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Protection from hypercholesterolemia and atherosclerosis by regular consumption of grape, apple or the extract

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Abstract: This study show how far regular consumption of either grape or apple dry matter or extract protects from hypercholesterolemia. Hypercholesterolemia was produced in animals by feeding high fat and cholesterol diet. The lipid parameters were followed after feeding on diets containing apple or grapes either dry matter or extract. The total polyphenols as tannic acid in apple was 464.77 mg/100g dry weight and for grapes it was 353.63 mg/100g dry weight The total isoflavonoids as catechin in apple was 1000 mg/100g dry weight and for grapes it was 1500 mg/100g dry weight.

Hyperchlesterolemic rats fed on diet containing 20% dry matter of apple or the methanol extract showed a change in lipid parameters towards normalization. There was a drop in cholesterol, LDL and VLDL contents, however not reaching normal level with slight difference between dry powder and the extract. An improvement in plasma triglyceride and lipid peroxide values was also noticed. The activities of SOD and catalase were also improved.

Rats fed on diet containing grape extract also showed improvement of lipid parameters towards normalization. There was a decrease in plasma cholesterol, LDL and VLDL contents. An improvement in plasma triglyceride, lipid peroxide and activities of each of SOD and catalase was noticed.

The HPLC analysis of the methanol extract of each of apple or grapes showed the presence of different polyphenol compounds with different concentrations.

The conclusion is that apple and grapes either dry matter or extract contain a lot of polyphenol compounds with antioxidant power that can protect from the harmful effect of consuming high fat and cholesterol diet. Either the dry matter or the extract exerts this health value on consumers.

Key words: Hypercholesterolemia, apple, grape, polyphenols, antioxidants, lipids.

Introduction

Several investigations have reported the link between oxidative stress and occurrence of each of atherosclerosis and cardiac diseases. Increased oxidant stress, resulting from both increased oxygen free radical production and decreased nitric oxide generation, appears to play an important role in the chronic inflammatory responses to hypercholesterolemia and atherosclerosis (1), (2).

Phenols are among the compounds that contribute to the antioxidant mechanisms in animals and participate in protection against diseases. Fruits such as apple and grapes are good source of nutrients such as vitamin C and folic acid, yet they are also excellent sources of polyphenols and dietary fiber (3). Grapes next to apples have been crowned the queen of fruits. It was stated that Grapes are good for all dyspeptic conditions,

febrile conditions, liver and kidney troubles, tuberculosis of the lungs and bones, hemorrhoids, varicose veins, osteomyelitis, gangrene, cancer, a great many other malignant diseases (4). Apple contains flavonols and flavanoids, querectin, rutin, epicatachin and catachin (5). Whole grapes with the skin and seeds contain the polyphenolic compounds resveratrol which proved to lower serum lipids and prevent cancer activity (6), (7), (8).Grape seed particularly the slimy films that surround the seed are rich in procyanidins or condensed tannins that were found to scavenge superoxide radical and trap hydroxyl radicals thus prevent both iron promoted and ultrasound-induced lipid peroxidation in vitro (9).

It is thus expected that regular consumption of these fruits can participate in protection from several diseases particularly those occurring due to oxidative stress as most chronic diseases. However, still it is needed to explore the type, variety, quantity and form of these food items. Example, whether an extract from the food source given as dietary supplement be more effective than the main source. It has been reported that apple polyphenols contributed less than 20% to the total antioxidant capacity of aqueous apple than extract (10).

The present study tries to explore the possibility of preventing or treating metabolic abnormalities due to high fat and cholesterol consumption through regular intake of dried powdered apple or grape extract fruits. In order to clarify the possible advantage contributed by concentrating the effective compounds in dried matter and the role of extraction. Apple meal was extracted with methanol and the effect of the extract was compared with that of the dried powder.

Material and methods.

Fruits namely apple (Molus punike) and grape (Vitis Vinifera) were purchased from the local market. Diet ingredients either casein vitamin or salt components were all extra pure grades. Kits used for the estimation of the biochemical parameters were obtained from Stainbo laboratory, Boerne Texas 78006 USA.

The apple fruit was cut into slices then placed over a perforated tray, put in the air drying oven at 60° C and left till complete dryness then ground into fine powder. The powder was kept in polyethylene bags, sealed and kept in the deep freeze at – 20° C till used. Grapes were squeezed into a fruit mixer, the whole juice including the skin and seeds were spread on trays and dried the same way as apple samples.

Extraction of the apple was done with ethanol in a soxhelt apparatus as described by (11).

Preparation of diets.

The composition of the standard diet prepared according to (12), and the composition of the experimental high fat and cholesterol diet is shown in table (1), (2). Salt and vitamin mixtures were prepared (13). The soluble vitamins A, E, D & k were prepared as indicated in (12). Animals used in this experiment were female Sprague dawley albino rats of body weight ranging between 80-100 g obtained from the Central animal house of the National Research Center.

Table (1): Composition of Control diet.

Ingredient	Amount g/kg
Casein	120
Sucrose	100
Hydrogenated fat	100
Oil	50
Salt mixture	35
Vitamin mixture	10
L-cystin	1.8
Choline chloride	2.5
Starch	580.7

Table (2): Composition of high fat and high cholesterol diet.

Ingredient	Amount	
	HF/HC	HF/HC containing 20% dry matter
Casein	120	120
Sucrose	100	100
Cow fat	200	200
Cholesterol	20	20
Salt mixture	35	35
Vitamin mixture	10	10
L-cystin	1.8	1.8
Choline chloride	2.5	2.5
Starch	510.7	200g dry matter

Dry matter was added on the expense of starch.
HF: High Fat.
HC: High Cholesterol.

Experimental design.

Rats were divided into 3 groups

Group 1: 8 animals given the normal standard diet.
Group 2: 24 animals given the high fat and cholesterol diet.

The feeding process lasted for 4 weeks. Blood samples were then taken from each rat after an overnight fasting (0.5 ml) from the suborbital vein using small fine capillary tubes. Serum cholesterol level was estimated to ensure hypercholesterolemia. Animals in this group were subdivided into 3 subgroups each of 8 litters.

Subgroup 1: given diet contain 20% apple dry matter.
Subgroup 2: given diet contain Apple extract equivalent 20% apple dry matter.
Subgroup 3: given the diet that contains 20% of the dried grapes.

At the end of the experiment that lasted for10 weeks rats in all groups were fasted overnight, blood was collected into clean heparinized centrifuge tubes kept to stand at cold for 15 minutes then centrifuged at 3500 rpm for 15 minutes. Plasma samples were kept in the deepfreeze at -20° C till analysis.

Methods of Analysis.

Plasma triacylglycerol level was determined according to (14), total cholesterol according **to (**15**)** .HDL cholesterol according to the method of **(**16**).** LDL cholesterol by enzymatic colorimetric method according to (17). Lipid peroxide was estimated according to the method of (18) RBCs catalase activity according to (19) and superoxide dismutase determined according to the method described by (20).

Determination of polyphenols as tannic acid was done according to the procedure described in the AOAC (21). Isoflavonoids as catechin was done according to the method of (22).

Phenolic compounds of plant samples were extracted according to the method outlined by (23).

Identification of individual Phenolic compounds of the samples was performed *on* JASCO HPLC apparatus, using hypersil C reversed-phase column (250 x 4.6 mm) with 5u particle size.

Results

The general analysis of apple showed moisture content of 86%. Ash content 2.19%, fat content was 1.37% and protein content 1.75%.

The total polyphenols present as tannic acid is 464.77 mg/100g dry weight, (table 3). The total isoflavonoids determined as catechin (table 5) is 1000 mg/100g dry weight.

Table (3): The Average Composition of Apple and Grape.

English Name	Water Content g%	Ash g%	Fat g%	Protein g%
Apple	86	2.19	1.37	1.75
Grape (Fauomy)	89.5	3.11	0.44	1.31

Table (4): Total Polyphenol as Tannic acid.

English Name	Total polyphenol as Tannic acid	
	Dry Weight mg /100g	Fresh weight mg/100g
Apple	464.77mg	65.07mg
Grape	363.63mg	49.89mg

Table (5): Total Isoflavonoid as Chatechin.

English Name	Total Isoflavonoid as Chatechin	
	Dry Weight mg/100g	Fresh Weight mg/100g
Apple	1000mg	140mg
Grape	1500mg	157.5mg

The general analysis of grape showed moisture content of 89.5%, ash content 2.11%, fat content 0.44% and protein content 1.3 l% of edible portion (table 3.).

The total polyphenols present as tannic acid was 353.63 mg/100g dry weight (table 4). The total isoflavonoids determined as catechin (table 5) was 1500 mg/100g dry weight.

The values of the biochemical parameters determined in this study for groups of control rats are shown in tables (6&7). Rats fed on diet containing 20% dry matter of apple or the methanol extract showed a change in lipid fractions towards normalization. There was a drop in T.cholesterol, LDL and VLDL contents. The values obtained were 224.88 ±14.03, 70.00 ±5.10 and 18.58 ±1.89 mg/dl respectively for rats given dry matter and 217.75 ±12.98 and 59.75 ±2.05 and 19.01 ±1.67 mg/dl respectively for rats given the extract equivalent. The high density lipoprotein (HDL) was 52.5 ±6.1 mg/ dl for rats given the dry powder and 44.o ±2.06 mg /dl for those given the extract. These values were still far from normal control with slight difference between dry powder and the extract (table 8). An improvement in plasma triglyceride and lipid peroxide values was noticed (table 9) when apple dry matter or its extract equivalent were given with the diet. The activities of SOD and catalase were also improved. The values turned towards normal particularly the activity of superoxide dismutase.

Table (6) Lipid fractions of the control groups.

Group		Total Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control I	**M**	67.63	22.75	30.00	13.35
	+S.E	2.07	1.05	1.77	0.828
Control II	**M**	401.86	26.04	108.29	36.74
	+S.E	13.99	1.95	13.61	1.56

*Control I: Rats fed on normal diet.

*Control II: Rats fed on high cholesterol –high fat diet

Table (7) Triglycerides and lipid peroxide as well as SOD and Catalase activity for control groups.

Groups		Triglyceride mg/dl	Lipid Peroxide Mmol/ml	SOD U/ml	Catalase Activity U/ml
Control I	M ±S.E.	76.13 3.96	1.949 0.113	489.87 15.89	9.97 0.19
Control II	M ±S.E.	184.00 7.83	4.94 0.13	120.66 12.87	1.92 0.12

*Control I: Rats fed on normal diet.
*control II: Rots fed on high cholesterol –high fat diet.

Table (8): Lipid Fractions in Plasma of rats fed on diet containing 20% apple dry matter or its extract.

Groups		Total cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Group 1 Apple (dry matter)	M ±S.E P1 P 2	224.88 14.03 0.000 0.000	52.50 6.10 0.014 0.001	70.00 5.10 0.000 0.016	18.00 1.89 0.000 0.000
Group 2 Apple (extract)	M ±S.E P1 P2	217.75 12.98 0.000 0.044	44.00 3.06 0.012 0.001	59.75 2.05 0.000 0.002	19.01 1.67 0.009 0.000

*Group 1:Rats fed on dry matter.
*Group2:Rats fed apple extract.
*P1: Significant against Control I.
*P2: Significant against Control II.

Table (9): Triglyceride and Lipid Peroxide, as well as and Catalase activates in rats fed on diet containing 20% apple dry matter or its extract.

Groups		Triglyceride mg/dl	Lipid Peroxide mmol/ml	SOD Activity U/ml	Catalase Activity U/ml
Group 1 Apple (dry matter)	M ±S.E P1 P2	92.88 9.45 0.124 0.000	2.45 0.14 0.007 0.000	315.28 13.12 0.000 0.000	8.73 0.11 0.000 0.000
Group 2 Apple (extract)	M ±S.E P1 P2	96.38 8.15 0.04 0.000	2.72 0.13 0.000 0.000	383.69 10.92 0.000 0.000	7.30 0.17 0.000 0.000

*Group 1:Rats fed on dry matter.
*Group2:Rats fed apple extract
*P1: Significant against Control I.
*P2: Significant against Control II

Table (10): Lipid fractions in plasma of rats fed on diet containing 20% grape extract.

Groups		Total cholesterol Mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Group 3 Grape (extract)	M ±S.E P1 P2	161.38 18.14 0.000 0.000	34.00 4.46 0.018 0.000	47.38 6.47 0.021 0.000	13.88 0.97 0.687 0.000

*Group 3: Rats fed on grape extract.
*P1: Significant against Control I.
*P2: Significant against Control II

Table (11): Triglyceride and Lipid peroxide, as well as SOD and Catalase activities in rats fed on diet containing 20% grape extract.

Groups		Triglyceride Mg/dl	Lipid Peroxide mg/dl	SOD Activity mg/dl	Catalase Activity mg/dl
Group 3 Grape (extract)	M ±S.E P1 P2	96.38 3.61 O.300 0.000	2.12 0.23 0.001 0.000	439.25 13.12 0.000 0.000	8.38 0.22 0.028 0.000

*Group 3: Rats fed on grape extract.
*P1: Significant against Control I.
*P2: Significant against Control II

As shown in table (10) rats fed on diet containing grape extract equivalent to 20% dry powder caused a change in lipid fractions towards normalization. There was a decrease in plasma T.cholesterol, LDL and VLDL contents, the values obtained were 161.38 ±18.14, 47.38 ±6.47 and 13.88 ±0.97 mg/dl respectively. These values are still far from normal values.

An improvement in plasma triglyceride and lipid peroxide values was noticed table (11) when grape extract was given with the diet. The activities of SOD and catalase were also improved.

The HPLC analysis of the methanol extract of each of apple or grapes (table 12) showed the presence of different polyphenol compounds with different concentrations. Phenol, galangin, catechin, daidzin, genstin, dazein, euganol and chrysin were detected in apple extract. P-hydrpxy-benzoic acid, vanillin and 3, 5 dimethyl-benzene, were detected in grape. Rutin and salicylic acid was detected in both apple grape extract while p-coumaric acid was found in apple. Pinostrobin was found in apple and grape. Apple contained relatively high level of cinnamic, salicylic, genstin, phenol and catachin 4.80, 265, 285, 110 and 110 mg/100g. The grape major polyphenol compounds were catechin (461 mg/100g), daidzin (250mg/100g) and vanillin (175mg/100g).

Discussion

The health benefits of either apple or grapes are attributed to certain compounds found in these fruits, such as pectin, sugars and phytochemicals, mainly flavonoids. Regarding the effect on lipid metabolism, the fiber and flavonoids seems to be most contributing. The effect of fiber resides in its capacity to absorb dietary fat, thus avoid its hazard on health, while that of flavonoids is due to its antioxidant capacity (24), (25).

The HPLC analysis of either grape or apple extract revealed the presence of a group of polyphenols and anthocyanines as shown in table (12) Figs. (1&2). The presence of such compounds in either apple or grape juice with antioxidant character was reported before in several articles (26), (27).

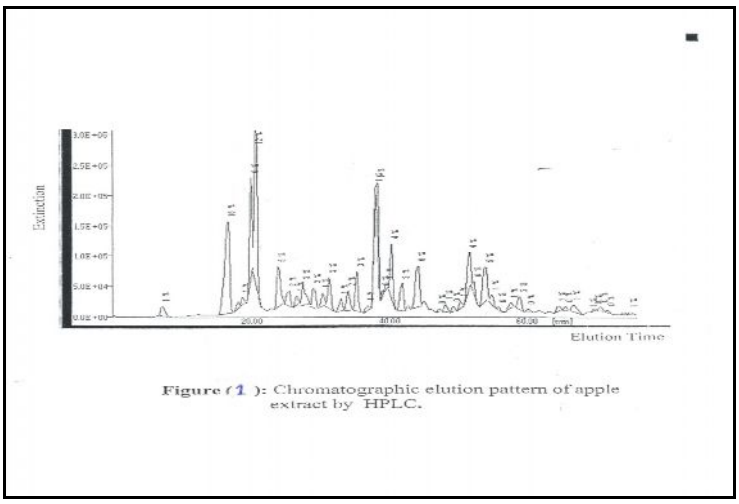

Figure (1): Chromatographic elution pattern of apple extract by HPLC.

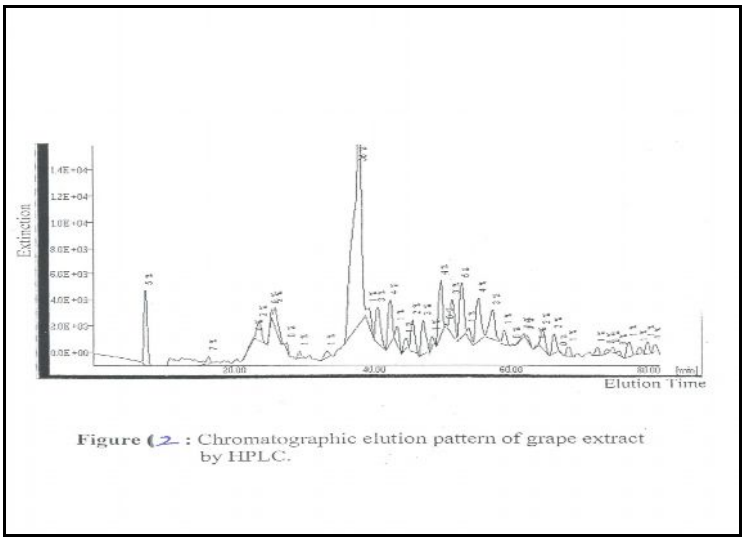

Figure (2 : Chromatographic elution pattern of grape extract by HPLC.

Table (12): HPLC analysis of polyphenols in methanol extract of apple and grape.

NO.	Ingredient	Apple mg/100g	Grape mg/100g
1	Pyro-gallic	---	---
2	Gallic acid	---	---
3	P-oH-benzoic acid	---	---
4	Catachin	100	4610
5	Caffic acid	---	---
6	phenol	110	95
7	Daidzin	50	250
8	Vanillin	---	175
9	Rutin	85	2
10	P-Coumaric	130	---
11	Genstin	285	15
12	3,5-dimethoxybenzel	---	---
13	Salicylic	265	25
14	Fuerulic	190	---
15	Dadzein	15	0.5
16	Cinnamic	480	---
17	Quercetin	45	4.5
18	Genstein	95	---
19	Kaempherol	---	---
20	Euganol	1	0.2
21	Chrysin	5	---
22	Galangin	1.5	0.05
23	Pinostrobin	20	2

The nutritive value of grapes is due to its content of simple carbohydrates, vitamins and minerals, however, ancient Egyptians praised it for its medicinal qualities. Most research articles done in this area are concentrating on the health benefits of wine made from grape fermentation particularly red wine usually taken with food. This is usual and familiar in Western countries, where wine consumption is prevalent. However, in Eastern countries, particularly in Moslem ones, the grape fruit is commonly consumed fresh. There are few articles attributed some health benefits of wine to the alcohol content. However, Vinson et. al., (28) found that grape juice had a significant benefit at a much lower dose of polyphenols than the wine. The benefit is related to the antiatherogenic effect, lipid improving action and antioxidant power. It is important to stress that grapes has to be taken as a whole since it was reported in a previous study that phytocompounds are distributed in all parts of the plant, seeds contain 65% of the polyphenols of the bunch, the stem 22%, the skin 12% and the pulp just 1% (29). Apple is also a rich source of polyphenols and other health compounds that proved to be beneficial in several health problems (30).

The improvement in lipid parameters of hyperlipedimic rats included in this study due to inclusion of either apple (dried or extract) or dried grape in their diet can thus be attributed to the same compounds present in these sources that exert the health improving effect.

It has been reported that oxidized LDL is formed due to free radical, generated oxidant reactions (31) .Generation of such oxidant reactions is believed to be pronounced under conditions of high lipid and cholesterol consumption. This oxidized LDL has the tendency to bind to beta$_2$-glycoprotein I and form a complex (oxLDL-β_2GPI) (32), that initiate a process which leads to atherosclerosis (33). A process of initiation of atherosclerosis was reported in these rats .However the results were reported before (34). In another study it was found that apple intake, specifically boiled apple, might reduce the risk of coronary heart disease by inhibiting post occlusion steps, such as myocardial injury after artery occlusion, as well as pre occlusion steps, such as atherosclerotic plaque formation (35). Besides, it was reported that Annurca apple fresh polyphenols enhanced low-density lipoproteins (LDL) receptor binding activity by 40% and led to an increase in Apo lipoprotein A1 (ApoA1) cell expression of 33.3% above control levels confirming a significant hypolipidemic potential thus leading to hypothesise a helpful role in the prevention and care of diseases in subjects affected by metabolic syndrome (36).

Determination of the total polyphenol content of apple as tannic acid showed that it is relatively higher than that of grape, while the isoflavone content of grape determined as catachin is higher than that of apple. Parallel to this, the lowering effect of grape on lipid parameters of rats fed on the diet containing grape is more marked than that in case of rats fed on the diet containing apple powder or extract. This is regarding total cholesterol, LDL and VLDL.The effect on triglycerides, and catalase was more or less similar. However, the improvement in the activity of SOD and the value of lipid peroxide is better in case of grape powder. This may point to a more specific effect of isoflavones, being more effective in ameliorating the action of hyper lipedemia. This does not exclude the finding that the effects of apples likely results from this fruit's content of a variety of antioxidant phytochemicals, especially those from polyphenol (37).Those authors reported that apples have a high percentage of free polyphenols and that this lack of binding to other molecules may produce relatively good polyphenol absorption. The improvement of the activity of superoxide dismutase enzyme due to inclusion of either apple dry or extract and dry grape was reported before (38). They mentioned that the increase in superoxide dismutase 1 activities can be a possible mechanism for apple effects on oxidized LDL., based on other finding that eating one apple per day for about a month raise erythrocyte superoxide dismutase 1 activities which is thought to inhibit LDL oxidation .

Comparison between the dry matter and the extract of apple did not show marked difference with regard to the effect on lipid or the antioxidant parameters. The lipid peroxide was better improved in case of dry matter consumption, the SOD activity was also better. The level of LDL was lower for hyperlipidemic rats fed on apple extract. This shows that the effect of either the dry matter or the extract is variable on different parameters. In general, it can be stated that both treatment either the dry matter or the extract proved to be effective for prevention or treatment of hyperlipidemia and in turn possible atherogenesis that may occur which was mentioned before (34). The advantage of the extract is that it can be used for preparation of dietary supplement that can be of value to provide sufficient quantity of the polyphenols present in this source.

It may be also remarked that no adverse effect was reported for either drying or extraction of apple with regard to the health compounds they contain and this confirm previous similar finding (39).

In conclusion, this study confirm that regular consumption of apple or grapes can improve health, however the dry matter or the extract is another effective means of providing health compounds in concentrating form. It is a form of dietary supplement that can provide the body with several polyphenol compounds with health value and protect from atherosclerosis that occur due to hypercholesterolemia prevalent among people who consume high fat and cholesterol diet.

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