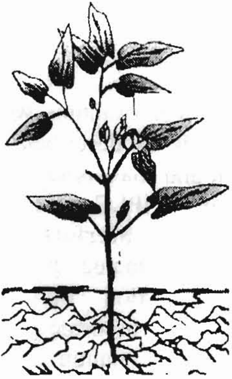

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ANTIOXIDANTS CONTENT OF CHICORY LEAVES EXTRACT AND ITS EFFECT AS HYPOLIPIDEMIC AGENT IN EXPERIMENTAL RATS

[14]

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Keywords: Chicory, antioxidant activity; natural antioxidant; hypolipidemic

ABSTRACT

Alcoholic chicory leaves extract was studied for its content of antioxidant components (total phenols, total flavonoids, carotenoids, total chlorophyll A & B, tannins, vit. C and E) and mineral elements. As well as hypolipidemic effects of chicory extract in male albino rats fed high-fat (HF) diet was also investigated. Three groups of rats were fed on HF diet treated with chicory extract at concentrations of (1, 2 and 5%) and compared to normal control (NC) and HF diet groups. The results revealed that, the chicory extract had high content of important compounds which act as antioxidants e.g., phenolic compounds (58.1 mg/g), flavonoids (7.23 mg/g), Tannins (0.53 mg/g), carotenoids (0.52 mg/g) and Mg (3.75 mg/g). The antioxidant activity of chicory extract was more effective compared to BHA and tannic acid. The biological experimental indicated a significant decrease in serum triglycerides, total cholesterol, atherogenic index, while an increase in HDL was detected when compared to the normal control and high fat diet groups. Also, significant increase in body weight gain and liver organ was found in the HF group at the end of the experiment.

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INTRODUCTION

Chicory (*Cichorium intybus* L., Asteraceae) as an important medicinal herb has been used in folk medicine for liver disorders, gallstones and for inflammations of the urinary tract since the 17th century. It is well-known from the literature that the main active compounds of chicory are: inulin, fructooligosaccharides, caffeic acid derivatives, flavonoids and polyphenols. Chicory fructooligosaccharides have been investigated in studies on the gastrointestinal system especially because of the inulin and dietary fiber content of this medicinal plant. Some oligosaccharides have functional effects similar to soluble dietary fiber such as enhancement of a healthy gastrointestinal tract, improvement of glucose control, and modulation of the metabolism of triglycerides **(Roberfroid 2000 and Roberfroid & Slavin 2000)**.

Chicory extract significantly decreased cholesterol absorption by 30% ($P < 0.05$) in rats jejunum and by 41% ($P < 0.05$) in the ileum, compared with control. Addition of inulin to the diet resulted in a significant reduction of cholesterol absorption from jejunum by 39% ($P < 0.05$) and from ileum by 51% ($P < 0.05$) **(Meehye 2000)**.

It is well known that the medicinal plant, chicory contains dietary oligofructose compounds which have beneficial effect on carbohydrate and lipid metabolism, other compounds e.g. polyphenol type derivatives could be responsible for the antioxidant properties. **Kok *et al* (1996)** measured

Polyphenols, flavonoids and caffeic acid derivatives in chicory extract by spectrophotometric and chromatographic methods. Antioxidant property of the plant extract was determined in vitro and the biological activity of antioxidant compounds of chicory was investigated in vivo by a luminometric technique Male Fischer rats were kept on normal and lipid rich diet supplemented with chicory extract. The effects of bioactive molecules of chicory extract influenced the lipid metabolism and the redox balance of pancreatic tissue of rats in experimental dislipidemia.

So, the main goal of our subject was to focus on cheap price plant "Chicory", its chemical and nutritional properties.

MATERIALS AND METHODS

1. Materials

Fresh chicory leaves were obtained from Horticultural Research institute-Agricultural Research Center, Egypt.

Total cholesterol, triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were obtained from biodiagnostic Co. Dokki, Egypt. While, HDL kit was obtained from Biosystem Co. Spain.

2. Methods

2.1. Preparation of chicory extract

Dried slices of chicory leaves were ground into powder then ethanol (85%) was added to the powder and soaked overnight to obtain 1, 2 and 5 % (W/V) of extract. This suspension was filtered and the residue was resuspended in an equal volume of 85% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvents were evaporated in a rotary evaporator at 40-50°C under vacuum to concentrate the extract.

2.2. Determination of antioxidants content

The most effective antioxidant components were determined in chicory extract. Phenols were determined calorimetrically as described by **Swain and Hillis (1959)**. Flavonoids were determined according to the method of **Miliauskas *et al* (2004)**. Chlorophyll-A, chlorophyll-B and carotenoids were extracted according to the method of **Fedtke (1973)** and calculated according to the method of **Wettstian (1957)**. Total tannins were also determined calorimetrically as described by **AOAC (1995)**. Polyphenol and flavonoid fractions were determined using HPLC (Hewlett Packard series 1100) according to **Merfort *et al* (1997)**. Ascorbic acid was estimated by the method based on the reduction of 2,6-dichlorophenol indophenol by ascorbic acid **(AOAC, 1995)**. Determination of vitamin E was carried out according to **Kirk and Sawyer (1991)**.

Mineral elements of Mn, Zn, Cu, and Mg were determined in samples as sulphate using the wet ashing method with acid mixture (nitric: phosphoric: sulphuric acids) at the ratio of (8:1:1) according to **Galvao *et al* (1976)** using atomic absorption spectrophotometer FMD_3 Zesis. Selenium was determined according to the method of **Diaz-Alarcon *et al* (1994)**.

2.3. Antioxidants activity

Antioxidant activity was determined against autoxidation according to **Nergiza (1991)**. Ten grams of dried sample was exhaustively extracted with ethanol (100ml). Twenty five ml of the extract were mixed with 25g of corn oil in a flask and the mixture was placed in an oven at 60°C for 3h daily, the experiment was repeated on seven consecutive days. Oil samples were taken at the beginning of experiment and daily at the end of the three hours of heating. The peroxide value was determined according to the method described in **AOAC (1995)**.

2.4. Biological experiments

Twenty five albino rats, with an initial body weight of 150 g were housed in screen-bottomed aluminum cages in rooms maintained at 25±1°C with alternating cycles of light and dark of 12h duration. Rats were randomly allocated into two groups, the first group (normolipidemic) contained five animals and the second group (hyperlipidemic) contained twenty animals with a mark on their tails as a mean of differentiation. In the normolipidemic group, rats were fed on normal diet. In the hyperlipidemic group, rats were fed on high fat diet (HF). The compositions of these diets are shown in **Table (1)**.

After arising the cholesterol level to ≥240 mg/dl, fifteen animals from the hypercholesterolemic rats was divided into four groups (with five rats each), three groups were treated oral by gastric tube with the chicory extract at 1, 2 and 5%

Table 1. Compositions of the experimental diets (%)

Composition %	Normal diet	Hyperlipidemic diet
Corn starch	65	50
Casein	15	15
Corn oil	10	10
Cellulose	5	5
Salt mixture	4	4
Vitamin mixture	1	1
Cholic acid	-	0.2
Lard	-	14.8

daily for 30 days while the fourth group was fed on high fat diet (HF). The changes in body weight were recorded weekly. Blood samples were also obtained from the retro-orbital plexus of the eyes from all animals of each group at the end of experiment; the organs were excised immediately after bleeding for weight. Serum was obtained from blood samples by centrifugation at 1500 rpm for 15 min at an ambient temperature. All the serum samples were stored under -20 °C before use.

Enzymatic determination of cholesterol was carried out according to **Allain (1974)**. Fully enzymatic determination of total triglycerides in plasma was measured colorimetrically at 546 nm, according to **Fossati and Principe (1982)**, While, the HDL was determined according to the method of **Burstein, *et al* (1980)**.

2.5. Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by **Snedecor and Cochran (1980)** and the statistical analysis system program **(SAS, 1996)** using Student t-test and factorial analysis.

RESULTS AND DISCUSSION

3.1. Antioxidant contents of chicory leaves extract

The data in **Table (2)** regarding the antioxidant components of chicory extract. It characterized by high content of total phenols, total flavonoids and vitamin C (58.10 mg/g, 7.23 mg/g and 191.04 mg/100g, respectively). On the other side, the extract had a moderate content of total chlorophyll (1.08 mg/g), while it contained low amounts of minor components which represented in tannins and carotenoids (0.53 and 0.52 mg/g). Furthermore, vitamin E content was 1.92 mg/100g. These results are agreed with those of **Peschel *et al* (2006)**.

Table 2. Antioxidant contents of chicory leaves extract

Components		Concentration
Total phenols	(mg/g)	58.10
Total flavonoids	(mg/g)	7.23
Carotenoids	(mg/g)	0.52
Total chlorophyll		1.08
Chlorophyll-A	(mg/g)	0.80
Chlorophyll-B		0.28
Tannins	(mg/g)	0.53
Vitamin C	(mg/100g)	191.04
Vitamin E	(mg/100g)	1.92

The data in **Table (3)** show that both polyphenols and flavonoids contents were relatively high in chicory ethanol extract. It contained relatively high amount of kaempherol and cinnamic acid (34.14 and 10.25 µg/g, respectively), while the extract had moderate amounts of quercetin, protocatechuic acid and hesperidine (3.57, 2.62 and 1.53 µg/g, respectively). Minor constituents of rutin, P-OH benzoic acid and apignin (0.97, 0.11 and 0.04 µg/g, respectively) were observed. These results are in parallel with those of **Dimitrios (2006)**.

Table 3. Polyphenols and flavonoids contents of chicory extract (µg/g)

Components	Rt *	µg/g
Protocatechiuc acid	11.568	2.62
P-OH benzoic acid	14.146	0.11
Apignin	20.971	0.04
Rutin	24.391	0.97
Hesperidine	26.689	1.53
Cinnamic acid	31.187	10.25
Quercetin	32.293	3.57
Kaempherol	36.617	34.14

*Rt = Retention Time

From **Table (4)**, it could be observed that chicory extract had a high content of the macro-element Mg (3.75 mg/g), while the other micro-elements content arranged in ascending order as follows: Mn, (0.54), Zn (0.52 mg/g), Cu (0.37 mg/g) and Se (0.38 µg/g). Generally, it is well known that these minerals are active as antioxidant according to **WHO (2003)**.

Table 4. Mineral contents of chicory extract as antioxidant (mg/g)

Minerals	mg/g
Mg	3.75
Cu	0.37
Mn	0.54
Zn	0.52
Se*	0.38

* µg/g

3.2. Antioxidant activity

Data in **Figure (1)** emphasized that chicory extract at 100 and 200 ppm was more effective than butylated hydroxyl anisole (BHA) or tannic acid, because this extract contained multi-antioxidant components which play a synergistic role between antioxidant groups. It could be noticed that the mixture of several antioxidant in extract was more effective than individual natural or synthetic antioxidant. These data were adapted by **El-Hadidy (2004) and El-Hadidy *et al* (2007)**, they found the same trend when used Jew's mallow, parsley, rocket, Egyptian leek, dill, coriander and celery extracts at different concentration.

It could be concluded from the aforementioned results that chicory plant is an excellent source of antioxidants which may have the potential for prevention of oxidation damage in food.

3.3. Biological experiment

3.3.1. Effect on body weight and internal organs relative weight of male albino rats

Initial body weights of the five groups were not significantly different, however, after feeding four weeks; body weights were significantly higher in the HF fed group compared to with the normal control **(Table 5)**.

The relative weights of liver were significantly higher in the HF group than in the NC and chicory extract treated groups because of the fatty liver. However, the kidney and heart relative weights were not significantly different between all the groups **(Table 5)**. This trend has also been reported by **Lee *et al* (2006)**.

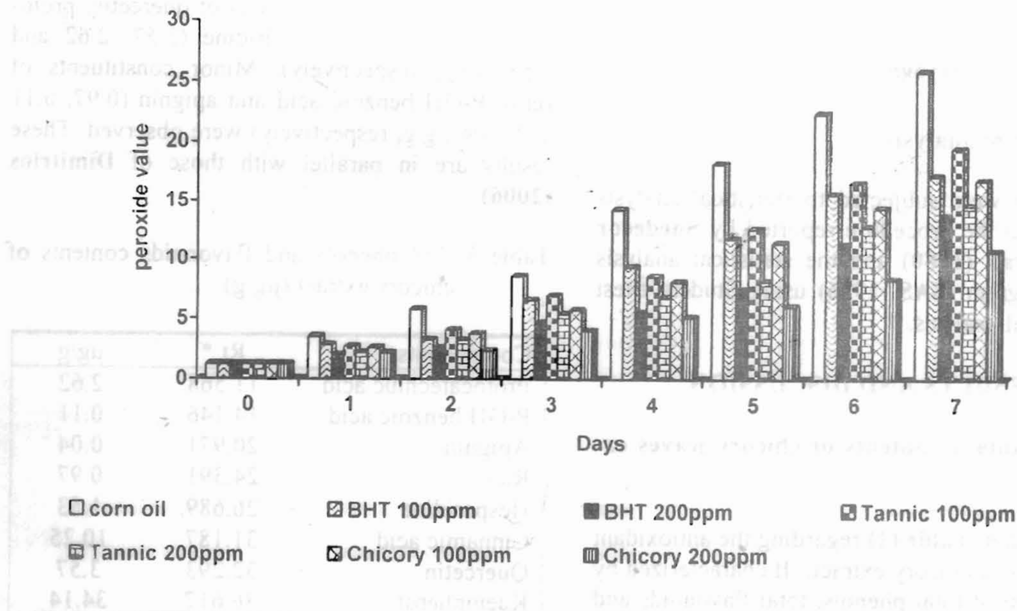

Fig. 1. Antioxidant activity of chicory extracts compared with BHA and tannic acid

Table 5. Effect of oral administration of chicory extract at different concentration on body weight gain and relative weight of the visceral organs in rats.

Parameters	NC[a]	HF[b]	Chicory extracts		
			1%	2%	5%
Body weight gain					
Initial	149.8± 0.4	151.2± 0.14	150.9±0.03	153.5± 0.1	149.4± 0.1
Final	170.7±0.3	177.1±0.8*	169.2±3.5	172.4±0.4	171.6±0.3
Relative weight of visceral organs (final) [% of body weight]					
Liver	3.33±0.13	4.15±0.1*	2.95±0.10	3.25±0.12	3.34±0.43
Kidney	0.68±0.03	0.71±0.01	0.70±0.01	0.71±0.02	0.73±0.02
Heart	0.40±0.03	0:50±0.05	0.44±0.03	0.40±0.04	0.42±0.07

* Statistical significant differences ($P < 0.05$)
[a] Normal control group
[b] High fat fed group

3.3.2. Effect on lipid profile parameters

Concentrations of plasma lipids are shown in **Table (6)**. The supplementation of chicory extract groups at concentrations 1, 2 and 5% significantly lowered plasma total cholesterol concentration by 27.64%, 32.84% and 44.8% compared to the HF group, and triglyceride concentration by 41.98%, 47.69% and 53.36% when compared to the HF groups, respectively. On the other hand the HDL-cholesterol concentration was significantly elevated in the chicory extract groups than in the NC or HF group, the ratio of HDL-C/Total-C exhibited the highest value in the chicory extract groups and the lowest value in the HF group. For this reason, atherogenic index (AI) was significantly higher in the HF group than in the NC and chicory extract groups.

In this study, chicory extract improved lipid profiles by lowering plasma total cholesterol and triglyceride concentrations compared with the HF and NC groups. The plasma HDL-cholesterol concentration was higher in the chicory extract groups than in the HF group, however, the ratio of HDL-cholesterol /Total- cholesterol was significantly increased by 37.58%, 61.67% and 79.95% of treated samples with 1, 2 and 5% chicory extract, respectively when compared with the HF group, these findings are in agreement with the results obtained by, **Kok *et al* (1996).**

3.3.3. Effect on liver function parameters

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) plasma levels were performed to assess liver function. As can be observed in **Table (6)**, no alterations were detected in the animal group treated with the HF diet while those treated chicory extract, significant decreases in AST and ALT enzymes during 4 weeks, when compared to the control group.

Table 6. Effect of oral administration of chicory extracts on plasma lipids profiles and liver function in rats.

Parameters	NC[a]	HF[b]	Chicory extracts		
			1%	2%	5%
Total cholesterol (mg/dl)	150.16 ± 0.32	166.02** ± 0.23	120.13*** ± 0.27	111.5*** ± 0.15	82.82*** ± 0.03
Triglycerides (mg/dl)	70.88 ± 0.06	107.63** ± 0.02	62.44 ± 0.05	56.3* ± 0.03	50.2** ± 0.02
HDL-cholesterol (mg/dl)	48.19 ± 8.68	45.29 ± [illegible].35	62.31** ± 2.6	73.22** ± 5.2	81.5*** ± 3.1
HDL-cholesterol/ Total cholesterol (%)	32.09 ± 0.46	27.28** ±0.45	51.87*** ±0.41	65.67*** ±0.26	98.41*** ±0.13
AI[c]	2.12 ±0.045	2.67** ±0.060	0.926*** ±0.01527	0.523*** ±0.006	0.016*** ±0.006
AST activity (U/L)	44.40 ± 14.41	41. 80 ± 18.3	35.61 ± 9.3	31.44* ± 6.4	27.36** ± 3.3
ALT activity (U/L)	50.07 ± 12.02	40.44 ± 3.95	39.84 ± 6.44	35.85* ± 3.35	31.51** ± 4.01

* Statistical significant differences ($P < 0.05$) ** Statistical significant differences ($P < 0.01$)
*** Statistical significant differences ($P < 0.001$) [a] Normal control group [b] High fat fed group
[c] Atherogenic index. (Total cholesterol - HDL-cholesterol)/HDL-cholesterol

CONCLUSION

From the aforementioned results, it could be concluded that, chicory extract could be used as source of dietary antioxidants included, bioactive plant phenols, and flavonoids such as kaempherol, cinnamic acid, carotenoids, chlorophyll (A and B), ascorbate and tocopherols. The health benefits of chicory extract are largely due to the antioxidant vitamins supported by the large number of phytochemicals, some with greater antioxidant properties and hypolipidemic effect. Therefore, we could recommend the consumption of this plant as raw or in addition form to food products.

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حوليات العلوم الزراعية
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محتوى مستخلص أوراق الشيكوريا من مضادات الأكسدة وتأثيره كعامل خافض لليبيدات فى فئران التجارب

[١٤]

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تم دراسة محتوى مستخلص أوراق الشيكوريا من مضادات الأكسدة (الفينولات الكلية، الفلافونويدات الكلية، الكاروتينات ، الكلوروفيلات الكلية، التانينات، فيتامين ج و ه ، وبعض العناصر المعدنية) كما تم دراسه تأثير مستخلص الشيكوريا على خفض ليبيدات الدم فى الفئران المغذاه على وجبه مرتفعة الدهن، حيث تم تقسيم الفئران إلى ثلاثة مجموعات مغذاه على غذاء مرتفع الدهن و عوملت بمستخلص الشيكوريا بتركيزات (1, 2 and 5%) و قورنت بالمجموعات التى غذيت على غذاء عادي و غذاء مرتفع الدهن.

وأظهرت النتائج ما يلى

١- إرتفاع المحتوى من المكونات الهامة التى تقوم بدور مضادات الأكسدة مثل الفينولات الكلية (٥٨,١ ملجم/جم و الفلافونويدات الكلية (١,٢٣ ملجم/جم) والتانينات (٠,٥٣ ملجم/حم) والكاروتينات (٠,٥٢ ملجم/جم) وأحتوى المستخلص على كمية مرتفعة من معدن الماغنسيوم (٣,٧٥ ملجم/جم).

٢- القوة الأحترالية والتى تعنى النشاط المضاد للأكسدة للمستخلص كانت أعلى من مضادات الأكسدة الصناعية والطبيعية.

٣- فى نهاية التجربة وجد إرتفاع معنوى فى معدل الزيادة فى وزن الجسم ووزن الكبد للمجموعة المغذاة على وجبة مرتفعة الليبيدات مقارنة بالمجموعة الضابطة.

٤- حدوث إنخفاض فى مستوى الكوليسترول الكلى والجليسريدات الثلاثية فى الفئران المعاملة بمستخلص الشيكوريا مقارنة بالمجموعة الضابطة والمجموعة المرتفعة فى ليبيدات الدم وبالتالى ادي ذلك الى تحسين مستوى الليبوبروتين مرتفع الكثافة فى المجاميع المعاملة وإنخفاض معامل تكوين الجلطات مقارنة بالمجموعة الضابطة والمجموعة المرتفعة فى ليبيدات الدم.

تحكيم: ا.د ماجدة حبيب علام
ا.د صلاح كامل محمد السماحي