Hepatoprotective Effect of Feeding Celery Seeds, Chicory Leaves and Barley Grains to Hypercholesterolemic Rats

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Abstract

This study aimed to investigate the effect of diet supplementation with celery, chicory and barley powder, alone and in combination, on body weight, feed conversion ratio, serum liver enzymes, total cholesterol, triglycerides and lipoproteins in rats fed cholesterol – enriched diet. The experiment was carried out 4 groups of Sprague Dawley rats fed 3% cholesterol supplemented diet for 4 weeks to induce hypercholesterolemia and one group was fed on cholesterol free basal diet which used as a negative control group. The dry powder of celery seeds, chicory leaves or barley grains was separately added to the basal diet at 10% concentration or in combination of three plants at 15% (5% from each) and fed to hypercholesterolemic rats for 4 weeks. At end of the experiment, body weight gains and food efficiency ratios were calculated. Biochemical analyses of serum liver enzymes, (AST, ALT and ALP), total cholesterol, triglycerides and lipoproteins as well as histopathological examination of liver were performed.The results showed that feeding plant combination of celery, chicory and barley at 15% concentration (5% from each) to hypercholesterolemic rats for 4 weeks increased body weight and improved food efficiency. It also decreased the elevated liver enzymes (AST, ALT & ALP), lowered serum total cholesterol, triglycerides, LDL and VLDL, but increased HDL in hypercholesterolemic rats. The histopathological lesions seen in the livers of hypercholesterolemic rats were ameliorated by feeding this plant mixture. It can be concluded that dietary intake of plant mixture of celery; chicory and barley at 15% (5% of each) concentration for 4 weeks may be beneficial to patients suffering from hypercholesterolemia and liver disease due to its hepatoprotective effect as it decreases the elevated serum liver enzymes, improves lipid profile and histology of liver.

Key words: Celery, Chicory, Barley, Biochemical analysis, Histopathology of liver Hypercholesterolemic rats

Introduction

Celery (*Apium graveolens*, Family *Apiaceae*) seeds are used around the world as a vegetable. The seeds contain a valuable volatile oil (celery oil) and an organic substance called apiol. Celery seeds are also used as a flavoring agent or spice either as whole seeds or mixed with salt. Each 100 g of celery contain 3 g carbohydrate; 0.7 g protein; 0.2 g fat; 2 g sugars and 1.6 g dietary fibers. Celery is valuable in weight loss diets as it provides low calorie fiber content.

Chicory (*Chicorium intybus*, Family *Astraceace*) leaves is a root vegetable whose green leafy part is often used in cooking or in salads and has a long history of herbal use. Previous studies on chicory extracts and/or formulations

containing the roots or leaves revealed that they produce hepatoprote (Mitera *et.al.*, 2001, Ahmed *et. al.*,2003 and Krylova *et.al.* 2006) and antiox effects (Sarawathy and Devi, 2001 and Rossetto *et al.*, 2005) Recently, et.al.(2007) concluded that inulin (fructans) extracted from chicory reg appetite and lipid/glucose metabolism. It has also promising effects on the weight and fat mass development.

Barley (*Hordeum vulgare* L, Family *Poaceae*) is an annual cereal grain v serves as a major animal feed crop. Small amounts of barley are use malting and in healthy food. Barley makes a natural choice for healthful be as it is rich in protein, carbohydrates, dietary fibers, chromium, fat, and cholesterol free Mahmoud (2002) Moreover, Jenkins *et.al.*(2003) concluded barley may be used as a part of vegetarian diet, because it decreases total and reduces the risk of developing liver disease as fatty liver.

The present study aimed to reveal the effect of diet supplementation with ce chicory and barley powder, alone and in combination, on the body weight, conversion ratio, serum liver enzymes and complete blood lipid profi hypercholesterolemic rats as well as on liver histology.

Material and methods

Material:

Plants:

Celery (*Apium graveolens*, Family *Apiaceae*) seeds and Chicory (*Chicc intybus*, Family *Astraceace*) leaves were obtained from a local market of H and Medicinal plants, Cairo Egypt. Barley (*Hordeum vulgare*, Family *Poac* grains (Giza 128 variety) were obtained from Agricultural Research Ce Giza, Egypt. The selected plant materials were air-dried, grinded in an elec blender into a fine powder which packed in air -tight plastic bags till use for t diet supplementation.

Cholesterol:

It was purchased from El-Gomhuryia Company for Chemical Industries; C Egypt as a pure white powder packed in bottles each containing 100 g.

Rats:

Adult male albino rats of Sprague Dawley strain weighing 150-160 g body w were used in this study. Rats were obtained from Laboratory Animal Co Helwan, Egypt, fed on basal diet and provided with water *ad libitum*.

Methods:

Preparation of basal diet:

Basal diet was prepared according to Reeves *et.al.* (1993). It consists of 2 protein (casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vit mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder was starch.

Induction of hypercholesterolemia:

It was induced by feeding rats on basal diet supplemented with 3 % choles for 4 weeks before start of the experiment according to Shinnick *et. al.* (19 After 4 weeks of feeding period, a random blood sample was withdrawn fron orbital sinus of the eye and serum total cholesterol was measured to insure hypercholesterolemia was induced.

Experiments and grouping of rats:

Forty two male albino rats were used in this experiment. Rats were divided into 6 equal groups of 7 animals each. Group (1) was fed on the basal diet and kept as a negative control, while the other groups were fed on 3 % cholesterol supplemented diet for 4 weeks for induction of hypercholesterolemia. Group (2) was left as a positive control (hypercholesterolemic), while groups (3), (4), (5) and (6) were fed on experimental diets containing 10% celery, 10% chicory, 10 % barley and 15 % mixture (5% from each) of celery, chicory and barley respectively for 4 weeks. During the feeding period, the rats of all groups were weighed weekly, food intake was recorded and body weight gains and feed conversion ratios were calculated according to Chapman *et.al.* (1959). At the end of experiment period (4 weeks), the rats were anaesthetized by ether and blood samples were collected from the portal vein into dry centrifuge tubes. Blood samples were centrifuged for 20 minutes at 3000 rpm to separate the serum which was kept at -10 °C till biochemical analysis of liver enzymes, total cholesterol, triglycerides and lipoprotein fractions. Livers of the sacrificed rats were removed and preserved in 10% neutral formalin solution till histopathological examination.

Biochemical analyses:

The collected serum samples from rats were used for estimation of aspartate and alanine aminotransferases (AST and ALT) enzymes according to the method described by Bergmeyer *et.al.* (1978) and alkaline phosphatase (ALP) according to the method described by King (1965). Serum total cholesterol was calorimetrically determined according to Allain *et.al.* (1974) and triglycerides according to Wahlefeld (1974). High density lipoprotein cholesterol (HDL) was calorimetrically determined according to Richmond (1973), Very low density lipoprotein cholesterol (VLDL) and low density lipoprotein cholesterol (LDL) were mathematically calculated.

Histopathological examination:

Livers of the scarified rats were dissected, removed and fixed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H & E) then examined microscopically according to Carleton (1980).

Statistical analysis:

Data were expressed as means ± S.D. Statistical analysis was carried out using computerized SPSS program (version 8.0, Chicago, IL, USA) with student "t" test for significance according to Snedecor and Cochran (1986).

Results and discussion

As shown in Table (1), feeding normal rats on basal diet supplemented with cholesterol for 4 weeks significantly increased body weight gain (BWG%) and food efficiency ratio (FER). Feeding hypercholesterolemic rats on diet supplemented with 10 % celery significantly decreased BWG% and FER, while diets supplemented with 10% chicory or 10% barley or 15% mixture of the three plants caused significant increases in BWG% and FER. This finding was similar to that reported by Urias *et.al.* (2007) who concluded that inulin (fructans)

extracted from chicory regulate appetite and has a promising effect on the weight.

It is clear from Table (2) that feeding of diet supplemented with celery, ch and barley plant powder, alone and combined, for 4 week hypercholesterolemic rats significantly decreased the levels of AST, ALT ALP enzymes in the serum, compared to the control positive group. This was similar to that reported by Tsi and Tan (200) for celery extracts ar Ahmed *et.al.* (2003) and Shimaa and Enarh (2008) for chicory leaves and ang *et.al.* (2003) for barley grains. Tsi and Tan (2000), Ahmed *et.al.* (2 Shimaa and Enarh (2008) and Yang et.al (2003) reported that different ex of celery seeds or chicory leaves or barley grains effectively lower the ele serum levels of AST, ALT and ALP enzymes.

Table (1):

Effect of diet supplementation with celery, chicory or barley and combination on food intake (FI), body weight gain (BWG %) and food conve ratio (FCR) in hypercholesterolemic rats. (n =7 rats)

Groups	FI (g)	BWG (%)	FCR
Negative Control	9.3 ± 0.40	10.2 ± 0.25	1.09
Positive Control	13.2 ± 0.50**	16.9 ± 0.32**	1.28
Celery (10%)	11.2 ± 0.10*	12.2 ± 0.13*	1.08
Chicory (10%)	13.9 ± 0.80*	17.0 ± 0.13*	1.22
Barley (10%)	14.4 ± 0.20*	18.1 ± 0.41**	1.25
Mixture of all (15%)	14.5 ± 0.10*	18.9 ± 0.32**	1.30

Values denote means ± SD. * Significant at $p < 0.05$ ** Significant at $p < 0.01$

Table (2):
Effect of diet supplementation with celery, chicory or barley and their combination on serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase(ALP)enzymes in hypercholesterolemic rats (n =7 rats)

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative Control	63.5 ± 2.6	26.5 ± 1.6	90.98 ± 1.4
Positive Control	75.4 ± 3.2 **	37.5 ± 2.2 **	104.95 ± 1.6 *
Celery (10 %)	72.6 ± 2.4 *	34.5 ± 3.3 *	100.97 ± 1.8 *
Chicory (10 %)	71.3 ± 2.3 *	33.7 ± 3.6 *	98.90 ± 1.2 *
Barley (10%)	66.9 ± 3.8 **	32.1 ± 3.9 **	96.16 ± 1.3 **
Mixture of all (15%)	64.5 ± 2.8 **	28.1± 3.9 **	92.00 ± 1.5 **

Values denote means ± SD. * Significant at $p < 0.05$ ** Significant at $p < 0.01$

As demonstrated in (Table 3), feeding experimental diets supplemented with celery, chicory and barley plant powder, alone and in combination, for 4 weeks to hypercholesterolemic rats significantly decreased levels of total cholesterol and triglycerides in the serum, as compared to the control positive group. The cholesterol lowering effect of celery seeds reported in this study was similar to that previously reported by Tsi and Tan (2000). The same authors concluded that the mechanism underlying the hypocholesterolemic activity of celery extracts (aqueous and butanol) could be possibly due to presence of sugar or amino acid side chains(s) compounds. The hypocholesterolemic effect of chicory that reported in the current study was similar to that obtained by Ahmed *et.al.* (2003) and Krylova *et.al* (2006).In addition, the same authors attributed the hepatoprotective effect of chicory extract to its hypocholesterolemic activity in CCl4 – intoxicated rats.

Concerning barley, Jenkins *et.al.* (2003) concluded that barley may be used as a part of the vegetarian diet because it decreases serum total lipids. Moreover, Yang *et.al.* (2003) attributed the hypocholesterolemic effect of barley grains to the presence of active component Beta – glucan. Accordingly, the marked decrease in total cholesterol and triglycerides reported in the present study by the plant mixture could be possibly attributed to the additive effect of the three plants used in this study.

As shown in Table (4), feeding hypercholesterolemic rats on d supplemented with celery, chicory and barley plant powder, caused a signific decrease in serum level of LDL and VLDL., but significantly increased the lev of HDL. As it is well known that LDLc is bad cholesterol so these plant mate when added to basal diet improved lipid profile. This finding is in agreement some extent, with those reported by Tsi and Tan (2000) for celery in rats and Ahmed *et.al.* (2003) and Krylova *et.al* (2006) and Kim and Shin (1998) chicory in rats and by Behall et.al. (2004) and Keenan *et.al.*(2007) for concentrated barley Beta- glucan in hypercholesterolemic men and women.

Table (3): Effect of diet supplementation with celery, chicory or barley and the combination on serum total cholesterol and triglyceride in hypercholesterolem rats. (n =7 rats)

Groups	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
Negative Control	94.28± 0.15	47.40 ± 0.15
Positive Control	116.70± 0.92**	84.50 ± 0.86**
Celery (10%)	102.00± 0.85**	70.50 ± 0.62**
Chicory (10%)	99.00± 0.85**	75.50 ± 0.62**
Barley (10%)	99.22± 0.95**	74.60 ± 0.33**
Mixture of all (15%)	96.56± 0.57**	73.80 ± 0.16**

Values denote means ± SD. ** Significant at $p < 0.01$

Table (4): Effect of diet supplementation with celery, chicory or barley and th combination on serum lipoprotein fractions in hypercholesterolemic rats (n rats)

Groups	Lipoproteins (mg/dL)		
	HDL	LDL	VLDL
Negative Control	46.60 ± 0.19	38.20 ± 0.21	9.48 ± 0.52
Positive Control	31.30 ± 0.67**	68.50 ± 0.75**	16.90 ± 0.86**
Celery (10%)	33.30 ± 0.82*	54.60 ± 0.61**	14.10 ± 0.76*
Chicory (10%)	35.30 ± 0.92**	48.60 ± 0.61**	15.10 ± 0.15*
Barley (10%)	39.10 ± 0.51**	45.20 ± 0.75**	14.92 ± 0.35*
Mixture of all (15%)	40.20 ± 0.79**	41.60 ± 0.88**	14.76 ± 0.52*

HDL: High density lipoprotein cholesterol
LDL: Low density lipoprotein cholesterol
VLDL: Very low density lipoprotein cholesterol
*Values denote means ± SD. * Significant at $p < 0.05$ ** Significant at $p < 0.01$*

The biochemical observations reported in this study were supplemented by histopathological examination of liver sections of hypercholesterolemic rats. The obtained results showed that examination of livers of the normal (negative control ve) rats fed on the basal diet had normal histological picture of hepatic lobule that consists of central vein surrounded by normal hepatocytes Fig. (1). Examination of liver of hypercholesterolemic rats showed severe fatty degeneration of the hepatocytes and infiltration of leucocytes in hepatic sinusoid (Fig. 2). Livers of hypercholesterolemic rats fed on diet containing celery 10% showed little vacuolar degeneration of hepatocytes and mild fatty degeneration of hepatocytes as shown in Fig. (3). Examination of livers of hypercholesterolemic rats fed on diet supplemented with chicory 10% showed only mild fatty degeneration of the hepatocytes as illustrated in (Fig.4).
Hypercholesterolemic rats fed on experimental diet containing barley 10% showed little vacuolar degeneration of hepatocytes and mild leucocytic infiltration around central vein (Fig. 5). Examination of livers obtained from hypercholesterolemic rats fed on diet supplemented with a mixture of celery, chicory and barley at 15% revealed almost normal hepatic lobules as illustrated in Fig.(6). Nearly similar histopathological findings were obtained by Jenkins *et.al.* (2003) and Kim and Shin (1998) in CCl4 - hepatotoxic rats when given orally chicory extracts. No available literatures on the effects of celery and barley on the histology of liver could be obtained.

In conclusion, this study suggests that dietary intake of plant mixture of celery, chicory and barley at 15 % (5% from each) for 4 weeks may be beneficial for patients suffering from hypercholesterolemia and liver disease as it lowers the elevated serum liver enzymes, total cholesterol and triglycerides, improves lipid profile in cholesterol fed rats. Moreover, diet supplementation with this plant mixture produces an excellent effect on the histology of liver as it ameliorates the hepatic damage seen in the liver of hypercholesterolemic rats.

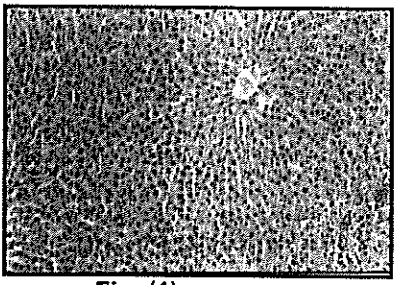

Fig. (1)

Fig. (2)

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Fig. (4)

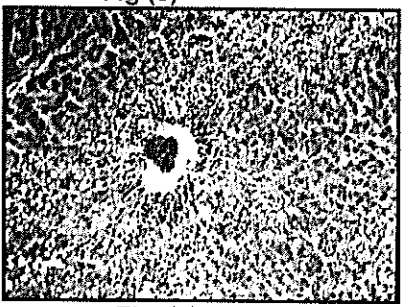

Fig. (5)

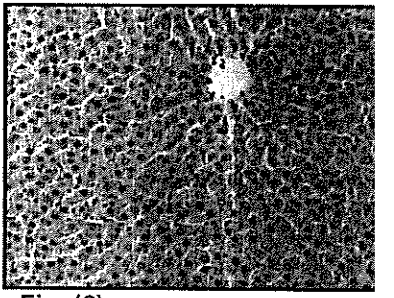

Fig. (6)

Fig. (1) Liver of control C–ve (normal) rats showing normal histology of hepatic lobule. H & E
Fig. (2) Liver of hypercholesterolemic (control C+ ve) rats showing severe fatty degener
hepatocytes and infiltration of leucocytes in hepatic sinusoid. (H & E X 100)
Fig. (3) Liver of rats fed on basal diet containing 10% celery powder showing little v
egeneration of hepatocytes mild fatty degeneration of hepatocytes. (H & E X 100)
Fig. (4) Liver of rats fed on basal diet containing 10% chicory powder showing only m
degeneration of hepatocytes (H & E X 100)
Fig. (5) Liver of rats fed on basal diet containing 10% barley powder showing little v
degeneration of hepatocytes and mild leucocytic infiltration around central vein. (H & E X 100)
Fig. (6) Liver of rats fed on basal diet containing 15% mixture of the three plant materials s
almost normal histology of hepatic lobule. (H & E X 100)

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ي العربى

اقى للكبد بتغذية بذور الكرفس، أوراق الشيكوريا وحبوب القمح للفئران المصابة الكوليسترول فى الدم

عباس شلبى - هشام يوسف الزربة - و نهال محمد عبد المجيد

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ف هذه البحث دراسة تأثيردعم الغذاء بمسحوق بذورالكرفس ،أوراق الشيكوريا وحبوب وخليط من الثلاثة معا على وزن الجسم، كفاءة تحويل الغذاء، صورة دهون الدم الكبد فى الفئران المصابة بارتفاع الكوليسترول فى الدم. وقد تم إجراء التجربة على م تغذيتها على غذاء أضيف اليه الكوليسترول بنسبة ٣ فى المائة لمدة أربعة أسابيع ارتفاع فى تركيز الكوليسترول فى الدم . وبعد ذلك تم تغذية الفئران المصابة بارتفاع ول الدم على غذاء مدعم بمسحوق بذور الكرفس أو أوراق الشيكوريا أوحبوب الشعير ١ فى المائة لكل منها أوعلى خليط من الثلاثة معا بنسبة ١٥ فى المائة (٥ فى المائة من) لمدة أربعة اسابيع . وفى نهاية فترة التجربة تم قياس بعض مكونات المصل الكيميائية الفحص الهستوباثولوجى لنسيج الكبد.

ت نتائج الدراسة أن تغذية الفئران المصابة بارتفاع كوليسترول الدم على غذاء مدعم ن مسحوق بذورالكرفس وأوراق الشيكوريا وحبوب الشعيرمعا بنسبة ١٥ فى المائة لمدة سابيع أدت إلى زيادة وزن الجسم وكفاءة تحويل الغذاء. كما أدت إلى نقص معنوى فى زيمات الكبد المرتفعة وكذا نقص فى تركيزات الكوليسترول والجليسريدات الثلاثية وتينات منخفضة الكثافة ومنخفضة الكثافة جدا، بينما أدت إلى زيادة فى يوبروتينات عالية الكثافة فى المصل. وأظهر الفحص الهستوباثولوجى لنسيج الكبد عظم التغيرات الهستوباثولوجية التى سببها الكوليسترول المرتفع فى الدم. وتوصى أن تناول خليط من مسحوق بذورالكرفس وأوراق الشيكوريا وحبوب الشعيرمعا بنسبة المائة (٥ فى المائة من كل نبات) لمدة أربعة اسابيع قد يكون مفيدا للمرضى الذين ن ارتفاع الكوليسترول فى الدم ومرضى الكبد وذلك لتأثيره الواقى للكبد حيث أنه يؤدى ن واضح فى أنزيمات الكبد وصورة دهون الدم وكذا تحسن فى نسيج الكبد فى الفئران بارتفاع الكوليسترول فى الدم.