Study the hypolipidemic effect of combination of pectin, niacin and apple cider vinegar supplied as Apple-cure effervescent tablets in rabbits.

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Abstract: In this study, was to investigate the hypolipidemic effect of Apple-cure drug on the hypercholesterolemic-induced new Zealand white rabbits by feeding the animals with normal chow (diet) enriched with 1% cholesterol and 10% corn oil for one month was investigated. Animals with normal and hypercholesterolemic diets through out the study were used as negative (A) and positive (B) control respectively. Moreover, hypercholesterolemic rabbits were treated with two different medications as follow: animals of group (C) were treated with standard antihyperlipidemic drug, Atorvastatin(2mg/Kg/day), while the animals of group (D) were treated with apple-cure drug (3ml/Kg/day of apple-cure tablet solution) daily via oral route for 4 weeks. The results showed that there was no significant change in the body weights of hypercholesterolemic rabbits (B,C) groups but there was a high significant reduction ($p < 0.005$) in the body weights of group (D) rabbits. Blood samples were collected at 0,1,2,3 and 4 weeks after treatment to investigate serum lipid profile. The daily administration of Apple-cure for hypercholesterolemic rabbits (group D) was led to a high significant reduction ($p < 0.005$) in both total cholesterol and triglycerides and significant reduction ($p<0.01$) in the LDL-cholesterol levels, while same administration led to significant increasing ($p<0.01$) in the HDL-cholesterol levels. These results indicate that the Apple-cure drug have a strong hypolipidemic effect through improving the serum lipid profile by decreasing total cholesterol, triglycerides and LDL-cholesterol levels and by elevating HDL-cholesterol level and it may be of greatful value in the treatment of dyslipidemias.

Key words: Apple-cure, atorvastatin, hypolipidemic, hypercholesterolemic rabbits, pectin, niacin, lipid profile.

Introduction

Blood lipid-lowering therapy had a great consideration in last few years due to the theories and clinical evidences that link elevated blood lipid, including cholesterol and triglycerides with up growing incidence of many diseases include; cardiovascular diseases and diabetes mellitus.[(1)]The importance of this issue led to develop a lot of lipid lowering agents were used efficiently in the clinical practice to reduce blood lipids, especially serum cholesterol; due to its connection with development of atherosclerosis [(2)]and atherosclerosis related disorders like coronary ,cerebral and peripheral vascular diseases. [(3)] Many drugs were developed for this purpose; one of famous medication class where HMG-CoA reductase enzyme inhibitors or what they called Statins, that ; found their way to be used widely in clinical practice; [(4)]although their chronic use was associated with side effects like GIT upset; myopathy;

rhabdomyolysis [5,6] and newly cardiomyopathy [7]. Other medications that used to treat hyperlipidemia: like fibrate derivatives which used for treatment of hypertriglyceridemia; Niacin and bile acids sequestering agents all also have a number of GITskin, and hepatic side effects occurred in variable degrees[8]. Due to many side effects of medications; especially for patients with cardiovascular disease that may use several medications at one time, that may leads to several undesirable side effects. Natural agents, especially if they are derived from our diet, that characterized by low or no side effects may take a great attention in next few years to be used for prophylaxis and treatment of major diseases like cardiovascular diseases. One of Such agents are dietary fibers .Many studies have shown the beneficial effects of soluble fibers on body weight management, [9] plasma cholesterol and lipoprotein levels [10].

Pectins consider as a type of dietary fibers that are fermented in the colon [11] are complex carbohydrate molecules and they are used mainly as gelling agents in the food industry, however pharmaceutical and cosmetic uses are also known. Usually Industrial pectins are extracted from citrus peels and apple Pomace[12]. In more recent studies, found that apple pectin may help lowered plasma and liver cholesterol, triglycerides, and apparent cholesterol absorption.[13]Apple pectin has demonstrated a better cholesterol lowering effect than other pectins including orange pectin[14].When a combined apple pectin with apple phenolic, the lipid-lowering effect will be much greater than either apple pectin alone or apple phenolics [13] .

Apple cider vinegar, is rich with organic acids, and phenolic compounds, in addition to amino acids, sugar, minerals [15] .Effervescent tablet of apple- Cure ®, a product of T&D Germany, contains both apple pectin and apple cider vinegar, we expect this product has pronounced effect on blood lipids , and the goal(s) of our study is found out that effect in rabbits .

Materials and methods

Drugs and Kits: Apple-cure® effervescent tablet was purchased from T&D Pharma GmbH, Germany. Total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride estimations were done using enzymatic method Kits, BioLabo, France.

Animal: Twenty male new Zealand white rabbits weighing 1-1.5 Kg were purchased from local markets at Basrah city. The animals were kept at animals house of Pharmacy college, Basrah University. Rabbits were acclimatized for a period of two weeks in the their cages under standard laboratory conditions(Temperature: 20-25 ˚C ; relative humidity: 50-60% and 12 hrs dark/light cycle). All animals were initially fed standard chow and water ad libitum (Table 1) [16,17].

Table 1:The standard chow of rabbits.

Ingredient	g/100g
Protein	13.5
Fat	3
Carbohydrates	50
Fiber	15
Minerals	7
Vitamins	0.25
Water	11.25

Study design: After accumulation period , rabbits were randomly divided into four groups of five animals each. The animals in the group A served as negative control and were fed standard diet (100 g/day); To prepare hyperlipidemic rabbits, the animals in group B served as positive control and were fed standard diet enriched with 1% cholesterol(induce hypercholesterolemia) and 10% corn oil (induce hypertriglyceridemia)[18]. Animals in the group C were fed cholesterol enriched diet and standard antihyperlipidemic drug, Atorvastatin in a dose 2mg/Kg/day by oral catheter; group D rabbits were fed the same cholesterol enriched diet and apple-cure drug in a dose 3ml/Kg/day via oral route after dissolving the apple-cure effervescent tablet in 200ml of water. The study period was for four weeks. The body weights of animals were determined weekly during study period.

Blood sample collection and lipid profile analysis: Overnight fasting blood samples were collected at times(0,1,2,3,4 weeks) from the marginal ear vein to analyze the lipid profile. After collection process, the blood samples were left at room temperature to complete blood coagulation, then serums were isolated from blood clot by centrifugation

Serum total cholesterol(TC), serum triglyceride (TG), high density lipoprprotein (HDL) and low density (LDL) was quantified by spectrophotometric method by using enzymatic method Kit. The absorbance of concerned samples was determined at 500 nm, where the value of total cholesterol was expressed in mg/dl .[19]

Statistical Analysis: Experimental results were mean ± S.D of 12 individuals. Statistical differences between the means of the various groups were evaluated by using one-way analysis of variance(ANOVA) followed

by S-Student test. Data were considered statistically significant only when p value < 0.05

Results

1- Effects on body weights: As shown in the Table(2), there was no statistically significant difference in weight gain for both positive (B) and standard drug (C) groups as compared with negative group (A). The reduction in the body weights was observed in the animals of apple-cure group beginning first week till the end of study period.

Table 2: Effect of apple-cure on body weight in cholesterol- rich diet induced hyperlipidemic rabbits.

Group	Time (week)				
	0	1	2	3	4
A	1180±60.5	1201±89.3	1198±44.2	1214±58.7	1222±32.9
B	1292±71.2	1364±49.5	1386±98.1	1394±72.5	1396±42.5
C	1223±48.8	1263±30.1	1288±72	1306±58.6	1320±44.7
D	1151±83.3	1056±79.4*	992±39.7**	923±66.7***	908±71.2***

Values are given as mean ± S.D for groups of five each

A: negative control; B: cholesterol-rich diet(positive) control; C: Atorvastatin group; D: apple-cure group.

* p <0.05 ; ** p <0.01 ; *** p <0.005

2- Effect on lipid profile:

2.1: Total Cholesterol TC

The sequential changes in the serum total cholesterol for all animals groups were summarized in Table (3) and Figure (1). Cholesterol-rich diet group (B) showed a statistically significant increasing in total cholesterol from first week (p < 0.05) to the end of study period (fourth week, p < 0.001).

The standard drug (Atorvastatin) group was showed a statistically significant reduction in the concentrations of total cholesterol beginning first week (p < 0.05) reaching to the fourth week (p < 0.01). Also, there was a significant reduction in the serum total cholesterol for animals group treated with Apple-cure (D) from the first week (p < 0.05) into fourth week (p <0.001) as compared with animals of positive group (B) .

2.2: Total Triglyceride TG

Elevation in a statistically significant manner was observed in the serum triglyceride levels for positive group (B) beginning first week ($p < 0.05$) reaching to the fourth week ($p < 0.001$) as compared with control (healthy) group (A) as shown in the Table 3 and Figure 2 .Also, the serum triglyceride levels of apple-cure animals group were showed statistically significant elevation at second and third week ($p < 0.01$) and high significant elevation at fourth week ($p < 0.001$).

As shown in the Table (3) there was no statistically significant differences in serum triglyceride levels for Atorvastatin group at 1,2,and 3 week time as compared with control group, but this difference would be significant reduction ($p < 0.05$) at fourth week.

2.3:HDL-Cholesterol

The HDL-C levels were decreased significantly ($p < 0.05$) at fourth week in the blood of high cholesterol-fed diet animals (B) with respect to animals (A) as observed in the Table (3) and Figure (3). The treatment of hypercholestrmic animals group with standard antihyperlipidemic drug, Atorvastatin (C) led to increase the HDL-C levels beginning from 2nd week ($p < 0.05$) to fourth week ($p < 0.001$) as compared with positive group (B). Also, the treatment of hypercholestrmic animals (D) with apple-cure drug was led to increase significantly the HDL-C levels from 1st week ($p < 0.05$) into 4th week ($p < 0.01$).

2.4:LDL-Cholesterol

The elevation of LDL-C levels in the untreated hypercholestrmic group (B) was statistically significant from first week ($p < 0.01$) reaching to the end period of study ($p < 0.001$) as compared with healthy animals (A) as observed in Table (3) and Figure (4). Receiving of Atorvastatin drug (C) was led to reduction the LDL-C levels in a significant manner beginning from second week ($p < 0.05$) into fourth week ($p < 0.01$) .

On the other hand, the animals which administrated the apple-cure drug (D) were showed a significant reduction in the LDL-C serum levels from first week ($p < 0.05$) reaching to the last week ($p<0.01$)as compared with hypercholestrmic animals (B).

Discussion

The essential components of apple-cure® drug are apple cider vinegar, there of polyols (polyphenols) and niacin, vitamin B_6. Apple cider vinegar is produced by fermentation of apple juice and the main content of it is acetic acid, which is present at concentration of 3-5%. Other contents of apple vinegar including some vitamins, minerals, amino acids, organic acids and soluble fibers(pectin).[20,21] The current study showed that daily oral administration of apple-cure solution decreased the elevated triglycerides levels in the hypercholestrmic rabbits. This is consistent with findings by Bender et al[22] and Shishehber et al[23] who reported that oral administration of apple cider vinegar to normal mice and rats has been induced a significant reduction in serum triglycerides levels. The hypotriglyceridemic effect of apple cider

vinegar might be due to the reduction of hepatic triglyceride storage [22] or due to further supported by findings of Fushismi et al [24], who reported that dietary apple vinegar has been reduced the serum triglyceride levels in rats through inhibition of lipogenesis in the liver, together with a concomitant enhancement of fatty acid ß-oxidation.

The observed reduction in serum triglycerides was accompanied with a significant reduction in both total cholesterol TC and low-density lipoprotein LDL-C and increasing in the high-density lipoprotein HDL-C. These results suggest that the hypocholesterolemic effect of apple cider vinegar may be due to its effect on the glycemic index[25], where it has been observed that the lower glycemic index diets are able to increase HDL-C and reduce TC and LDL-C levels.[26,27]

Another possible mechanism for the hypocholesterolemic activity of apple cider vinegar might be due to the presence of soluble fibers(pectins) in it, which are affect either its inhibition of cholesterol and bile acids absorption or due to increased excretion of these neutral and acidic sterols. In fact, the greater excretions of both fecal cholesterol and bile acids in the apple-cure animals group(D) were consistent at beginning from first week of the study. Thus, the reduced absorption of dietary cholesterol and increased excretion of bile acids was directly associated with a low blood cholesterol.[28-30]

Also, the results of present study might be due to the effect of polyphenols that are consisted of apple cider vinegar, where these compounds have been shown to decrease the serum LDL-C levels in healthy humans[31,32] and increase the HDL-C levels in rats and hamsters.[33,34]

Niacin comprises the oldest hypolipidemic drug, in use since 1955. Despite its established broad spectrum effect on lipid profile and the associated reduction in cardiovascular morbidity and mortality. Regarding the niacin-associated reduction in triglyceride and low density lipoprotein cholesterol levels,

suggested that niacin inhibits lipolysis and decreases free fatty acid (FFA) release both via activation of adipose tissue and via inhibition of hepatic triglyceride synthesis. Other mechanisms involving modulation of transcription and translation pathways.[35]

In addition, niacin has been demonstrated to affect high density lipoprotein (HDL) particles remodeling in a number of ways, including reducing cholesterol ester transfer protein levels and activity, increasing apolipoprotein A-I levels, eliminating HDL hepatic uptake, increasing cholesterol efflux via ATP-binding cassette A1, inhibiting hepatic lipase, thereby overall increasing

the plasma residence time of HDL .[35,36]

Group	Time(Week)	Total Cholesterol	Triglycerides	HDL-C	LDL-C
A	0	55±2.7	75±6.3	22±2.1	55±6.2
	1	102±7.5	81±3.3	26±2.4	48±4.8
	2	98±4.3	83±5.3	21±3.0	51±3.2
	3	89±5.2	72±2.2	28±2.6	50±4.4
	4	91±5.6	69±3.1	25±3.5	49±4.3
B	0	105±5.3	68±3.2	27±1.5	52±4.1
	1	210±6.5*	111±6.8*	21±2.0	116±6.7**
	2	330±12.8**	198±8.7**	19±2.1	261±14.7***
	3	486±21.2***	324±15.9***	18±1.8	389±22.0***
	4	612±23.5***	422±19.9***	17±0.7*	451±21.8***
C	0	101±5.2	77±3.8	26±0.8	60±4.8
	1	163±6.6*	126±6.3	28±2.5	109±8.1
	2	189±7.1*	175±11.1	32±3.3*	161±13.4*
	3	218±8.9*	234±17.2	34±2.9*	191±12.6*
	4	258±16.4**	259±12.8*	41±5.3**	202±16.1**
D	0	105±4.2	79±5.2	25±2.8	56±3.7
	1	125±3.5*	98±4.3	30±3.9*	71±5.5*
	2	149±7.5**	76±4.1**	31±3.4*	98±4.6**
	3	171±6.4**	68±2.9**	35±4.1*	110±6.2**
	4	178±8.7***	57±3.7***	38±2.6**	156±8.4**

Table 3: Lipid profiles for rabbits groups at different times.

Fig.1: Cholesterol levels of different experimental groups at study stages

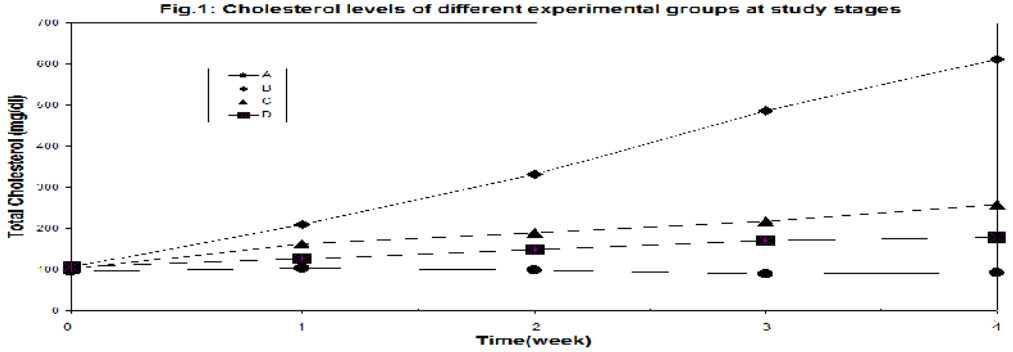


Fig.2: Triglycerides levels of different experimental groups at study stages

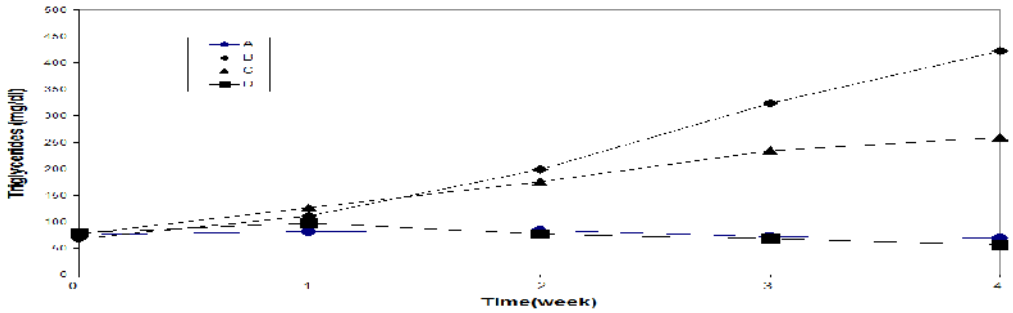


Fig.3: HDL-Cholesterol levels of different experimental groups at study stages

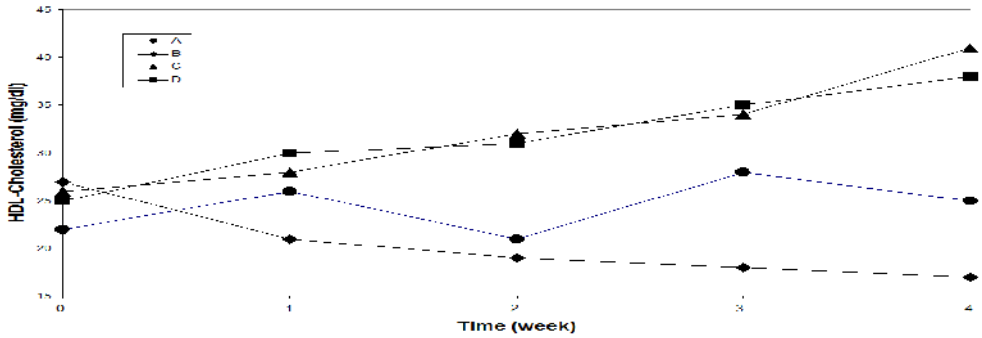
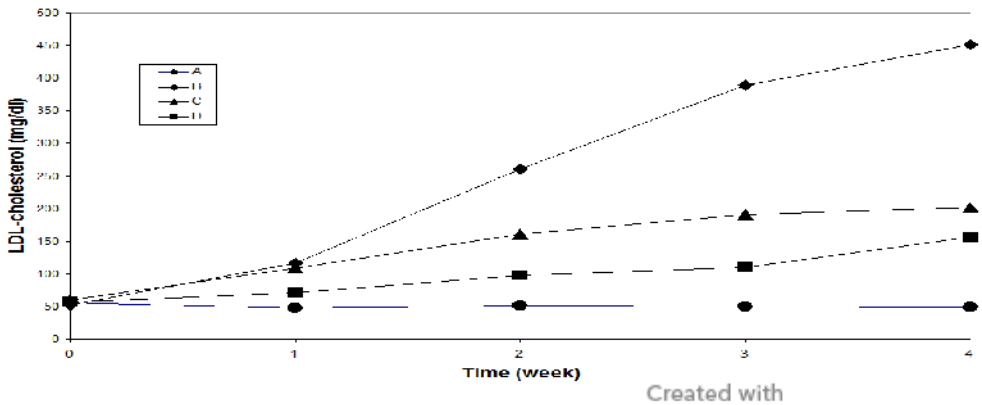


Fig.4: LDL-Cholesterol levels of different experimental groups at study stages



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دراسة التأثير المخفض للدهون في دم الأرانب للمزيج المتكون من البكتين والنياسين و خل التفاح والمجهز بشكل أقراص فوارة من دواء التفاح العلاجي .

باسم جاسم حميد/فرع الكيمياء الصيدلانية والعقاقير/كلية الصيدلة-جامعة البصرة

الخلاصة: تم دراسة التأثير المخفض للدهون لدواء التفاح العلاجي Apple-cure في هم الأرانب التي تعاني من حالة فرط في ارتفاع دهون الدم والمستحدثة من خلال تغذية هذه الحيوانات بعليقة طبيعية مضافا لها الكولسترول بنسبة 1% وزيت الذرة بنسبة 10% ولفترة زمنية قدرها شهر. تم تقسيم الحيوانات الطبيعية والحيوانات التي تعاني من فرط في ارتفاع دهون الدم إلى مجوعتين هما على التوالي المجموعة (A) التي سلكت كمجموعة قياسية سالبة والمجموعة (B) التي سلكت كمجموعة قياسية موجبة، والأكثر من ذلك، فأن الحيوانات التي تعاني حالة فرط في ارتفاع دهون الدم عولجت بنوعين من الأدوية ولمدة شهر هما الدواء القياسي المخفض لدهون الدم Atorvastatin و بجرعة (2 ملغم/ كغم/يوم) كما في المجموعة (C) إما النوع الثاني فكان دواء التفاح العلاجي Apple-cure وبجرعة (3مل/كغم/يوم) كما في المجموعة (D). النتائج المستحصل عليها من هذه الدراسة بينت بان أوزان الأرانب في المجموعتين (B,C) لم يتغير بفارق معنوي، بينما انخفضت أوزان الحيوانات في المجموعة (D) بفارق معنوي كبير($p < 0.005$) . جمعت عينات الدم من الحيوانات عند الفترات الزمنية 0، 1، 2، 3، 4 أسبوع من بدء عملية المعالجة. النتائج أظهرت بأن التجريع اليومي لدواء التفاح العلاجي قد أدى الخفض كلا من الكولسترول والدهون الثلاثية بفارق معنوي كبير ($p < 0.005$) وأدى أيضا إلى خفض الكولسترول وطئ الكثافة LDL بفارق معنوي ($p < 0.01$) بينما أدى نفس التجريع إلى رفع نسبة الكولسترول العالي الكثافة HDL وبفارق معنوي معتد ($p < 0.01$) . أوضحت نتائج هذه الدراسة بأن دواء التفاح العلاجي يمتلك تأثير قوي في خفض دهون الدم في الأرانب، الأمر الذي يؤهله بأن يمتلك قيمة علاجية كبيرة في معالجة الحالات المرضية المصاحبة لارتفاع نسب الدهون في الدم.