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المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد الثالث - العدد الأول - مسلسل العدد (٥) - الجزء الأول، يناير ٢٠١٧

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

ISSN-Print: 2356-8690 ISSN-Online: 2356-8690

موقع المجلة عبر بنك المعرفة المصري <https://jsezu.journals.ekb.eg>

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Protective effect of Globe Artichoke (*Cynara Scolymus* L.) on Growing Male Rats Fed on High Fat and Fructose diet

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Abstract:

The present study was carried out to investigate the effect of globe artichoke on high fat and fructose (HF/HFr) diet on the onset of the characteristics of the the levels of growing male. Also the possible protective effects of artichoke (AK) against (HF/HFr) diet induced-metabolic disturbances. Thirty sex male albino rats were randomly assigned into 4 groups and were fed for 6 weeks the following diets group 1 basal diet, negative control (C) group 2 basal diet containing 20 g AK/kg diet (C+AK), group 3 high fat diet (15% beef tallow + 5% corn oil) combined with fructose (13% W/V) in drinking water (HF/HFr), group 4 HF/HFr diet containing 20 g AK/kg diet (HF/HFr+AK). HF/HFr diet induced elevated levels of blood glucose, serum TG, TC, LDL-C, liver, renal function and (NO) with a decrease in (HDL-C) and total antioxidant capacity compared to control. These effects were more pronounced in HF/HFr diet fed on. Globe artichoke reinstated most of the altered measured parameters. So, HF/HFr diet developed characteristics of metabolic syndrome in growing male rats. Artichoke were protected against some metabolic disturbances of HF/HFr diet. The inclusion of artichoke consumption has a beneficial effect in control and management of diabetes and diabetes associated complications with no risk of hypoglycemic effect.

Key words: *Cynara scolymus* L., Metabolic syndrome, Phenolic compounds, HF/ HFr diet.

Introduction:

Many facts of the metabolic syndrome (MS) are often linked to the macronutrient content of the diet, and there is evidence that excessive consumption of macronutrients such as carbohydrates, fats, and even protein may eventually lead to the development of insulin resistance

(Felizola, 2015). High dietary (HF) intake appears to be an important determinant of obesity and has been shown to induce perturbations in insulin signaling and rates of lipid synthesis via increased hepatic free fatty acid flux and triglycerides (TG) accumulation (Falkner and Cossrow, 2014). Metabolic syndrome can also be induced by consumption of refined carbohydrates, and excessive intake of these may be particularly deleterious with respect to increasing the risks of insulin resistance (Beltrán-Sánchez *et al.*, 2013). A global change in dietary habits has occurred over the last few decades resulting from the introduction of sweeteners such as fructose and sucrose by the food industries (Felizola, 2015).

Globe artichoke, *Cynara Scolymus*, is widely cultivated in the Mediterranean area. The edible parts of artichoke are the heads, the large immature inflorescences with edible fleshy inner and intermediate leaves (bracts), which are a good source of health-promoting polyphenols and therefore encourage a nutraceutical use of these species (Alamanni and Cossu, 2003; Romani *et al.*, 2006 and Scaglione *et al.*, 2016). Two major compounds in globe artichoke are the salts of chlorogenic acid and cynarin, phenolic compounds that are derivatives of caffeic acid. Extracts containing cynarin (1,5-dicaffeoylquinic acid) have effects on hepatobiliary diseases, hyperlipidaemia and cholesterol metabolism (Sharaf-Eldin *et al.*, 2007). Also, globe artichoke is a rich source of Fructooligosaccharides (FOS), inulin, isomalto-oligosaccharides (IMO), polydextrose, lactulose and resistant starch are considered as the main prebiotic components through their fermentation in the colon to yield including short chain fatty acids (SCFAS) (Wider *et al.*, 2013). The physiological effects of (FOS) which are indigestible carbohydrates, especially mixtures of different sugar length such as 1-kestose, nystose and fructofuranosyl-nystose in which, they are safe for diabetic and improve the intestinal flora (Lattanzio *et al.*, 2009 and Ceccarelli *et al.*, 2010). In this study, the effect of eating a diet high in both fat and fructose (in drinking water) on the development of metabolic syndrome and its association to the levels of leaves on blood glucose in rats. Second, we studied the impact of artichoke on HF/HFr diet induced metabolic dysregulation in experimental animals.

Materials and Methods:

Materials:

Globe artichoke:

(*Cynara Scolymus* L.) was obtained from the local market in Cairo, Egypt.

Fructose:

Was purchased from the International Company for Scientific and Medical Supplies, Cairo, Egypt.

Experimental rats:

Thirty six male albino rats (Sprague Dowlay) weighing 150 ± 10 g. The rats were purchased from Agriculture Research Center, Giza, Egypt. The animals were kept under observation and fed standard diet for a week as adaption period before using the experimental diets.

Methods:

Preparation of artichoke powder:

Artichoke was weighed, cut into small pieces, and dried till complete dryness at 60°C . Moisture contents were determined such of meatloured (AOAC, 1980). The dried samples were ground to fine powders, sifted through a 16-mesh sieve and packed in well sealed polyethylene bags and stored at room temperature $25\pm 5^{\circ}\text{C}$ until use.

Preparation of Diets :

Two types of diets were used in this study basal diet was based on AIN-93 recommendations (Reeves *et al.*, 1993). The HF/HFr diet, consisted of basal diet contain 20% fat (15% beef tallow + 5% corn oil) combined with fructose added in drinking water at a level of 13% w/v which is the concentration range reported for soft drinks (Light *et al.*, 2009).

Biological Assays:

There were composed of 36 male rats were divided into the following groups of 9 rats each:

Group 1: Control group (-ve): normal control rats, received basal diet.

Group 2: Artichoke group (C+AK): artichoke -treated normal rats, received basal diet contained 20 g artichoke /kg diet

Group 3: High fat high fructose-fed group (HF/HFr): fat- fructose fed rats, received high fat diet and fructose in drinking water.

Group 4: High fat high fructose + artichoke group (HF/HFr+AK): artichoke group-treated fat-fructose fed rats, received high fructose high fat diet contain 20 g artichoke /kg diet.

Body weight (BWG) and the amount of food intake (FI) for each rat were measured once per week during the experimental period (6 weeks). BWG and FI were also calculated, food efficiency ratio (FER) was calculated at the end of experiment as follows:

Food efficiency ratio "FER" = body weight gain/food intake.

Oral glucose test (OGTT):

Twelve hours prior to day 40, rats were fasted and were subject to OGTT. Fructose-supplemented drinking water in HF/HFr and HF/HFr + CN groups was replaced with water for the overnight fasting period for the measurement of basal blood glucose concentrations. Basal blood glucose concentrations were measured in tail vein blood using a Medisense Precision Q.I.D glucose meter (Abbott Laboratories). The rats were given 2 g/kg body weight of glucose as a 40% solution via oral gavage. Tail vein blood samples were taken at 0, 30, 60, 90, and 120 min following glucose administration. At the first, each two weeks and the end of experimental period, the rats were anaesthetized by diethyl ether and sacrificed. Blood samples were collected in clean test tubes, blood were left for coagulation then centrifuged at 3000 rpm for 15 minutes to obtain serum to assay the biochemical analysis. Heart, liver, spleen and kidney for rats were collected and weighted.

Biochemical analysis of serum:

Blood glucose levels were measured according to **Barham and Trinder (1972)**. Total cholesterol (TC), and Triglycerides (TG) were determined according to **Schettler and Nussel (1975)**. High density lipoprotein cholesterol (HDL) according were determined to **Gordon and Amer (1977)**. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated according to

Lee and Nieman (1996). Serum alanine and aspartate aminotransferase enzymes activities (AST & ALT) were determined according to the method of **Reitman and Frankel (1957)**. Total protein, bilirubin, uric acid and creatinine were determined according to **Josephson et al. (1957)**, **Jendrassik et al. (1938)**, **Barham and Trinder (1972)** and **Henry (1974)**. Superoxide dismutase (SOD) enzyme activity and total antioxidants capacity (TAC), nitric oxide (NO) were determined according to **Oyanagui (1984)**, **Cao et al. (1993)** and **Nagi et al. (2010)** respectively.

Statistical analysis:

All obtained data were statistically analyzed using analysis of variance using computer program of **SAS (2000)**. Duncan multiple range test **Duncan (1955)** was used to determine the significant differences among dietary treatments.

Results and Discussion:

Body Weight Gain (BWG), Feed Intake (FI) and Feed Efficiency Ratio (FER)

Data present in Table (1) showed that FI was significantly ($p \leq 0.05$) decreased while body weight gain was significantly ($p \leq 0.05$) increased in rats fed on HF/HFr diet compared to control group. FI and BWG were not significantly affected by artichoke treatment (Table 1) may be to artichoke leaves are rich in phenolic compounds, such as mono- and dicaffeoylquinic acids and flavonoids, which improve health status **Abrams et al. (2007)**. FER in group (2) was more than group (4) and group (1). Also **Jimenez-Escrig et al. (2003)**, **Wang et al. (2003)** and **Abrams et al. (2007)** reported that there are a significant difference in daily FI, BWG and FER between experimental rats consumed with artichoke than the animals not fed with artichoke.

Table (1): Effect of artichoke on Body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) in rats fed on high fat and fructose diets.

Variables Groups	BWG (g)	FI (g)	FER (%)
Group 1: Control (ve-)	100.29± 0.71 ^{cd}	15.99± 1.40 ^a	0.02± 0.05 ^c
Group 2: C + AK	108.14± 0.22 ^c	15.12± 1.06 ^a	0.07± 0.02 ^c
Group 3: HF/HFr	143.36± 0.60 ^a	16.68± 1.20 ^a	0.18± 0.03 ^a
Group 4:HF/HFr+AK	122.36± 0.60 ^b	17.66± 0.85 ^a	0.09± 0.03 ^b

Values are expressed as means ± SD. Values with the same letters indicate of raw and no significantly difference of $P \leq 0.05$

Serum glucose :

Serum glucose levels were significantly ($p \leq 0.05$) higher in the HF/HFr-fed rats as compared to those in the control rats. The effects of HF/HFr diet on these parameters were quite different in was observed at week 6 for all groups the reduction are less. In HF/HFr group, artichoke supplementation significantly ($p \leq 0.05$) reduced fasting serum glucose levels as compared with the groups fed on HF/HFr (Table 2). Fructooligosaccharides (FOS) are a group of glycosyl-fructosyl polymer with DP (3–5) occur in artichoke, these results are in accordance with those reported by **Bornet et al. (2012)**. Also, **Sandoval et al. (2004)** **Schutz et al. (2004)**; **Nazni et al. (2006)** and **Nomikos (2007)** reported that the decrease in blood glucose levels probably attributed to a inulin in artichoke influence the formation of blood glucose.

Table (2): Effect of artichoke on serum blood glucose (mg/dl) in rats fed on high fat and fructose diets.

Variables Groups	Blood glucose (mg/dl)			
	Weeks (1)	Weeks (2)	Weeks (4)	Weeks (6)
Group 1: Control (ve-)	100.00±0.00 ^a	99.67±3.09 ^d	99.87±5.31 ^d	99.33±4.50 ^c
Group 2: C + AK	100.00±0.00 ^a	116.67±4.19 ^c	107.67±5.32 ^c	102.33±5.44 ^b
Group 3: HF/HFr	100.00±0.00 ^a	198.00±4.62 ^a	263.00±3.37 ^a	279.75±4.65 ^a
Group 4: HF/HFr +AK	100.00±0.00 ^a	138.67±1.89 ^a	151.67±3.30 ^b	144.33±3.30 ^b

Values are expressed as means \pm SD. Values with the same letters indicate of raw and non significantly difference of $P \leq 0.05$.

Serum lipid profile:

Table (3) demonstrated that the consequences of HF/HFr diet feeding on serum lipids levels were markedly different between rats group. HF/HFr diet resulted in significant ($p \leq 0.05$) elevation in serum levels of TG, TC and LDL, as compared with the control group. It has been suggested that fructose reduces insulin sensitivity by providing a direct source of intrahepatic triglyceride via *de novo* lipogenesis (Stanhope *et al.*, 2009). Serum HDL level was significantly ($p \leq 0.05$) decreased in group 2 and group 4 as compared with control group. Group 2 (C + AK) had no effect on serum TG in rats fed on HF/HFr diet, whereas in the (-ve) control, serum TG level was significantly ($p \leq 0.05$) decreased and normalized compared to HF/HFr group.

The levels of TC and LDL tended to be lower and the level of HDL tend to be higher but not significantly in groups 4 fed on HF/HFr + AK compared with the HF/HFr group. Results demonstrated the hypolipidemic effects of artichoke by reducing the levels of TC, TG, VLDL and LDL. These combined effects can subsequently play a vital role in preventing the incidences of premature occurrence of coronary heart diseases. This is further strengthened by the increase in the levels of high density lipoprotein cholesterol (HDL). This effect may be due to certain chemical constituents such as polyphenols in artichoke which

possess good oxygen radical scavenging potential (**Rodríguez-Cabezas et al., 2010**).

Table (3): Effect of artichoke on total cholesterol (TC), triglyceride parameters (TG), HDL-c, LDL-c and VLDL-c in rats fed on high fat and fructose diets.

Variables Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Group 1: Control (ve-)	102.80±6.65 ^c	99.20±3.54 ^c	41.50±6.27 ^c	34.46±8.26 ^c	16.84±3.60 ^b
Group 2: C + AK	100.00±2.87 ^d	97.70±4.11 ^d	47.00±5.25 ^a	37.46±4.70 ^b	16.34±5.44 ^b
Group 3: HF/HFr	158.00±3.79 ^a	120.60±6.65 ^a	39.40±2.55 ^b	53.48±6.45 ^a	21.12±5.12 ^a
Group 4: HF/HFr+AK	112.00±3.11 ^b	100.40±4.97 ^b	48.20±2.89 ^a	34.92±4.74 ^c	17.88±6.80 ^b

Values are expressed as means ± SD. Values with the same letters indicate of raw and non significantly difference of $P \leq 0.05$.

Lupattelli et al. (2004) said that inulin of artichoke reduce the levels of cholesterol and serum lipids. **Brown and Rice-Evance (1998)**; **Zapolska-Downar et al. (2002)** and **Jimenez-Escrig et al. (2003)** reported that artichoke heart reduce and prevent LDL-c oxidation. **Sandoval et al. (2004)** and **Schutz et al. (2004)** reported that decrease in total cholesterol, total triglyceride and LDL-c, LDL/HDL ratio and a decrease reduced ox-LDL-c. **Lattanzio et al. (2009)** said that globe artichoke have the ability to inhibit cholesterol biosynthesis and LDL oxidation. It is clear that artichoke under this study has been shown to induce cholesterol and lipids lowering effect in both normal and diabetic rats.

Liver function:

Table (4) demonstrated that the consequences of HF/HFr diet feeding on serum aminotransferase enzymes activity (ALT and AST) levels were markedly different amongst rat groups. HF/HFr diet resulted in significant ($p \leq 0.05$) increasing elevation in serum levels of ALT, AST, total protein and bilirubin, as compared with the control group. The levels of ALT, AST, total protein and bilirubin tended to be lowered and the level tend to be highest significantly in group 3 fed on HF/HFr compared

with the corresponding group 4 HF/HFr + AK and C + AK group 2. Other studies are in consistence with the present results (**Kostogryns and Pisulewski 2010**) found reduction in levels of ALT, AST, total protein and bilirubin.

Table (4): Effect of artichoke on serum aminotransferase enzymes activity (ALT and AST, μ /ml) and serum levels total protein (g/dl) and bilirubin (g/dl) in rats fed on basal or high fat high fructose diets.

Variables Groups	AST (μ /ml)	ALT (μ /ml)	Total protein (g/dl)	Bilirubin (g/dl)
Group 1: Control (ve-)	43.75 \pm 2.50 ^c	37.00 \pm 3.46 ^c	7.53 \pm 0.21 ^c	0.25 \pm 0.06 ^c
Group 2: C + AK	36.00 \pm 7.07 ^b	39.00 \pm 2.83 ^b	7.30 \pm 0.41 ^c	0.25 \pm 0.05 ^c
Group 3: HF/HFr	58.75 \pm 4.79 ^a	47.00 \pm 2.00 ^a	16.35 \pm 0.42 ^a	0.48 \pm 0.05 ^a
Group 4: HF/HFr + AK	44.75 \pm 4.79 ^c	40.50 \pm 3.00 ^b	9.10 \pm 0.36 ^b	0.42 \pm 0.05 ^b

Values are expressed as means \pm SD. Values with the same letters indicate of raw and nonsignificantly difference of $P \leq 0.05$.

The present results are in agreement with **Miccadei et al. (2004)** and **Schutz et al. (2004)** who reported that artichoke heart have a hepatoprotective activity and improvement liver. **Stoev et al. (2000)** and **Lattanzio et al. (2009)** mentioned that globe artichoke have exhibited hepatoprotective and decrease in total protein this effect may be due to certain chemical constituents such as polyphenols or terpens in globe artichoke which possess good oxygen radical scavenging potential.

Renal function:

Ingestion of HF/HFr diet resulted in significant ($p \leq 0.05$) higher values of serum uric acid (mg/dl) and creatinine as compared with the control group. The higher values of serum uric acid (mg/dl) and creatinine in HF/HFr group were significantly ($p \leq 0.05$) reduced and nearly normalized in group 4 HF/HFr + AK and C + AK group 2 treated rats (Table 5). The present results agreed with **Kaur and Gupta (2002)** who reported that inulin and oligofructose are effective in lowering the blood urea and uric acid levels. **Lattanzio et al. (2009)** said that globe

artichoke have urinate activities. **Stoev et al. (2000)** reported increase in uric acid, serum urea and creatinine are considered as significant markers of renal dysfunction **Chibbar and Baga (2004)**. However, feeding on artichoke with different levels normalizes these metabolic changes in high fat high fructose diets rats groups. The observed decrease in these parameters by feeding the rats with artichoke suggests its potency in management of these ailments. This could be attributed to vitamin C and minerals content.

Table (5): Effect of artichoke on serum uric acid (mg/dl) and creatinine (mg/dl) in rats fed on high fat and fructose diets

Variables Groups	Uric acid (mg/dl)	Creatinine (mg/dl)
Group 1: Control (ve-)	2.01± 0.15c	0.87± 0.10 c
Group 2: C + AK	2.13± 0.09 b	1.17± 0.12 b
Group 3: HF/HFr	4.48± 0.33a	2.08± 0.10 a
Group 4: HF/HFr + AK	2.19± 0.17 b	1.20± 0.14 b

Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of $P \leq 0.05$.

Serum total antioxidant , SOD and NO:

Table (6) demonstrated that total antioxidant capacity and (SOD) were significantly decreased while NO was significantly increased in rats fed on HF/HFr diet compared with the corresponding control. Supplementation of group 4 (HF/HFr + AK) and group 2 (C + AK) significantly increased the total antioxidant capacity and SOD, and significantly ($p \leq 0.05$) reduced the (NO) as compared to those fed on HF/HFr diet. The chemical components of artichoke are rich in phenolic compounds, such as mono- and dicaffeoylquinic acids and flavonoids, which have been extracted, isolated and identified as major chemical components ((**Llorach et al., 2002 and Wang et al., 2003**). The observed improved endothelial reactivity could be due to the antioxidant contents of artichoke leaves. Artichoke extracts exhibit antioxidant properties in cultured endothelial cells and monocytes, mainly antagonizing lipid peroxidation (**Zapolska-Downar et al., 2002**).

Table (6): Effect of artichoke on total antioxidant capacity (TAC), superoxide dismutase (SOD) and nitric oxide (NO) in rats fed on high fat and fructose diets.

Variables Groups	TAC (mmol/L)	SOD (U/MI)	NO (μ mol/l)
Group 1: Control (ve-)	3.10 \pm 0.22 ^a	70.13 \pm 5.22 ^a	2.68 \pm 0.33 ^c
Group 2: C + AK	3.13 \pm 0.09 ^a	70.04 \pm 7.91 ^a	2.78 \pm 1.21=
Group 3: HF/HFr	1.37 \pm 0.15 ^c	21.25 \pm 3.47 ^c	6.45 \pm 1.44 ^a
Group 4: HF/HFr + AK	2.98 \pm 0.12 ^b	69.76 \pm 7.81 ^a	3.07 \pm 1.21 ^b

Values are expressed as means \pm SD. Values with the same letters indicate of raw and nonsignificantly difference of $P \leq 0.05$.

Conclusion:

The deleterious effect of feeding HF/HFr diet were more pronounced in impairment glucose dyslipidemia associated with higher levels of serum lipid ,liver, renal function and total antioxidant and oxidant capacities. The inclusion of artichoke in the diet was effective in modulating these metabolic disturbances. Therefore, consumption of artichoke could be beneficial for control of diabetes and diabetes associated complications.

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التأثير الوقائي للخرشوف على ذكور الفئران المتنامية المغذاه على حمية عالية في الدهون والفركتوز

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الملخص :

تهدف الدراسة الحالية الى استكشاف تأثير الخرشوف على الحمية العالية في الدهون والفركتوز (HF/HFr) من حيث ظهور خصائص متلازمة التمثيل الغذائي ومستوياتها في نمو الذكور. وكذلك دراسة التأثيرات الوقائية للخرشوف ضد الاضطرابات الأيضية التي تسببها هذه الحمية، استخدمت الدراسة ثلاثين فأر من ذكور الفئران قسمت إلى أربعة مجموعات التالى : المجموعة الاولى تغذت على الوجبة القياسية كمجموعة ضابطة سالبة (C) المجموعة الثانية تغذت على الوجبة القياسية بالإضافة إلى ٢٠ جم من الخرشوف /كجم/ من الوجبة (C+AK). والمجموعة الثالثة تغذت على الوجبة القياسية بالإضافة الحمية العالية في الدهون (١٥% شحم الخنزير + ٥% زيت الذرة) مع الفركتوز (١٣% في مياه الشرب) (HF/HFr) ، والمجموعة الرابعة تغذت على الوجبة القياسية بالإضافة إلى الحمية العالية في الدهون والفركتوز بالإضافة إلى ٢٠ جم من الخرشوف/كجم/ من الوجبة (HF/HFr+AK) واستمرت التجربة لمدة ٦ اسابيع.

اظهرت النتائج ان المجموعة الثالثة التي تغذت على الحمية العالية في الدهون والفركتوز (HF/HFr) لقد احدثت ارتفاع في مستويات الجلوكوز في الدم ، والجليسيريدات الثلاثية والكوليسترول الكلي وكوليسترول البروتين الشحمى منخفض الكثافة ووظائف الكبد و الكلى و اكسيد النيتريك مع انخفاض في نسبة كوليسترول البروتين الشحمى عالي الكثافة وإجمالي القدرة المضادة للأكسدة مقارنة بالمجموعة الضابطة السالبة. وكانت هذه التأثيرات الضارة أكثر وضوحا في المجموعة الثالثة التي تغذت على الحمية العالية في الدهون والفركتوز. إضافة الخرشوف إلى الحمية العالية في الدهون والفركتوز حسن من معظم القياسات التي تغيرت. أدى تناول حمية عالية في الدهون و الفركتوز إلى حدوث تغيرات شبيهة بمتلازمة الأيض في تزايد الفئران الذكور.

ولذلك توصى الدراسة بتناول الخرشوف له دور فعال في التحكم في مرض السكري ومضاعفاته.

الكلمات الاسترشادية: الخرشوف، متلازمة التمثيل الغذائي، المركبات الفعالة، الحمية العالية في الدهون والفركتوز.