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A comparative study on the beneficial effects of garlic *(Allium sativum* Linn), amla (*Emblica Officinalis* Gaertn)and onion (*Allium cepa* Linn) on the hyperlipidemia induced by butter fat and beef fat in rats

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Three months feeding of butter fat (BUF) and beef (BF) separately as components of diet at a level of 21% by weight for albino rats, significantly raised their serum and tissue lipids, lipid peroxidation and activities of certain enzymes. BUF was found to be more atherogenic than BF. On incorporation of 5% garlic, amla or onion separately in the above diets, each of them ameliorated the deleterious effects of the animal fats. A higher hyperlipidemic effect of BUF as compared to that of BF may be due to the fact that the ratio of unsaturated to saturated fats is lower for the former (0.56) than for the latter (0.75) and also that the former is richer in cholesterol content than the latter. The order of the curative effects of the vegetables are garlic>amla>onion. The better hypolipidemic effects and correction of elevated levels of certain enzymes shown by garlic and amla may be due to the facts that they contain comparatively better active principles than that found in onions.

The hypolipidemic effects of garlic *(Allium sativum* Linn) and onion (*Allium cepa* Linn) were demonstrated simultaneously by Mathew and Augusti[1]in India and Itokawa *et al*[2] in Japan in the year 1973. Later Augusti[3] demonstrated the hypocholesterolemic effect of garlic in humans. Several other effects of these spices are also reported viz; antioxidant,[4,5] hypotensive,[6,7] antiplatelet aggregation [8,9,] fibrinolytic[10,11] and antibiotic[12,13] effects. However prolonged and excessive use of these vegetables may induce hymolysis, anaemia, weight loss and failure to grow.[14] Their mechanism of action is attributed to the presence of disulphides and sulphoxide substances in these vegetables and their reaction with thiol group substances including cysteine and related enzymes.

Amla or Indian gooseberry (*Emblica Officinalis Gaertn)* is being used against a variety of conditions like liver injury, atherosclerosis [15] and diabetes. [16] It possesses very good hypocholesterolemic effect also.[17] Chavanaprasa and Brahma rasayana contain amla and they are being used for rejuvenating body [18] and also as immunomodulators[19]. Amla paste applied on forehead helps to lower body temperature, bleeding from nose, head aches and certain psychiatric condition [20]. Amla was also found to enhance interferon production[21]. Maharishi Ayurveda herbel mixtures M_4 and M_5 containing amla were reported to have anticarcinogenic property[22]. A very recent finding about amla is its antioxidant action.[23]

We know that animal fats are atherogenic in nature. However we wanted to learn which of the two fats BUF with a comparatively low amounts of long chain saturated and unsaturated fatty acids and also with a fairly high amount of short chain fatty acids (i.e 46%, 36% and 18% respectively) or BF with high amounts of long chain saturated and unsaturated fatty acid content (i.e. 57% and 43% respectively)[24(a)] is more harmful than the other. More over BUF contains more cholesterol than BF i.e 727 and 455 mg/100g fat respectively[24(b)]. The vegetariants mostly think that only fats from meats are harmful and BUF is quite harmless. With this idea they consume any preparation in BUF with out any limit which in due course may make them suffer from CHD. In order to understand the reasons for this we designed the following experiments in which we have used these two fats at high level in the diets for two groups of rats for a period of three months. Further we also wanted to know which of the three vegetables, garlic, onion and amla could effectively ameliorate the deleterious effects of the two animal fats on the serum and tissue parameters. Therefore we incoorperated each of them along with the high fat diets and conducted the experiments.

Materials and Methods

The various chemicals employed for different procedures were of analytical grade supplied by BDH (Glaxo Laboratories), E. Merck and Sigma Diagnostic (India) Pvt. Ltd. Beef fat was bought from a butcher's a shop. It was heated on a pan and the melted fat was filtered through a cloth and collected for the use. Commercially available BUF was purchased for the present work from a shop. Locally bought garlic, onions and amla were used. The two former were skinned, sliced and dried in an oven and powdered. Amla was also sliced, dried and powdered as above.

Experimental animals—Adult albino rats (Sprague Dawley Strain) 9-12 months old and weighing around 250g were selected and divided into nine groups containing 5 rats in each group, for different dietary studies.

Diet preparation—Normal rat feed (GoldMohur) supplied by Lipton India Ltd Calcutta was fed to control of group1, in measured quantities and it was found that a rat consumed an average weight of 14 g feed daily. The normal rat feed was powdered and mixed with each type of fat so as to get 21% fat in the diet for control rats of groups 2 and 6, and similar high fat diets were mixed with 5% garlic/amla/onion powder as the case may be for the test groups 3,4 and 5 in BUF series and for the test groups 7, 8 and 9 in the BF series. The mixture of feeds were wetted with a little water and made into balls and dried in an oven for feeding it daily. Water was supplied in bottles to each group. Only 70g feed of any type was given to each group so that the controls and tests were pair fed. Details of the groups are given below:

Group 1	Normal control	Only normal rat feed and water *ad libitum.*
Group 2	Butter fat control	21% BUF + 79% normal rat feed mixture and water *ad libitum*
Group 3	Garlic test	21% BUF + 74% normal rat feed + 5% garlic powder mixture and water *ad libitum.*
Group 4.	Amla test	21% BUF + 74% normal rat feed + 5% Amla powder mixture and water *ad libitum*
Group 5	Onion test	21% BUF + 74% normal rat feed + 5% Onion powder mixture and water *ad libitum*
Group 6	Beef fat control	21% BF + 79% normal rat feed mixture and water *ad libitum*
Group 7	Garlic test	21% BF + 74% normal rat feed + 5% garlic powder mixture and water *ad libitum*
Group 8	Amla test	21% BF + 74% normal rat feed + 5% amla powder mixture and water *ad libitum*
Group 9	Onion test	21% BF + 74% normal rat feed + 5% Onion powder mixture and water *ad libitum*

After three months of feeding the rats were sacrificed after over night fasting. Their blood was collected in centrifuge tubes by cutting the jugular vein and the serum was separated after an hour. It was used for the estimation of lipid parameters and enzyme activities. Their liver, heart and kidneys were also collected and preserved in ice cooled beakers for various estimations. Kits provided by Sigma Diagnostics Pvt. Ltd were used for lipid and enzyme estimations according to standard methods. Extraction of tissues were carried out as mentioned below for various estimations.

Extration for cholesterol and triacyl glycerol (TAG)—Accurately weighed (0.5g) tissue was ground with 4g of anhydrous sodium sulphate using mortar and pestle. An extract using chloroform methanol mixture (1:1) was made by 1:5 volume and diluted to 20ml and centrifuged. 2ml of this supernatant was evaporated and redissoleved in 1ml acetic acid. 0.05 ml of this extract was used for the estimation of total cholesterol. 0.01 ml of the extract was used for the estimation of TAG. Serum VLDL +LDL cholesterol was determined by subtracting HDL cholesterol from total cholesterol.

Extraction for thiobarbituric acid reacting system (TBARS)—Accurately weighed 0.5g tissue was ground in a mortar with a pestle. 4.5ml of 0.5% cold TCA was added and mixed well. 1.0ml of this homogenate was used for TBARS which is that part of the products of lipid peroxidation viz; malondialdehyde (MDA) that reacts with thiobarbituric acid.

Extraction for HMGCoA reductase enzyme (EC1.1.1.34)—Accurately weighed 0.5g tissue was ground in a mortar with a pestle under cold conditions. A 10% homogenate was prepared by adding 4.5ml of saline arsenate (1g of sodium arsenate/L of normal saline). This homogenate was used for the assay of the enyzme.

Extraction for AST (EC 2.6.1.1) and ALP (EC3.1.3.1)—Accurately weighed 0.5g tissue was ground in a mortar with pestle under cold conditions. 2ml of phosphate buffer (*p*H 7.4) was added and centrifuged in a referigerated centrifuge at 2000g. The supernatant was used for the assay of enzymes. Serum lipid parameters such as triacylglycerol (TAG)[25], total cholesterol,[26] HDL cholesterol and VLDL + LDL cholesterol[27] and serum enzymes such as aspartate transainase (AST)[28] and alkaline phosphatase (ALP)[29] were estimated by standard methods.

Tissue homogenates from liver, heart and kidney prepared as above were used for the estimations of lipid parameters,[25,26] TBARS[30] and enzymes such as AST[28], ALP[29] and HMGCoA reductase [31]. A modification for expressing the activity of the last enzyme was used by which the ratio of Mevalonate/HMGCoA is calculated which gives the activity of the enzyme directly [32].

Results and Discussions

The results on the lipid profile and two enzymes AST and ALP in serum are given in Table 1. It is observed that the diets containing BUF and BF increased very significantly the lipid profile i.e total cholesterol, TAG and VLDL +LDL cholesterol and also the two marker enzymes i.e. AST for CHD and ALP for liver diseases. The incorporation of any of garlic/amla/onion in the high fat diets significantly ameliorated the deleterious effects of these animal fats and in addition the former two vegetables significantly increased HDL cholesterol which has a protective action against CHD. The atherogenic index i.e the ratio of total cholesterol/HDL cholesterol in both the fat fed groups increased more than double i.e from a normal value of 1.7 to 3.7. However as anticipated the incorporation of any of the above vegetables in the diet decreased this index towards normal i.e 2-2.6 range. We may note that the damage is not completely prevented by any of the above vegetables but their use may lessen the atherogenic effects of the animal fats in our diets. It appears that

Table 1 — Serum TAG, cholesterol, VLDL +LDL,HDL cholesterol (mg/dl) and AST and ALP activities (IU./L, KAU/L)

[Values are mean ± SD of 5 rats in each group]

	Groups	TAG	Total Cholesterol	HDL Chol.	VLDL+LDL Chol.	AST (IU/L)	ALP (KAU/L)
1	Normal control	7.02±0.151	72.27±2.016	42.47±0.540	29.80±0.891	26.59±0.773	9.79±0.430
2	BUF	16.91±0.434*	166.0±3.673*	42.68±1.279 (N.S)	123.27±2.973*	57.13±1.127*	13.97±0.592*
3	BUF + garlic	12.34±0.234†	125.42±2.324*	48.153±0.633*	77.27±2.298*	32.21±0.697*	10.43±0.380*
4	BUF + amla	12.08±0.264†	128.98±1.804*	43.929±1.426‡	84.85±2.243*	43.44±1.044*	11.50±0.301*
5	BUF + onion	14.86±0.401†	142.96±3.20†	42.508±1.064 (N.S)	100.45±2.84†	46.34±1.26*	12.29±0.275†
6	BF	14.18±0.306*	160.17±2.94*	42.325±0.515 (N.S)	117.85±3.009*	41.79±0.156*	12.68±0.253*
7	BF + garlic	7.58±0.199*	81.53±2.127*	49.098±1.064*	32.43±0.512*	21.66±0.571*	10.43±0.309*
8	BF+amla	8.88±0.217*	89.06±0.848*	45.479±0.326*	43.58±0.476*	24.19±0.64*	10.92±0.292†
9	BF + onion	12.88±0.270 (N.S)	109.70±2.240	41.362±0.963 (N.S)	65.85±1.707*	34.27±1.003†	11.241±0.243†

Student's t-test

‡ $P<0.05$, † $P<0.01$, * $P<0.001$ as compared to their control i.e, normal is control for BUF and BF groups and these are controls for the corresponding test groups 3,4,5, and 7,8,9 respectively. Values of atherogenic index are given in the text. The order of activity is garlic >amla>onion.

BUF is more harmful than BF in their hyperlipidemic and related effects i.e with respect to the two enzymes studied. The order of the beneficial effects of the vegetables used is garlic>amla>onion.

The results on lipid profile, lipid peroxidation in terms of TBARS and the enzymes AST, ALP and HMGCoA reductase of liver are given in Table 2. Here also we get a some what similar effects of the animal fats as observed above and also on addition of any of the three vegetables in the diet.

Most of the altered parameters are ameliorated significantly and the lipid profile was better controlled than the enzyme levels on incorporation of any of the three vegetables in the high fat diets. In treated groups

Table2—Lipid parameters (mg/100g wet tissue), TBARS (μ mol MDA/g wet tissue), HMGCoA reductase, AST and ALP enzyme activities (Mevalonate / HMGCoA ratio, IU and KAU/100g wet tissue respectively) in Liver

[Values are mean ± SD of 5 rats in each group]

Groups	Total cholesterol*	TAG*	TBARS*	HMGCoA reductase	ALP	AST
1 Normal	345.6±9.702	389.41±10.25	12.56±0.352	0.476±0.022	5.568±0.154	40.32±1.18
2 BUF	776.84±23.30	826.57±24.89	27.62±0.740	1.06±0.033*	8.231±0.236-*	62.83±1.84*
3 BUF +garlic	594.67±15.84	663.02±18.20	18.94±0.560	0.909±0.023†	5.899±0.168*	50.33±1.48*
4 BUF+amla	643.59±19.36	699.80±20.90	19.59±0.540	0.913±0.024‡	6.42±0.182*	53.59±1.58*
5 BUF+ onion	690.82±20.79	757.40±22.72	21.3±0.624	1.01±0.032‡	7.37±0.208†	57.35±1.69†
6 BF	613.29±17.39	702.14±18.62	23.75±0.685	1.06±0.042*	7.63±0.215*	59.27±1.74
7 BF+garlic	375.93±10.92	460.13±12.24	16.43±0.471	0.95±0.031‡	6.090±0.178*	49.47±1.42*
8 BF+amla	402.65±11.75	475.21±14.18	17.81±0.487	0.93±0.025‡	6.28±0.175*	51.056±1.473*
9 BF+onion	472.22±13.57	560.71±14.87	19.37±0.557	0.96±0.022‡	6.97±0.182†	54.60±1.59†

Students t-test:- *All these 3 parameters are very significantly altered ($P<0.001$) by fats and drugs. Other parameters are altered at various levels only.

‡$P<0.05$, † $P<0.01$, * $P<0.001$ as compared to their control i.e. Normal is control for BUF and BF groups and these are the controls for the corresponding test groups 3,4,5 and 7,8,9 respectively. In columns 2-4 all the values are significantly different from their control at a significant level of $P<0.001$. For the other values in columns 5-7 different notations are given for significant changes of each value. The order of activity is garlic>amla>onion

Table 3—Lipid parameters (mg/100g wet tissue) TBARS (μ moleMDA/g wet tissue) and AST and ALP enzyme activities IU and KAU/100g. wet tissue of heart respectively.

[Values are mean ±SD of 5 rats in each group]

Groups	TBARS*	Total Cholesterol*	TAG*	AST*	ALP*
1 Normal	9.56±0.146	150.35±2.5	44.72±2.6	45.27±1.7	3.12±0.23
2 BUF	24.52±0.56	286.83±5.7	72.79±2.3	81.14±2.3	7.11±0.32
3 BUF+Garlic	15.5±0.30	196.83±8.5	55.50±3.7	54.72±1.6	5.26±0.50
4 BUF +Amla	16.86±0.32	197.74±5.8	57.04±1.7	58.22±1.68	5.39±0.16
5 BUF+Onion	19.73±0.26	243.43±7.3	60.30±1.7	65.98±1.8	6.03±0.18
6 BF	20.35±0.24	250.32±7.4	63.23±1.8	74.46±4.4	6.07±0.23
7 BF+Garlic	14.5±0.20	168.91±5.05	41.81±2.6	60.91±2.1	4.56±0.35
8 BF+Amla	15.68±0.17	168.74±5.01	40.72±1.22	65.46±1.3	5.23±0.13
9 BF+Onion	18.38±0.26	222.67±6.6	48.83±4.2	71.22±1.6	5.59±0.16

Student's t-test:- *$P<0.001$ as compared to their control i.e. Groups 2 and 6 vs 1 and groups 3,4,5, vs 2. and groups 7,8,9 vs. 6 The order of activity is the same as in other tables

Table 4—Lipid parameters (mg/100g. wet tissue), TBARS (μmol MDA/g wet tissue) and HMGCoA reductase (Mevalonate/ HMGCoA ratio)/100g wet tissue of kidney
[Values are mean ± SD of 5 rats in each group]

Groups	Total Cholesterol*	TAG*	TBARS*	HMGCoA reductase*
1 Normal	358.42±10.75	373.62±11.21	17.01±0.51	0.60±0.02
2 BUF	575.47±17.26	1124.65±33.74	31.40±0.942	1.58±0.04
3 BUF+Garlic	440.05±13.2	501.02±15.03	20.45±0.614	0.99±0.03
4 BUF+Amla	466.67±14.0	685.11±20.55	22.71±0.681	1.05±0.05
5 BUF+Onion	494.10±14.82	998.34±29.95	23.47±0.704	1.20±0.06
6 BF	434.01±13.02	716.40±21.49	28.22±0.847	1.15±0.05
7 BF+Garlic	369.23±11.08	431.20±12.94	18.95±0.569	0.826±0.03
8 BF+Amla	385.25±11.5	500.12±15.0	19.90±0.597	0.980±0.04
9 BF+Onion	405.32±12.16†	654.28±19.63†	22.42±0.673†	1.08±0.05†

Student's t-test:- †$P<0.05$ as compared to their controls. In all the columns except the BF+onion values all other values are highly significantly different from the corresponding controls as in previous tables. *$P<0.001$. The order of activity is as in other tables.

a significantly reduced level of HMGCoA reductase, the rate limiting enzyme in cholesterol synthesis may account for the fall in serum and tissue cholesterol. Here also the order of the curative effects of these vegetables is the same as stated above.

In Table 3 the effects of the two animal fats and the ameliorating effects of the above vegetables on lipids, lipid peroxidation and two enzymes of heart tissue viz; AST and ALP are given. The effects of the two fats on heart tissue are similar to that in liver i.e the lipid profile, TBARS and enzyme activities are increased very significantly and on incorporation of any of the above vegetables in the diets at 5% level significantly counteracted the deleterious effects of the fats and the order of the curative actions of the vegetables is in the same manner as stated earlier. An addition of garlic at 5% level to a high sucrose diet of rabbits was found to be beneficial in a previous work to counteract the deleterious effects of sucrose[33], that is why we also fixed the 5% level of the vegetables in the diets of the rats for getting a readable effect.

In Table 4 the lipid profile, TBARS and HMGCoA reductase activity of the kidney tissues of the above groups of rats are given. As is the case with other tissues both the high fat diets increased significantly all the above parameters in the kidney also and all the three vegetables when incorporated separately as components of the high fat diets, each of them counteracted the deleterious effects of the fats significantly.

In all the combinations of the vegetables with BUF and BF except that with onion and BF the curative effects of them were highly significant ($P<0.001$). However in the case of onion with BF the effects were just significant ($P<0.05$). All these data clearly demonstrate that all the three vegetables are antiatherogenic in action and the order of their action is garlic>amla>onion.

The hyperlipidemic and particularly the hypercholesterolemic effects of the animal fats may be due to the higher percentage of saturated fatty acids. i.e. 57-64% in them. A richer content of cholesterol in BUF as compared to BF may account for a greater hypercholesterolemic effect of the former. An enhancement in the activities of the three enzymes we measured in fat fed rats may be due to various reasons, viz; as a result of stimulation of different metabolic pathways leading to the synthesis of TAG and cholesterol from dietary fats and also from the interconversion of aminoacids and breadkdown of phospholipids and related compounds under a stress of high fat diets. Under such conditions lipid peroxidation may have also increased leading to a rise in TBARS. Such a rise in TBARS is partly due to the high percentages of unsaturated fatty acids, say

36% in BUF and 43% in BF. The vegetables used here are rich sources of hypolipidemic and antioxidant agents, viz, alkyl derivatives of cysteine sulphoxide, disulphides, polysulphides and their sulphoxides, e.g. allicins and ajoene type compounds in garlic and onions[34,35] and flavonoids, gallotannins, ellagic acids, phyllemblic acids and related polyphenols in amla (Emblica officinalis) are strong antioxidants. More over amla contains ascorbic acid also which is also a strong antioxidant. However more than a source of vitamin C, amla is reputed for the other antioxidants mentioned above which show many medicinal effects as reported elsewhere.[23] All these results emphasise the need for incorporation of these vegetables in our daily diet as a prophylactic measure to protect our body from atherosclerosis and related diseases.

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