Evidence of glycemia-lowering effect by a *Cynara scolymus* L. extract in normal and obese rats

Noemi Fantini, Giancarlo Colombo, Antonella Riva, Paolo Morazzoni, Ezio Bombardelli, Mauro A.M. Carai

► To cite this version:

Noemi Fantini, Giancarlo Colombo, Antonella Riva, Paolo Morazzoni, Ezio Bombardelli, et al.. Evidence of glycemia-lowering effect by a *Cynara scolymus* L. extract in normal and obese rats. *Phytotherapy Research*, 2010, 10.1002/ptr.3285 . hal-00613789

HAL Id: hal-00613789

<https://hal.science/hal-00613789>

Submitted on 6 Aug 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Evidence of glycemia-lowering effect by a *Cynara scolymus*
L. extract
in normal and obese rats**

Journal:	<i>Phytotherapy Research</i>
Manuscript ID:	PTR-10-0189.R3
Wiley - Manuscript type:	Short Communication
Date Submitted by the Author:	02-Jul-2010
Complete List of Authors:	Fantini, Noemi; C.N.R. Institute of Neuroscience Colombo, Giancarlo; C.N.R. Institute of Neuroscience Riva, Antonella; Indena SpA Morazzoni, Paolo; Indena SpA Bombardelli, Ezio; Indena SpA Carai, Mauro; C.N.R. Insitute of Neuroscience
Keyword:	Artichoke (<i>Cynara scolymus</i> L.), Extract of flowering heads, Glycemia, Zucker rats, Nutraceutical

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Evidence of glycemia-lowering effect by a *Cynara scolymus L.* extract
in normal and obese rats

Noemi Fantini¹, Giancarlo Colombo¹, Andrea Giori², Antonella Riva², Paolo Morazzoni², Ezio Bombardelli², Mauro A.M. Carai¹

¹C.N.R. Neuroscience Institute, Viale Diaz 182, I-09126 Cagliari, Italy, and ²Indena S.p.A., Viale Ortles 12, I-20139, Milan, Italy.

Corresponding author:

Mauro A.M. Carai

C.N.R. Neuroscience Institute

Viale Diaz 182

I-09126 Cagliari (CA)

Italy

Phone: +39 070 302227

Fax: +39 070 302076

e-mail: caraimam@tiscali.it

Abstract

Several recent preliminary clinical studies have suggested that artichoke (*Cynara scolymus* L., *Asteraceae* family) preparations may be capable of lowering post-prandial glycemia. The present study was designed to test this hypothesis in laboratory rats. To this aim, non-selected Wistar and genetically obese Zucker rats were acutely treated with a purified extract of *Cynara s.* flowering heads (500-1500 mg/kg by gavage) immediately prior to 1-hour access to a fixed amount of food. Glycemia was recorded 60, 120, and 360 min after food presentation. Treatment with *Cynara s.* flowering head extract resulted in a significant decrease of post-prandial glycemia in both rat strains. The lack of any fiber content in this *Cynara s.* flowering head extract excludes the involvement of dietary fibers in glycemia reduction. The results obtained constitute the first evidence of a hypoglycemic effect of an artichoke preparation in laboratory rodents and confirm previous observations made in humans.

Key-words: Artichoke (*Cynara scolymus* L.); Extract of flowering heads; Glycemia; Zucker rats; Nutraceutical.

1
2 1 **Introduction**
3
4
5 2
6
7 3
8
9 4 Artichokes (*Cynara scolymus L.*, *Asterceae* family) are an ancient crop and medicinal plant, the
10
11 5 therapeutic potential of which was known to the ancient Egyptians, Greeks, and Romans
12
13 6 (Lattanzio et al., 2009). In the remote past artichoke preparations were used as a digestive aid. In
14
15 7 recent times, reports from folk medicine as well as preliminary data from scientifically designed
16
17 8 clinical studies have suggested that artichokes may have cholorectic, hypocholesterolemic, and
18
19 9 hypolipidemic properties (Gebhart, 1998; English et al., 2000; Bundy et al., 2008; Wider et al.
20
21 10 2009; Küskü-Kiraz et al., 2010).
22
23 11 Based on the notion that dietary fibers may be beneficial in carbohydrate metabolism a recent
24
25 12 study (Vinik et al., 1998) found that consumption of an artichoke food supplement for three
26
27 13 consecutive months reduced fasting and post-prandial glycemia in type-2 diabetic patients (Nazni
28
29 14 et al., 2006). Consistently, an independent study found that a meal of bread and wild artichokes
30
31 15 (*Cynara cardunculus L.*) attenuated post-prandial increase in glycemia in healthy subjects
32
33 16 (Nomikos et al., 2007).
34
35 17 The present study was designed to assess the ability of artichoke extract to reduce glycemia via a
36
37 18 mechanism other than the action of its fibers (Vinik et al., 1998). For this purpose, the present
38
39 19 study tested the capacity of a standardized, purified, fiber-free extract of *Cynara scolymus L.* to
40
41 20 lower post-prandial glycemia in laboratory rats. This extract was of particular interest with regard
42
43 21 to the study aims as it was completely fiber-free. Two different strains of rats were used:
44
45 22 unselected Wistar rats and genetically selected, obese Zucker rats; notably, Zucker rats display
46
47 23 fasting hyperglycemia and have been proposed as a possible animal model of pre-diabetes
48
49 24 (Romanovsky et al., 2008). The effect of the acutely administered artichoke extract on glycemia
50
51 25 was evaluated after the consumption of a large meal comprising a given amount of food. Rats were
52
53
54
55
56
57
58
59
60

1 fasted before the meal to ensure consumption of an equal amount of food by rats from each
2 experimental group (vehicle- or extract-treated rats) within the scheduled 1-hour access period.
3
4
5
6
7
8
9
10

11 **Materials and Methods**

12
13
14
15
16 7 The experimental procedures employed in the present study were in accordance with the European
17
18 8 Communities Council Directive (86/609/EEC) and the subsequent Italian Law on the Protection of
19
20 9 animals used for experimental and other scientific reasons.
21
22
23
24
25

26 *Animals*

27
28
29
30 13 Adult male Wistar (Experiment 1) and Zucker (Experiment 2) rats (Charles River Laboratories,
31
32 14 Calco, LC, Italy) were used. In both experiments rats were individually housed in standard plastic
33
34 15 cages with wood chip bedding. The animal facility was under an inverted 12:12 hour light-dark
35
36 16 cycle (lights on at 11:00 p.m.), at a constant temperature of $22\pm 2^{\circ}\text{C}$ and relative humidity of
37
38 17 approximately 60%. Rats were extensively habituated to handling, intragastric infusion, and blood
39
40 18 drawing from the tail tip.
41
42
43
44
45
46

47 *Extract preparation*

48
49
50
51 22 *Cynara scolymus* flowering heads, Sardinian spiny variety, were chopped and frozen at the time of
52
53 23 harvesting. The biomass, maintained at -30°C until extraction, was cryoground and immediately
54
55 24 submitted to alcoholic extraction. 1 kg of *Cynara scolymus* flowering heads was extracted in a
56
57 25 percolator with ethanol/water (70:30), using a total volume of 7 L. The hydroethanolic extract was
58
59 26 concentrated under vacuum, to remove ethanol, obtaining 1 L of an aqueous concentrate, a volume
60

corresponding to that of extracted plant material. Subsequently, the concentrate was purified on an absorption resin through loading into a column packed with 900 mL polymeric resin (Amberlite™); the resin was thoroughly washed with 1 L water, the purified extract eluted with 2 L ethanol, and was subsequently concentrated to dryness to yield 8.5 g of powder. Separation and determination of caffeoylquinic acid derivatives and flavonoids in the extract was performed simultaneously by a reversed phase HPLC method, using a stainless steel column (250 x 4.6 mm), packed with octadecylsilyl silica gel for chromatography (5 µm), kept at 20°C, and with gradient elution at a constant flowrate of 1.0 ml/min. Mobile phase A was composed by a mixture of trifluoroacetic acid (0.01 % v/v, in water); mobile phase B was composed by a mixture of trifluoroacetic acid (0.01 % v/v, in acetonitrile); the injection volume for all samples was 10 µl. Detection was obtained with a UV spectrophotometer at 263 nm. Samples and standards were dissolved in methanol (40 % v/v, in water), filtered by a 0.45-µm polytetrafluoroethylene syringe filter and then injected directly into the HPLC system. The extract obtained had a high caffeoylquinic acid (≥50%), and flavonoid content, expressed as luteolin glycosides, of approximately 2%.

Experimental procedure

In both experiments, rats were kept under a regimen of unlimited access to regular rodent chow [Harlan, Global Diet 2018, Mucedola, Settimo Milanese, MI, Italy; main composition: 18.9% crude protein, 5.7% crude oil, 3.8% crude fibre, 5.9% ash, 57.3% carbohydrate (42.2% starch, 4.9% sugar); digestible energy: 3.35 cal/g, metabolisable energy: 3.27 cal/g] and water for 24 hours/day. Rats were fasted 12 hours before the start of the experiment (this 12-hour period coincided with the entire light phase of the light-dark cycle). In Experiment 1, on the test day Wistar rats were divided into four groups of $n=7-9$, matched for body weight and glycemia at time 0, and treated with 0, 500, 1000, and 1500 mg/kg *Cynara s.* flowering head extract. In Experiment

2, on the test day Zucker rats were divided into three groups of $n=6-7$, matched for body weight and glycemia at time 0, and treated with 0, 500, and 1500 mg/kg *Cynara s.* flowering head extract. Doses of *Cynara s.* flowering head extract were selected on the basis of the results of previous experiments and literature data (Küskü-Kiraz et al., 2010). In both experiments, *Cynara s.* flowering head extract was suspended in distilled water with a few drops of Tween 80 and administered intragastrically by a metal gavage at an infusion volume of 4 ml/kg, 30 min before lights off. At lights off, rats were given 8 g/kg food pellets; this food amount was chosen on the basis of preliminary experiments demonstrating how fasted Wistar and Zucker rats consumed this entire amount in less than 60 min (this laboratory, unpublished results). Glycemia was determined 0, 60, 120, and 360 min after food presentation. A small (0.05 ml) blood sample was collected from the tip of the tail of each rat and analyzed enzymatically by means of GL5 Analox (Analox Ltd, London, United Kingdom).

Statistical analysis

In both experiments, data on the effect of *Cynara s.* flowering head extract on time-course of glycemia were analyzed by a 2-way (dose; time) ANOVA with repeated measures on the factor “time”. Data on the effect of *Cynara s.* flowering head extract on the area under the curve of the time-course of glycemia [expressed in mg*min/100 ml; calculated using GraphPath Prism 3.03 (GraphPath Software, La Jolla, CA, USA)] were analyzed by a 1-way ANOVA.

Results

Experiment 1 (Wistar rats)

Glycemia in fasted Wistar rats averaged approximately 70 mg/dl in all rat groups (Figure 1, top panel). In vehicle-treated rats, meal consumption resulted in an increase of glycemia at both the 60- and 120-min recording times, when it averaged approximately 130 mg/dl (Figure 1, top panel). At the 360-min recording time, glycemia in vehicle-treated rats approached basal levels (Figure 1, top panel).

ANOVA revealed a highly significant effect of treatment with *Cynara s.* flowering head extract on post-prandial glycemia over the 6-hour recording period [$F_{\text{dose}}(3,30)=7.44$, $P<0.001$; $F_{\text{time}}(2,60)=129.31$, $P<0.0001$; $F_{\text{interaction}}(6,60)=1.22$, $P>0.05$]. At the 60- and 120-min recording times, all three doses of *Cynara s.* flowering head extract produced a marked decrease in glycemia (Figure 1, top panel). Glycemia increase (expressed as percentage of values recorded in fasted rats at time 0) averaged approximately 85%, 65%, 60%, and 50% in 0, 500, 1000, and 1500 mg/kg *Cynara s.* flowering head extract-treated rats, respectively, with negligible differences between the 60- and 120-min recording times (Figure 1, top panel).

In close agreement with the above results, treatment with *Cynara s.* flowering head extract also reduced the area under the curve of glycemia time-course [$F(3,30)=7.23$, $P<0.001$] (Figure 1, bottom panel).

Experiment 2 (Zucker rats)

Glycemia in fasted Zucker rats averaged approximately 105 mg/dl in all rat groups (Figure 2, top panel). In vehicle-treated rats, meal consumption resulted in an increase of glycemia at all three recording times; it rose to 150 mg/dl at the 60-min recording time and declined slowly, being approximately 135 mg/dl at the 360-min recording time (Figure 2, top panel).

ANOVA revealed a significant effect of treatment with *Cynara s.* flowering head extract on post-prandial glycemia over the 6-hour recording period [$F_{\text{dose}}(2,16)=5.22$, $P<0.05$; $F_{\text{time}}(2,32)=1.09$, $P>0.05$; $F_{\text{interaction}}(4,32)=0.06$, $P>0.05$]. At the 60-min recording time, both doses of *Cynara s.*

flowering head extract tended to reduce the glycemia (Figure 2, top panel). At the following time intervals (120 and 360 min) a decrease in glycemia was observed only in the rat group treated with 1500 mg/kg *Cynara s.* flowering head extract (Figure 2, top panel). Accordingly, treatment with *Cynara s.* flowering head extract reduced the area under the curve of glycemia time-course [$F(2,16)=4.60$, $P<0.05$] (Figure 2, bottom panel).

Discussion

The results of the present study indicate that the acute administration of a standardized extract of *Cynara s.* flowering heads effectively reduced post-prandial increase in glycemia in two different strains of rats, unselected Wistar and genetically obese Zucker rats. The experiment was designed to oblige rats to consume large amounts of food (a regular rat chow made up of 57.3% carbohydrates) in a single meal; to this end, rats were initially fasted and then given the maximum amount of food that rats of both strains were able to consume entirely over a 1-hour period. In Wistar rats the effect of *Cynara s.* flowering head extract produced a negligible dose-dependence relationship. Additional studies should be undertaken to investigate whether alternative vehicles may improve extract absorption resulting in a higher degree of separation between the effects of the different doses.

To our knowledge, the results of the present study constitute the first experimental evidence of the reducing effect of an artichoke preparation on glycemia in laboratory rodents. These results are consonant with recent human studies reporting the capacity of dietary artichoke (both *Cynara scolymus L.* and *Cynara cardunculus L.*) to lower post-prandial rise of glycemia in humans (Nazni et al., 2006; Nomikos et al., 2007).

In the present study, potency and efficacy of *Cynara s.* flowering head extract tended to be higher in Wistar than Zucker rats. The reason for this difference is unknown at present and may require further investigation.

The present investigation did not specifically address the mechanism(s) of action and/or the active principle(s) by which *Cynara s.* flowering head extract exerted its reducing effect on glycemia. At present, we can however rule out the hypothesized contribution of fibers contained in dietary artichokes (Nazni et al., 2006; Nomikos et al., 2007), as the extract used in this investigation was completely purified and devoid of any fiber content. Additional studies are needed to investigate mechanism(s) of action, active principle(s), and changes in levels of hormones in specific tissues, including pancreas, liver, gut, muscle, and adipose tissue.

Acknowledgements

The present study was funded by Italian National Research Council [*Consiglio Nazionale delle Ricerche* (CNR)], and Indena S.p.A., Milan, Italy.

References

- 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10
 - 11
 - 12
 - 13
 - 14
 - 15
 - 16
 - 17
 - 18
 - 19
 - 20
 - 21
 - 22
 - 23
 - 24
 - 25
 - 26
 - 27
 - 28
 - 29
 - 30
 - 31
 - 32
 - 33
 - 34
 - 35
 - 36
 - 37
 - 38
 - 39
 - 40
 - 41
 - 42
 - 43
 - 44
 - 45
 - 46
 - 47
 - 48
 - 49
 - 50
 - 51
 - 52
 - 53
 - 54
 - 55
 - 56
 - 57
 - 58
 - 59
 - 60
- Bundy R, Walzer AF, Middleton RW, Wallis C, Simpson HC. 2008. Artichoke (*Cynara scolymus*) reduces plasma cholesterol in otherwise healthy hypercholesterolemic adults: a randomized, double blind placebo controlled trial. *Phytomedicine* **15**: 668-675
- Englisch W, Beckers C, Unkauf M, et al. 2000. Efficacy of Artichoke dry extract in patients with hyperlipoproteinemia. *Arzneimittelforschung* **50**: 260–265.
- Gebhardt R. 1998. Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by Artichoke (*Cynara scolymus* L.) extracts. *J Pharmacol Exp Ther* **286**: 1122-1128.
- Küskü-Kiraz Z, Mehmetçik G, Dogru-Abbasglu S, Uysal M. 2010. Artichoke leaf extract reduces oxidative stress and lipoprotein dyshomeostasis in rats fed on high cholesterol diet. *Phytoter Res* . **24**: 565-570.
- Lattanzio V, Kroon PA, Linsalata V, Cardinali A. 2009. Globe artichoke: A functional food and source of nutraceutical ingredients. *J Function Foods* **1**: 131-144.
- Nazni P, Poongodi Vijayakumar T, Alagianambi P, Amirthaveni M. 2006. Hypoglycemic and Hypolipidemic Effect of *Cynara scolymus* among Selected Type 2 Diabetic Individuals. *Pakistan J Nutr* **5**: 147-151.
- Nomikos T, Detopulou P, Frogopulou E, Pliakis E, Antonopoulou S. 2007. Boiled wild artichoke reduces postprandial glycemic and insulinemic responses in normal subjects but has no effect on metabolic syndrome patients. *Nutr Res* **27**: 741-749.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Romanovsky D, Walker JC, Dobretsov M. 2008. Pressure pain precedes development of type 2 disease in Zucker rat model of diabetes. *Neuorsci Lett* **445**: 220-223.

Vinik AI, Jenkins DJ. 1998. Dietary fiber in management of diabetes. *Diab Care* **11**: 160-173.

Wider B, Pittler MH, Thompson-Coon J, Ernst E. 2009. Artichoke leaf extract for treating hypercholesterolaemia. *Cochrane Database Syst Rev* **4**: CD003335.

Figure captions

Figure 1 – Effect of the acute administration of a *Cynara s.* flowering head extract on time-course of glycemia (top panel) and area under the curve of the time-course of glycemia (bottom panel) in Wistar rats given a 1-hour (corresponding to the 0-60 min time interval) access to regular rat chow and water. Each bar or point is the mean $\pm$ S.E.M. of $n=7-9$ rats. *: $P<0.05$ and +: $P<0.005$ with respect to the rat group treated with 0 mg *Cynara s.* flowering head extract.

Figure 2 – Effect of the acute administration of a *Cynara s.* flowering head extract on time-course of glycemia (top panel) and area under the curve of the time-course of glycemia (bottom panel) in Zucker rats given a 1-hour (corresponding to the 0-60 min time interval) access to regular rat chow and water. Each bar or point is the mean $\pm$ S.E.M. of $n=6-7$ rats. *: $P<0.05$ with respect to the rat group treated with 0 mg *Cynara s.* flowering head extract.

Carai et al., Figure 1

190x254mm (96 x 96 DPI)

Carai et al., Figure 2

190x254mm (96 x 96 DPI)