EFFECT OF CYNARA SCOLYMUS L. (ARTICHOKE) EXTRACT ON LIPID PROFILE OF HYPERLIPIDEMIC MALE RATS

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Abstract:

Introduction: *Cynara scolymus L.* (Artichoke) grows in Egypt and other countries. It is used as foods and has medicinal properties. Artichoke extracts have been shown to produce various pharmacological effects, such as the inhibition of cholesterol biosynthesis and low density lipoprotein (LDL) oxidation.

Purpose: To study the effect of *Cynara scolymus L*, and its extract on lipid profile of hyperlipidemic male rats.

Study Design: Eighty male albino rats, Sprague-Dawley strain, weighing (204.0 ± 10) were housed individually in wire-mesh cages. Induction of hyperlipidemia was carried out on all rats except negative control group by addition of cholesterol to the basal diet (2%) +0.25 bile salts (<u>taurocholic</u>) for 4 weeks. After that the rats were divided into 8 groups (10 rats each), the first (1^{st}) and 2^{nd} groups was negative (normal) and positive control groups (hyperlipidemic), groups from G 3: G8, they were fed on basal diet supplemented with 2 level of extract (4 & 8 %) of either heads, heads, or leaves extract. At the end of the experimental period (6 weeks treatment) rats were fasted over night before sacrificing, blood was collected, centrifuged; serum or plasma was stored at - 20 ^oC until analysis. Lipid profile and triacylglycerol were measured.

Results and Discussion: Artichoke extracts (plant, head, and leaves) significantly reduced cholesterol, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triacylglycerol. No change was observed with high density lipoprotein cholesterol (HDL-C). Heads extract being more powerful. This effect may be due to its content of mono- and di-caffeoylquinic acids, flavonoids and other contents or through inhibition of LDL oxidation.

Conclusion: artichoke extract seems to be positively modulate hypercholesterolemia and can provide a protection from cardiovascular diseases.

Keywords: Cynara scolymus L. (artichoke), Atherosclerosis; Antioxidant, Lipid profile.

Introduction:

The use of plants for treating various ailments dates back several centuries. Usually, plants (herbal) medicine has relied on tradition that may or may not be supported by empirical data. The belief that natural medicines are much safer than synthetic drugs has gained popularity in recent years and led to tremendous growth of phytopharmaceutical usage (**Bhattaram** *et al.*, 2002).

Atherosclerosis is a complex multicellular process, resulting in an unstable atherosclerotic plaque that ultimately bursts, causing mvocardial infarction. Botanical dietary supplements (herbs) can ameliorate this process and prevent cardiovascular disease at many steps in the process. Many herbs have antioxidant activity and can reduce low lipoprotein density oxidation. Some phytosterols found in botanicals can inhibit cholesterol absorption (Heber 2001). Hypercholesterolaemia is directly associated with an increased risk of coronary heart disease (CHD) (Holme 1990). Standard drug therapy includes bile

acid sequestrants, nicotinic acid, fibric acids and HMG-CoA reductase inhibitors (statins) [Expert Panel, 2002]. None of the pharmacological options is free of adverse events and some have been associated with potential carcinogenicity (Expert Panel, **2002**). A harmless yet effective treatment option would therefore be of considerable interest. Artichoke leaf extract (ALE) has been suggested as such an option. Effective non-pharmacological treatment consists largely of dietary interventions and physical increased activity and is considered the treatment of choice for primary and secondary prevention of CHD (Pyörälä et al., 1994 a & b). A favorable effect on serum lipids may be achieved with herbal medicinal products, if they are administered at sufficient high dosages and sufficient long term use. Their efficacy is not so strong than that documented for chemically defined products, however their tolerability is superior (Wegener, 2002). A harmless yet effective treatment option would therefore be of considerable interest. Artichoke leaf extract (ALE) has been suggested as such an option.

Cynara scolymus L. (artichoke) is an important crop of ancient Greece, grows in Egypt, Mediterranean area and other countries. It has been known by the ancient Egyptians, Its green leaves and head are used as foods due to their high nutritive value. Artichoke (Cynara scolymus L.) is one of the world's oldest medicinal plants. It has medical properties. It is used in traditional folk medicine. It is a good source of natural antioxidants such as vitamin C, hydroxycinnamic acids. and flavones (Jimenez et al., 2003). Artichoke and its byproduct contain many compounds as caffeoylquinic acid derivatives and flavonoid. Caffeic acid derivaties are the main phenolic compounds in artichoke heads with a wide range of caffeoylquinic acid derivatives. Apigenin-7-rutinoside and narirutin were found to be unique to artichoke heads (Wang et al., 2003). Cervellati et al., 2002 and Taylor 2002 found that cynarin are used to mobilize fatty stores in the liver and detoxify it.

Content of artichoke:

From the leaves of Cynara scolymus the following substances where isolated: apigenin, luteolin, luteolin-4'-

glucoside, cynaroside, scolimoside, cosmoside, quercetin, rutin, chloro-genic acid, caffeic acid, isochlorogenic acid, luteolin-7-gentiobioside, along with the more uncommon scopoletin, hesperitin, hesperidoside, esculetin-6-O-betamaritimein, glucoside, sesquiterpenes (cynaropicrin, aguerin B, and grosheimin), sesquiterpene glycosides (cynara-scolosides A, B, and C) (Shimoda et al., 2003, Schütz **2006**). The anthocyanin of artichoke heads was cyanidin 3, 5-diglucoside, cyanidin 3glucoside, cvanidin 3. 5malonyldiglucoside, 3-(3"cyanidin malonyl) glucoside, and cyanidin 3-(6"malonyl) glucoside which represent the maior anthocvanin. two peonidin derivatives and one delphinidin derivative. Total anthocyanin content ranged from 8.4 to 1,705.4 mg / kg dry mass (Shimoda et al., 2003, Schütz 2006). Cynarine (1.5-dicaffeoyl-D-quinic acid) is the principal active component of artichoke. The flavonoid luteolin has a role in the cholesterol synthesis inhibition of (Gebhardt 1997).

AIM OF THE WORK: To study the effect of *Cynara scolymus L*. (balady artichoke) and its extract on lipid profile of hyperlipidemic male rats.

Materials and Methods:

Preparation of artichoke extract (whole plant, head, and leaves (Wang et al., 2003; and Wagner et al., 1984): a known weight of artichoke was air dried at the room temperature and grinded using a blinder into fine powder. The powdered plant material was macerated in 70% methanol. Successive addition of aqueous methanol to plant material was carried out till complete exhaustion of the plant. The aqueous methanolic extract was concentrated under reduced pressure using rotatory evaporator till dryness and then weighed. The same was done on the leaves and heads.

Experimental design:

Eighty male albino rats, Sprague-Dawley strain, weighing $(204.0 \pm 10 \text{ g})$ were housed individually in wire-mesh cages. All rats were initially fed basal

(control) diet for 10 days before starting the experiment (adaptation period). The control diet was prepared according to National Research Council (NRC) Committee on Animal Nutrition. (1978) and Reeves et al., 1993. The water and diets were given ad libitum. Induction of hyperlipidemia was carried out on all rats except negative control group by addition of cholesterol (2 %) +0.25 % bile salts to the basal diet for 4 weeks. After that the rats (80) were divided into 8 groups (10 rats each), the first (1st, G 1) second (2nd, G 2) groups was negative (normal) and positive control groups (hyperlipidemic), the third (3rd, G 3) and fourth (4th, G 4) group fed on diet supplemented with artichoke extract (whole plant) in a dose of 4% and 8% of the diet respectively; the fifth $(5^{th}, G 5)$ and sixth (6th, G 6) groups fed on basal diet supplemented with artichoke heads extract in a dose of 4% and 8% of the diet respectively; the seventh (7th, G 7) and eighth (8th, G 8) groups fed on basal diet supplemented with artichoke leaves extract in a dose of 4% and 8% of the diet respectively. Second group was scarified at the beginning (after being hyperlipidemic) to act as base line. At the end of the experimental period (6 weeks, 45 days after feeding the artichoke and its extract)¹, rats were fasted over night before sacrificing, blood was collected, centrifuged; serum was stored at - 20 °C until analysis.

THE ANALYTICAL METHODS

Total cholesterol, TC, (Bio Mérieux kits-Richmond 1973; and Allain *et al.*, 1974), total triacylglycerol, TG, (Bicon kits- Bucolo & David 1973), serum HDL (Bio Mérieux kits- Burstein *et al.*, 1970, and Lopes Virella *et al.*, 1977), serum LDL (Bio Mérieux kits- Friedewald *et al.*, 1972; Levy *et al.*, 1981) contents were determined using suitable kits reagents. VLDL-C was determined by using the following equation: VLDL-C=total cholesterol - (HDL-C + LDL-C).

STATISTICAL ANALYSIS

Data are expressed as Mean \pm SE. Data were assessed by paired t-test (Avram **1964; and Steel & Torrie 1960).** Anova and Tukey as post hoc analysis was done using SPSS version 11.

Result:

Data of table (I) reveal that group 2 (+ve control, hypercholesterolemic rats) had significantly higher cholesterol, LDL-C, VLDL-C and triacylglycerol levels than normal control, while HDL-C showed significantly lower levels. Data of table (I) also reveal that artichoke extract (4, 8 %) significantly reduced cholesterol, LDL-C, VLDL-C and triacylglycerol concentration of G 3 & 4. The reduction of cholesterol, LDL-C, VLDL-C and triacylglycerol levels being more in G 4 than G3 with using higher extract concentration. Cholesterol reduction reaches 18.18% in (G 4), while VLDL-C and triacylglycerol LDL-C. reduction reach 26.03, 13.23 and 9.73% in G 4 respectively (table II). Data of table (I) reveal that artichoke head extract (4, 8 %) significantly reduced cholesterol, LDL-C, VLDL-C and triacylglycerol concentration of G 5, 6 and the reduction being more in G 6 than G 5. Cholesterol, LDL-C, VLDL-C and triacylglycerol reduction reach 19.59, 23.72, 32.28 and 31.81% in G 6 respectively (table 2). Data of table (I) reveal that artichoke leaves extract (4, 8 %) significantly reduced cholesterol, LDL-C, VLDL-C and triacylglycerol concentration of G 7, 8. The reduction being more in G 8 than G 7 with using higher extract concentration. Cholesterol, LDL-C, VLDL-C and triacylglycerol reduction reach 22.18, 28.69,29.09 and 23.07 % in G 8 respectively (table II). Leaves extract seems to be more powerful in reducing cholesterol and LDL-C concentration while head extract shows more reduction in VLDL-C and triacylglycerol concentration. The atherosclerotic indices (chol/HDL-C) are affected by the extract, especially by the The reduction reaches leaves extract. 26.16% with using higher extracts concentration.

Discussion:

Atherosclerosis is a complex multicellular process involving oxidation of cholesterol and the intracellular accumulation of oxidized cholesterol. This

¹The whole experiment since the adaptation, control diet lasted around three months.

accumulation causes a cascade of inflammatory processes, resulting in an atherosclerotic plaque unstable that ultimately bursts. causing mvocardial infarction. Botanical dietary supplements (herbs) can ameliorate this process and prevent cardiovascular disease at many steps in the process. Many herbs have antioxidant activity and can reduce lowdensity lipoprotein oxidation. Some phytosterols found in botanicals can inhibit cholesterol absorption.

The results of this study shows that aqueous organic extract of artichoke (whole plant, heads, and leaves) can significantly reduce cholesterol, LDL-C, VLDL-C, and triacylglycerol concentration of hypercholesterolemic rats and have no effect on HDL-C level. The results are in agreement with **Pittler** *et al.*, **2002** (leaves extract lowers cholesterol levels); and **Lupattelli** *et al.*, **2004** (cholesterol and HDL-C); and **Englisch** *et al.*, **2000** (lowering cholesterol, LDL-C levels using artichoke dry extract).

There are several possible mechanisms that's through it artichoke extract can cause а significant effect on hypercholesterolemia. Artichoke extracts have been shown to produce various pharmacological effects, such the inhibition of cholesterol as biosynthesis and of LDL oxidation. Artichoke dietary supplementation positively modulate seems to hypercholesterolemia. These therapeutic properties may be attributed to mono- and di-caffeoylquinic acids and cynarin (one of the caffeoylquinic acid family) content of artichoke

(Speroni et al., 2003)

Artichoke contain at least 22 major compounds, 11 caffeoylquinic acids and 8 flavonoids as apigenin 7-O-glucuronide which is considered as the major flavonoid, 1,5-Di-O-caffeoylquinic acid which represent the major hydroxycinnamic acid, narirutin, and cynarin (Schutz *et al.*, 2004). Heckers *et al.* (1977) stated that

cynarin in a dose of 250 mg and 750 mg daily, as treatment of familial Type IIa or Type IIb hyperlipoproteinaemia, has no hypolipidaemic effect since mean serum cholesterol and triglyceride concentrations were not significantly changed within 3 months. The oxygen functional groups at the 3- and 8-positions and exo-methylene moiety in alpha-methylene-gammabutyrolactone ring were found to be essential for the anti-hyperlipidemic activity of guaiane-type sesquiterpene. In addition, inhibition of gastric emptying was shown to be partly involved in anti-hyperlipidemic

activity (Shimoda et al., 2003). Aqueous organic artichoke leaf extract (ALE) increased the activity of the human endothelial nitric-oxide synthase (eNOS) promoter which produce nitric considered oxide that is as antiatherosclerotic principle in the vasculature thus could provide protection against cardiovascular diseases. Aqueous organic artichoke leaf extract increase the activity of eNOS mRNA expression and eNOS protein expression. The flavonoids luteolin and cynaroside increased eNOS promoter activity and eNOS mRNA expression. The increase in eNOS gene transcription may also contribute to ALE beneficial cardiovascular profile. Artichoke flavonoids are likely to represent the active ingredients mediating eNOS up-regulation (Li et al., 2004). Li et al., 2004 found that cynarin acid did not increase eNOS mRNA expression.

ALE also inhibited LDL oxidation (Brown and Rice-Evans, 1998) and reduced the production of intracellular reactive oxygen species by oxidized LDL in cultured endothelial cells and monocytes

(Zapolska-Downar et al., 2002). Artichoke leaves extract (ALE) inhibits the incorporation of ¹⁴C-labelled acetate into the non-saponifiable lipid fraction and thus reduces cholesterol biosynthesis at the level

hydroxymethylglutaryl-CoA-reductase (HMG-CoA-reductase) through indirect HMG-CoA-reductase modulation of activity (Gebhardt 1995; Gebhardt 1996 a; and Gebhardt 1998). Furthermore, insulin stimulation of acetate incorporation was efficiently reduced by artichoke extracts. The reduction of HMGCoAreductase activity by the artichoke extracts might be responsible for the selective effect on acetate incorporation. Other studies suggested indirect inhibitory effects exerted at the level of HMG-CoA-reductase, a key enzyme cholesterol biosynthesis in (Fintlemann, 1996 a; Gebhardt, 1996 a; and Gebhardt, 1997). All of this might be

due to some regulatory mechanism HMGCoA-reductase, which of is influenced. This influence could possibly involve: 1) inhibition of activating mechanisms and/or 2) stimulation of inactivating mechanisms of the enzyme. Artichoke extracts effectively blocked insulin-dependent stimulation of HMGCoA-reductase without affecting insulin effects in general. Quantitative measurements show that artichoke extract inhibits cholesterol biosynthesis in a concentration dependent manner (Artner-Dworzak, 2000, Gebhardt, 1996 b). More recent findings indicate a role for the avonoid luteolin in inhibiting effects of cholesterol synthesis (Gebhardt, 1997). Because artichoke extracts may also enhance biliary cholesterol excretion as a result of the choleretic influence (Kirchhoff et al., 1994), both mechanisms (physiologically through indirect mechanism and enhance biliary cholesterol excretion) may contribute to the clinically known reduction of blood cholesterol levels (Gebhardt, 1996 b; and Gebhardt, 1998). Our results are in agreement with **Bundy** et al. (2008).

Data of table (I) reveal that aqueous organic extract from artichoke (*Cynara scolymus L.*) plant, heads and leaves did not significantly affect HDL-C levels. They have no effect on HDL-C concentration despite the little increase. Head Extract cause 6.09 increase in HDL-C levels, which is higher than that caused by leaves extract (5.91%).

Data of table (I) reveal that aqueous organic extract from artichoke leaves **References:**

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(Cynara scolymus L.) significantly reduced triacylglycerol levels due to presence of certain compounds in the artichoke as sesquiterpenes (cynaropicrin, aguerin B, and grosheimin) and sesquiterpene glycosides (cynarascolosides A, B, and C) (Shimoda et al., 2003) and also the presence of oxygen functional groups at the 3- and 8-positions and exo-methylene alpha-methylene-gammamoiety in but vrolactone ring are essential for the antihyperlipidemic activity of guaiane-type sesquiterpene. Our results for triacylglycerol are in agreement with (Shimoda et al., 2003) and are in disagreement with (Lupattelli et al., 2004). The traditional use of a plant-based

relative remedv implies safety. for artichoke. Held, 1992, Fintelmann, 1996 b; 1997; 1999 reported the absence of serious adverse events which means that ALE is relatively well tolerated but Ernest et al., 2001; and Pittler et al., 2002 stated that these limited evidence, which is available is not sufficient to recommend ALE treatment option as а for hypercholesterolaemia.

CONCLUSION:

study This prospective could contribute evidence beside the other published ones to recommend artichoke extract for aiding in treating hyperlipoproteinemia since artichoke extract seems positively modulate to hypercholesterolemia.

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		Cholesterol mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	Triacylglycerol mg/dl	<u>Chol</u> HDL-C
G 1	Μ	92.15	42.31	35.03	14.81	80.28	2.18
	±SE	1.95	0.85	1.04	0.35	0.94	0.01
G 2	Μ	183.85 ^{1,3-8 *}	35.28 ^{1*}	114.40 ^{1, 3-8*}	34.17 ^{1,6,8} *	179.27 ^{1,6,8 *}	5.24 ^{1,3-8*}
	±SE	1.93	0.90	1.86	0.75	4.39	0.12
G 3	Μ	164.71 ^{1,2,4,6,8*}	36.12 ^{1*}	97.05 ^{1,2,4,6,8 *}	31.54 ^{1,2,6,8} *	169.43 ^{1,2,6,8 *}	4.59 ^{1,2,4,6,8*}
	±SE	1.77	1.10	1.09	0.99	2.54	0.11
G 4	Μ	150.42 ^{1,2,3,5,6,7,8*}	36.15 ^{1*}	84.62 ^{1,2,3,5,7 *}	29.65 ^{1,2,6,8} *	161.82 ^{1,2,6,8 *}	4.17 ^{1,2,3,5,6,7 *}
	±SE	2.25	0.67	1.28	1.51	6.22	0.09
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G 5	Μ	166.61 ^{1,2,4,6,8*}	36.06 ^{1*}	98.37 ^{1,2,4,6,8 *}	32.18 ^{1,6,8 *}	171.99 ^{1,6,8 *}	4.70 ^{1,2,4,6,8 *}

Table (I): Lipid profile of rats fed on extracts of artichoke (whole plant, head, and leaves).

Effect of Cynara

	±SE	2.88	1.77	1.42	1.21	5.70	0.19		
G 6	Μ	143.07 ^{1,2,3,4,5,7 *}	37.26 ^{1*}	81.58 ^{1,2,3,5,7,8 *}	24.23 ^{1,2,3,4,5,7 *}	137.92 ^{1,2,3,4,5,7,8 *}	3.87 ^{1,2,3,4,5,7} *		
	±SE	2.49	1.22	1.21	1.23	6.21	0.11		
G 7	Μ	163.22 ^{1,2,4,6,8*}	35.98 ^{1*}	96.63 ^{1,2,4,6,8 *}	30.61 ^{1,2,6,8*}	160.71 ^{1,2,6,8 *}	4.57 ^{1,2,4,6,8} *		
	±SE	2.70	1.28	0.99	1.40	6.42	0.11		
G 8	Μ	147.84 ^{1,2,3,5,7 *}	37.43 ^{1,2*}	87.27 ^{1,2,3,5,6,7 *}	23.14 ^{1,2,3,4,5,7 *}	122.25 ^{1,2,3,4,5,6,7 *}	3.96 ^{1,2,3,5,7 *}		
	±SE	1.49	0.69	1.54	0.60	1.95	0.06		
One	Way	ANOVA							
	F	149.536*	4.001*	305.878*	35.564*	48.62*	68.944*		
	P <	0.000	0.001	0.000	0.000	0.000	0.000		
Superscript numbers refer to the group number, which of this group is significant with (Superscript refer to									
Tukey test as post-hoc analysis). Using the Tukey procedure, this table lists the pair wise comparisons of the									
group means for post hoc procedures. Mean difference lists the differences between the group means.									

Table (II): Percent (%) change of the tested groups from their respective controls

		Cholesterol	HDL-C	LDL-C	VLDL-C	TG	<u>Chol</u> HDL-C		
G 3	а	78.74	-14.63	177.05	112.96	111.05	110.70		
	b	-10.41	2.38	-15.17	-7.70	-5.49	-12.35		
G 4	а	63.23	-14.56	141.56	100.20	101.57	91.58		
	b	-18.18	2.47	-26.03	-13.23	-9.73	-20.30		
G 5	а	80.80	-14.77	180.81	117.29	114.24	115.71		
	b	-9.38	2.21	-14.01	-5.82	-4.06	-10.26		
G 6	а	60.43	-11.53	149.13	56.25	52.28	81.76		
	b	-19.59	6.09	-23.72	-32.28	-31.81	-24.38		
G 7	а	77.12	-14.96	175.85	106.68	100.19	109.73		
	b	-11.22	1.98	-15.53	-10.42	-10.35	-12.75		
G 8	a	55.26	-11.94	132.89	63.61	71.80	77.50		
	b	-22.18	5.61	-28.69	-29.09	-23.07	-26.16		

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تأثير مستخلص الخرشوف على صورة الليبيدات في الفئران المصابة بارتفاع الليبيدات أ أغادة زع سليمان ، ²تامر م مسعد 1: المعهد القومي للتغذية ، ^{2:} وحدة الابحاث الطبية، هيئة الطاقة الذرية

الملخص

مقدمة : ينمو الخرشوف في مصر وبلدان أخرى. و هو يستخدم في الأطعمة ، و له خصائص طبية. ثبت ان مستخلص الخرشوف في مصر وبلدان أخرى. و هو يستخدم في الأطعمة ، و له خصائص طبية. ثبت ان مستخلص الخرشوف له تأثيرات دوائية ، مثل تثبيط تخليق الكولسترول والبروتين الدهني منخفض الكثافة. الغرض : لدراسة تأثير الخرشوف البليدي ومستخلصه على صورة الدهون في ذكور الفلران.

مناقشة النتائج : مستخلصات الخرشوف (النبات كاملا ، والرأس ، الاوراق) قد ادت الى انخفاض كبير معنوي للكولسترول ، والكولسترول منخفض الكثافة ، و الكولسترول منخفض الكثافة جدا والدهون الثلاثية. لا تغيير لوحظ مع الكولسترول الدهني العالي الكثافة . الرأس أكثر قوة. هذا التأثير قد يكون بسبب محتواه من أحماض الكافولينك الأحادية والثنائية، فلافونويدس ومحتويات أخرى أو عن طريق تثبيط أكسدة الكوليسترول منخفض الكثافة.

الاستنتاج : يبدو ان اضافة مستخلص الخرشوف يبدو إيجابيا في التأثير على ارتفاع الكوليسترول و كذلك يمكن . تـــــوفير الحمايـــــة مــــــن أمــــراض القلـــــب والأوعيــــــة الدمويـــــة.

الكلمات الرئيسية : الخرشوف ، تصلب الشرايين ، المواد المضادة للأكسدة ، صورة الدهون.