyceride content in liver and kidney was significantly increased in the rats fed HID as compared to the controls ($p < 0.001$). Similarly, when colocasia leaves were fed along with HID, there was an increase in the triglyceride content in both the tissues and was found to be higher than the HID group (Table 4). The total cholesterol content was increased in the HID group as compared to the controls (Table 4). The increase was to the tune of 12 times. When colocasia leaves were fed to rats along with HID, an increase in total cholesterol content in both the organs was noticed and the mean values were similar to HID group. In the case of kidney the mean value was lower than that of HID group but the difference was statistically non-significant.

Discussion

During the past few years considerable interest has developed regarding the

Table 4. Effect of colocasia leaves on tissue total lipids, phospholipids, triglycerides and cholesterol in rats[1] (Mean ± SD (mg/g))

	Groups	Total Lipids		Phospholipids		Triglycerides		Cholesterol	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Liver	Kidney
I.	Control	35^{a}	44^{a}	18^{a}	19^{a}	8^{a}	15^{b}	2^{a}	3.7^{a}
		±4	±3.2	±3	±5	±1.1	±2.2	±0.6	±0.4
II.	Cholester	107^{b}	58^{b}	22^{a}	21^{a}	35^{b}	24^{c}	45^{b}	5.2^{b}
	control	±9	±4.8	±3	±3	±3.2	±1.7	±6.4	±1.9
III.	Cholesterol + 10%	40^{a}	42^{a}	18^{a}	19^{a}	13^{a}	13^{a}	3^{a}	3.5^{a}
	colocasia	±5	±5.4	±5	±6	±2.9	±1.5	±0.8	±0.2
IV.	Control + 20%	40^{a}	43^{a}	19^{a}	19^{a}	12^{a}	14^{ab}	2^{a}	3.3^{a}
	colocasia	±5	±2.2	±5	±3	±3.2	±1.2	±0.8	±0.5
V.	Cholesterol + 10%	119^{b}	57^{b}	21^{a}	22^{a}	50^{c}	23^{c}	42^{b}	3.9^{a}
	colocasia	±14	±5.3	±4	±2	±12.6	±1.0	±4.5	±0.4
VI.	Cholesterol + 20%	114^{b}	58^{b}	22^{a}	20^{a}	47^{c}	25^{c}	40^{b}	4.0^{ab}
	colocasia	±13	±4.9	±3	±4	±13.4	±1.3	±2.9	±0.6

[1] Means sharing a common superscript letter (within a single column) are not significantly different.

etiological role of dietary fibre in the development of human diseases and also as an potential therapeutic role of fibre. The dietary fibres from different sources have been tested for their role in reducing the serum cholesterol in hypercholesterolemic conditions. Wheat bran did not show any significant decrease either in cholesterol or in any lipoprotein fraction [5–10], whereas oat bran, pectin, guargum showed significant decreases in cholesterol level [1–4 and 22]. Besides cereals, some pulses also have shown some hypocholesterolemic effect [23, 24]. The present study was therefore undertaken to investigate the effect of powdered colocasia leaves on the lipid metabolism in cholesterol fed rats.

The results of the present study suggest that incorporation of colocasia leaves at 10% and 20% level along with cholesterol showed a lipogenic effect, i.e. increase in the total lipid levels in both serum and in tissues namely liver and kidney. Similarly, increases were observed in serum and tissue levels of total cholesterol and tryglycerides. The increased levels of these contents are similar to other studies conducted by various workers [5, 25].

As a result of increased serum cholesterol level, there is production of β-VLDL to secrete out the cholesterol that has reached liver from the intestine. Concomittently there is an increase in triglyceride synthesis but the amount of triglyceride produced does not seem to be utilized and gets accumulated in the liver as revealed in the present study. Moreover, the β-VLDL that is produced may be taken up by liver due to the presence of macrophages in the kupffer cells, thereby leading to an accumulation of cholesterol in the liver.

Dried colocasia leaves seems to increase the liver triglyceride level but this change even though statistically significant is not of pathological level to affect the animals, whereas colocasia leaves when included along with cholesterol in the diet still increases thus aggravating the fatty liver condition. On the contrary a slight reduction in the cholesterol content of the liver is noticed whereas an increasing level is observed in the serum.

Thus, it may be possible that some component of the colocasia leaves is interfering with the lipid metabolism in general and specially with cholesterol metabolism thereby increasing hypercholesterolemic conditions in the serum and not aggravating the cholesterol content at the tissue level. It may be at the apo-protein level which are essential for the uptake of cholesterol and triglycerides or at the enzymatic level in the tissue that too mainly in liver and needs further detailed investigations.

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References

1. Kritchevsky D, Tapper SA, Goodman GT, Webber MM, Kurfeld DN (1984) Influence of cat bran and wheat bran on cholesterolemia in rats. Nutr Rep Int 29: 1353–1358
2. Sharma RD (1984) Hypocholesterolemic activity of some Indian gums. Nutr Res 4: 381–389
3. Ano A, Vusitoper, M, Youtilainen E, Konhonen T (1984) Effects of guar gum in male subjects with hypercholesterolemia. Am J Clin Nutr 39: 911–915
4. Jenkin DJA, Reynolds D, Slavin B, Leeds AR, Jenkins AL, Jepson EM (1980) Dietary fibre and blood lipids: treatment of hypercholesterolemia with guar crisp bread. Am J Clin Nutr 33: 575–581
5. Asp NG, Baues HG, Nilseon-Ehlep, Nyman M, Oste R (1981) Wheat bran increases high density lipoprotein cholesterol in rats. Br J Nutr 46: 385–390
6. Sharma S, Kishore N, Sharma RK, Sharma BB (1980) Hypocholesterolemic effect of dietary fibre. Ind J Physiol Pharma 24: 136
7. Goswamy S, Mani I, Mani UV (1985) Effect of wheat bran supplementation on tissue lipids in diabetic rats. Ind J Biochem Biophys 22: 240–243
8. Mani I, Mani UV (1987) Effect of wheat bran supplementation on blood sugar, glycosylated protein and serum lipids in NIDDM subjects. Plant Fds Hum Nutr 37: 161–168
9. Truswell AS (1984) Dietary fibre and lipid metabolism. In: Vichai Tanphaichitr (ed) Human nutrition, Better nutrition, Better life. Winai Dahlan, Varanunt Suphkaran and Aree Valyasevi. Publ by Aksoismai Press, Bangkok, Thailand, pp 478–488
10. Arvanitakis C, Stammes CL, Folscraft J, Bayer P (1977) Failure of bran to alter diet induced hyperlipidimia in the rat. Proc Soc Exp Biol Med 154: 550–552
11. Elliott J, Mulvihill E, Duncan C, Foraythe R and Kritchevsky D (1981) Effects of tomato pomace and mixed vegetables pomace on serum and liver cholesterol in rats. J Nutr 111: 2203
12. Chen WJL, Anderson JW (1979) Effects of plant fibre in decreasing plasma total cholesterol and increasing HDL-C. Proc Soc Exp Bio Med 162: 310–313
13. Chen ML, Chia DF, Run JQ (1984) Effect of dietary vegetable (water convolvulus) on cholesterol metabolism in rats. J Nutr 114: 503–507
14. Hawk PB, Oser BL (1976) Hawk's Physiological Chemistry, 14th edn. Tata McGraw-Hill Publishing Company Ltd., pp 803 and 1356
15. National Academy of Science (1972) National Research Council Nutritional Requirements of the Lab Animals. National Academy Science Publication
16. Folch J, Lees M, Stanley GMS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509
17. Christopher K, Frings CS, Fendly TW, Dunn RT, Queen CA (1972) Improved determination of total serum lipids by the sulph-phospho vanillin reaction.Clin Chem 18: 673–677
18. Foster LB, Dunn RJ (1973) Stable reagents for the determination of serum triglyceride by a colorimetric Hantssch condensation method. Clin Chem 19: 338–395
19. Marinetti CV (1962) Chromatographic separation, identification and analysis of phosphatides. J Lip Res 3: 1–4
20. Carr JJ, Drekter IJ (1956) Estimation of total cholesterol in the serum. Clin Chem 2: 353–368

21. Warnick GR, Mayfield CBS, Benderson JBS, Chem JS, Albert JJ (1982) HDL-cholesterol quantitation by phosphotungstate-Mg^{+2} polyethylene glycol precipitation. Both with enzymic cholesterol assay compared with the Lipid Research method. Am J Clin Pathol 78: 718–721
22. Fred LS, Melisea JL, Steven LI, Judith AM (1988) Oat fiber: composition versus physiological function in rats. J Nutr 118: 144–151
23. Indira M, Karup CA (1982) Effect of black gram fibre on ethanol induced hyperlipidimia in rats. Atherosclerosis 41: 241–247
24. Jenkins DJA, Jenkins A, Wang GS, Robert P, Josephine B, Hall M, Brackley GC, Valeria BA, Reichat R, Alicklittle J (1983) Leguminous seeds in the dietary management of hyperlipidemia. Am J Clin Nutr 38: 567–571
25. Sultan CD, Muir NM, Begvi JJ (1981) Effect of fibre on cholesterol metabolism in the coturnix quail. Poult Sci 60: 812–815