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

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2 1 **Evidence of glycemia-lowering effect by a *Cynara scolymus L.* extract**
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5 2 **in normal and obese rats**
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1
2 1 **Abstract**
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9 4 Several recent preliminary clinical studies have suggested that artichoke (*Cynara scolymus L.*,
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11 5 *Asterceae* family) preparations may be capable of lowering post-prandial glycemia. The present
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13 6 study was designed to test this hypothesis in laboratory rats. To this aim, non-selected Wistar and
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15 7 genetically obese Zucker rats were acutely treated with a purified extract of *Cynara s.* flowering
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17 8 heads (500-1500 mg/kg by gavage) immediately prior to 1-hour access to a fixed amount of food.
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19 9 Glycemia was recorded 60, 120, and 360 min after food presentation. Treatment with *Cynara s.*
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21 10 flowering head extract resulted in a significant decrease of post-prandial glycemia in both rat
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23 11 strains. The lack of any fiber content in this *Cynara s.* flowering head extract excludes the
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25 12 involvement of dietary fibers in glycemia reduction. The results obtained constitute the first
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27 13 evidence of a hypoglycemic effect of an artichoke preparation in laboratory rodents and confirm
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29 14 previous observations made in humans.
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42 18 **Key-words:** Artichoke (*Cynara scolymus L.*); Extract of flowering heads; Glycemia; Zucker rats;
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Introduction

Artichokes (*Cynara scolymus L.*, *Asterceae* family) are an ancient crop and medicinal plant, the therapeutic potential of which was known to the ancient Egyptians, Greeks, and Romans (Lattanzio et al., 2009). In the remote past artichoke preparations were used as a digestive aid. In recent times, reports from folk medicine as well as preliminary data from scientifically designed clinical studies have suggested that artichokes may have cholerectic, hypocholesterolemic, and hypolipidemic properties (Gebhart, 1998; English et al., 2000; Bundy et al., 2008; Wider et al. 2009; Küskü-Kiraz et al., 2010).

Based on the notion that dietary fibers may be beneficial in carbohydrate metabolism a recent study (Vinik et al., 1998) found that consumption of an artichoke food supplement for three consecutive months reduced fasting and post-prandial glycemia in type-2 diabetic patients (Nazni et al., 2006). Consistently, an independent study found that a meal of bread and wild artichokes (*Cynara cardunculus L.*) attenuated post-prandial increase in glycemia in healthy subjects (Nomikos et al., 2007).

The present study was designed to assess the ability of artichoke extract to reduce glycemia via a mechanism other than the action of its fibers (Vinik et al., 1998). For this purpose, the present study tested the capacity of a standardized, purified, fiber-free extract of *Cynara scolymus L.* to lower post-prandial glycemia in laboratory rats. This extract was of particular interest with regard to the study aims as it was completely fiber-free. Two different strains of rats were used: unselected Wistar rats and genetically selected, obese Zucker rats; notably, Zucker rats display fasting hyperglycemia and have been proposed as a possible animal model of pre-diabetes (Romanovsky et al., 2008). The effect of the acutely administered artichoke extract on glycemia was evaluated after the consumption of a large meal comprising a given amount of food. Rats were

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2 1 fasted before the meal to ensure consumption of an equal amount of food by rats from each
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4 2 experimental group (vehicle- or extract-treated rats) within the scheduled 1-hour access period.
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11 **Materials and Methods**

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16 7 The experimental procedures employed in the present study were in accordance with the European
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18 8 Communities Council Directive (86/609/EEC) and the subsequent Italian Law on the Protection of
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20 9 animals used for experimental and other scientific reasons.
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24 25 26 11 *Animals*

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30 13 Adult male Wistar (Experiment 1) and Zucker (Experiment 2) rats (Charles River Laboratories,
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32 14 Calco, LC, Italy) were used. In both experiments rats were individually housed in standard plastic
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34 15 cages with wood chip bedding. The animal facility was under an inverted 12:12 hour light-dark
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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
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38 17 approximately 60%. Rats were extensively habituated to handling, intragastric infusion, and blood
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40 18 drawing from the tail tip.
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45 46 47 20 *Extract preparation*

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51 22 *Cynara scolymus* flowering heads, Sardinian spiny variety, were chopped and frozen at the time of
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53 23 harvesting. The biomass, maintained at -30°C until extraction, was cryoground and immediately
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55 24 submitted to alcoholic extraction. 1 kg of *Cynara scolymus* flowering heads was extracted in a
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57 25 percolator with ethanol/water (70:30), using a total volume of 7 L. The hydroethanolic extract was
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59 26 concentrated under vacuum, to remove ethanol, obtaining 1 L of an aqueous concentrate, a volume
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2 1 corresponding to that of extracted plant material. Subsequently, the concentrate was purified on an
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4 2 absorption resin through loading into a column packed with 900 mL polymeric resin
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6 3 (Amberlite™); the resin was thoroughly washed with 1 L water, the purified extract eluted with 2
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8 4 L ethanol, and was subsequently concentrated to dryness to yield 8.5 g of powder. Separation and
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10 5 determination of caffeoylquinic acid derivatives and flavonoids in the extract was performed
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12 6 simultaneously by a reversed phase HPLC method, using a stainless steel column (250 x 4.6 mm),
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14 7 packed with octadecylsilyl silica gel for chromatography (5 µm), kept at 20°C, and with gradient
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16 8 elution at a constant flowrate of 1.0 ml/min. Mobile phase A was composed by a mixture of
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18 9 trifluoroacetic acid (0.01 % v/v, in water); mobile phase B was composed by a mixture of
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20 10 trifluoroacetic acid (0.01 % v/v, in acetonitrile); the injection volume for all samples was 10 µl.
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22 11 Detection was obtained with a UV spectrophotometer at 263 nm. Samples and standards were
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24 12 dissolved in methanol (40 % v/v, in water), filtered by a 0.45-µm polytetrafluoroethylene syringe
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26 13 filter and then injected directly into the HPLC system. The extract obtained had a high
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28 14 caffeoylquinic acid (≥50%), and flavonoid content, expressed as luteolin glycosides, of
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30 15 approximately 2%.
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40 17 *Experimental procedure*

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44 19 In both experiments, rats were kept under a regimen of unlimited access to regular rodent chow
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46 20 [Harlan, Global Diet 2018, Mucedola, Settimo Milanese, MI, Italy; main composition: 18.9%
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48 21 crude protein, 5.7% crude oil, 3.8% crude fibre, 5.9% ash, 57.3% carbohydrate (42.2% starch,
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50 22 4.9% sugar); digestible energy: 3.35 cal/g, metabolisable energy: 3.27 cal/g] and water for 24
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52 23 hours/day. Rats were fasted 12 hours before the start of the experiment (this 12-hour period
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54 24 coincided with the entire light phase of the light-dark cycle). In Experiment 1, on the test day
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56 25 Wistar rats were divided into four groups of $n=7-9$, matched for body weight and glycemia at time
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58 26 0, and treated with 0, 500, 1000, and 1500 mg/kg *Cynara s.* flowering head extract. In Experiment
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1
2 1 2, on the test day Zucker rats were divided into three groups of $n=6-7$, matched for body weight
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4 2 and glycemia at time 0, and treated with 0, 500, and 1500 mg/kg *Cynara s.* flowering head extract.
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6 3 Doses of *Cynara s.* flowering head extract were selected on the basis of the results of previous
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8 4 experiments and literature data (Küskü-Kiraz et al., 2010) . In both experiments, *Cynara s.*
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10 5 flowering head extract was suspended in distilled water with a few drops of Tween 80 and
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12 6 administered intragastrically by a metal gavage at an infusion volume of 4 ml/kg, 30 min before
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14 7 lights off. At lights off, rats were given 8 g/kg food pellets; this food amount was chosen on the
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16 8 basis of preliminary experiments demonstrating how fasted Wistar and Zucker rats consumed this
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18 9 entire amount in less than 60 min (this laboratory, unpublished results). Glycemia was determined
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20 10 0, 60, 120, and 360 min after food presentation. A small (0.05 ml) blood sample was collected
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22 11 from the tip of the tail of each rat and analyzed enzymatically by means of GL5 Analox (Analox
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24 12 Ltd, London, United Kingdom).
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33 14 *Statistical analysis*

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37 16 In both experiments, data on the effect of *Cynara s.* flowering head extract on time-course of
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39 17 glycemia were analyzed by a 2-way (dose; time) ANOVA with repeated measures on the factor
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41 18 “time”. Data on the effect of *Cynara s.* flowering head extract on the area under the curve of the
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43 19 time-course of glycemia [expressed in mg*min/100 ml; calculated using GraphPath Prism 3.03
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45 20 (GraphPath Software, La Jolla, CA, USA)] were analyzed by a 1-way ANOVA.
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54 23 **Results**

55 24 56 25 *Experiment 1 (Wistar rats)* 57 58 59 60 61

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2 1 Glycemia in fasted Wistar rats averaged approximately 70 mg/dl in all rat groups (Figure 1, top
3 panel). In vehicle-treated rats, meal consumption resulted in an increase of glycemia at both the
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5 3
6 60- and 120-min recording times, when it averaged approximately 130 mg/dl (Figure 1, top panel).
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8 At the 360-min recording time, glycemia in vehicle-treated rats approached basal levels (Figure 1,
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10 top panel).
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12 6 ANOVA revealed a highly significant effect of treatment with *Cynara s.* flowering head extract on
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14 post-prandial glycemia over the 6-hour recording period [$F_{\text{dose}}(3,30)=7.44$, $P<0.001$;
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16 $F_{\text{time}}(2,60)=129.31$, $P<0.0001$; $F_{\text{interaction}}(6,60)=1.22$, $P>0.05$]. At the 60- and 120-min recording
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18 times, all three doses of *Cynara s.* flowering head extract produced a marked decrease in glycemia
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20 (Figure 1, top panel). Glycemia increase (expressed as percentage of values recorded in fasted rats
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22 at time 0) averaged approximately 85%, 65%, 60%, and 50% in 0, 500, 1000, and 1500 mg/kg
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24 *Cynara s.* flowering head extract-treated rats, respectively, with negligible differences between the
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26 60- and 120-min recording times (Figure 1, top panel).
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28 14 In close agreement with the above results, treatment with *Cynara s.* flowering head extract also
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30 reduced the area under the curve of glycemia time-course [$F(3,30)=7.23$, $P<0.001$] (Figure 1,
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32 bottom panel).
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36 18 *Experiment 2 (Zucker rats)*

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38 20 Glycemia in fasted Zucker rats averaged approximately 105 mg/dl in all rat groups (Figure 2, top
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40 panel). In vehicle-treated rats, meal consumption resulted in an increase of glycemia at all three
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42 recording times; it rose to 150 mg/dl at the 60-min recording time and declined slowly, being
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44 approximately 135 mg/dl at the 360-min recording time (Figure 2, top panel).
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47 24 ANOVA revealed a significant effect of treatment with *Cynara s.* flowering head extract on post-
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49 prandial glycemia over the 6-hour recording period [$F_{\text{dose}}(2,16)=5.22$, $P<0.05$; $F_{\text{time}}(2,32)=1.09$,
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51 $P>0.05$; $F_{\text{interaction}}(4,32)=0.06$, $P>0.05$]. At the 60-min recording time, both doses of *Cynara s.*
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2 1 flowering head extract tended to reduce the glycemia (Figure 2, top panel). At the following time
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4 2 intervals (120 and 360 min) a decrease in glycemia was observed only in the rat group treated with
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6 3 1500 mg/kg *Cynara s.* flowering head extract (Figure 2, top panel).
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9 4 Accordingly, treatment with *Cynara s.* flowering head extract reduced the area under the curve of
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11 5 glycemia time-course [$F(2,16)=4.60$, $P<0.05$] (Figure 2, bottom panel).
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16 7 **Discussion**

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20 9 The results of the present study indicate that the acute administration of a standardized extract of
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22 10 *Cynara s.* flowering heads effectively reduced post-prandial increase in glycemia in two different
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24 11 strains of rats, unselected Wistar and genetically obese Zucker rats. The experiment was designed
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26 12 to oblige rats to consume large amounts of food (a regular rat chow made up of 57.3%
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28 13 carbohydrates) in a single meal; to this end, rats were initially fasted and then given the maximum
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30 14 amount of food that rats of both strains were able to consume entirely over a 1-hour period. In
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32 15 Wistar rats the effect of *Cynara s.* flowering head extract produced a negligible dose-dependence
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34 16 relationship. Additional studies should be undertaken to investigate whether alternative vehicles
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36 17 may improve extract absorption resulting in a higher degree of separation between the effects of
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38 18 the different doses.
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42 20 To our knowledge, the results of the present study constitute the first experimental evidence of the
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44 21 reducing effect of an artichoke preparation on glycemia in laboratory rodents. These results are
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46 22 consonant with recent human studies reporting the capacity of dietary artichoke (both *Cynara*
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48 23 *scolymus L.* and *Cynara cardunculus L.*) to lower post-prandial rise of glycemia in humans (Nazni
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50 24 et al., 2006; Nomikos et al., 2007).
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54 26 In the present study, potency and efficacy of *Cynara s.* flowering head extract tended to be higher
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56 27 in Wistar than Zucker rats. The reason for this difference is unknown at present and may require
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58 28 further investigation.
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2 1 The present investigation did not specifically address the mechanism(s) of action and/or the active
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4 2 principle(s) by which *Cynara s.* flowering head extract exerted its reducing effect on glycemia. At
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6 3 present, we can however rule out the hypothesized contribution of fibers contained in dietary
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8 4 artichokes (Nazni et al., 2006; Nomikos et al., 2007), as the extract used in this investigation was
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10 5 completely purified and devoid of any fiber content. Additional studies are needed to investigate
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12 6 mechanism(s) of action, active principle(s), and changes in levels of hormones in specific tissues,
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14 7 including pancreas, liver, gut, muscle, and adipose tissue.
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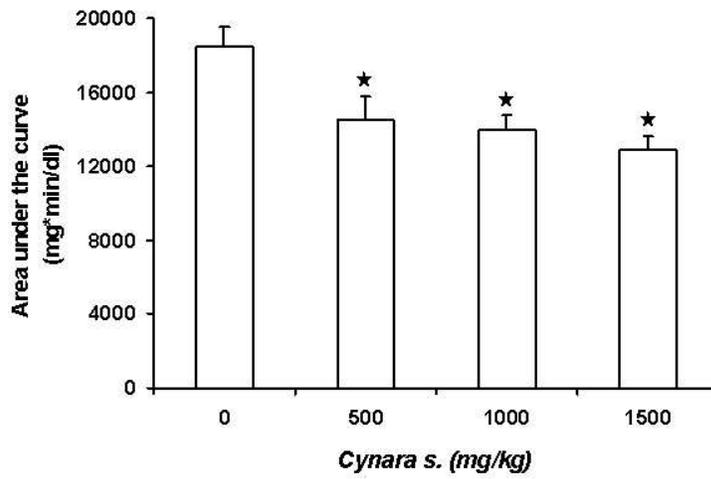
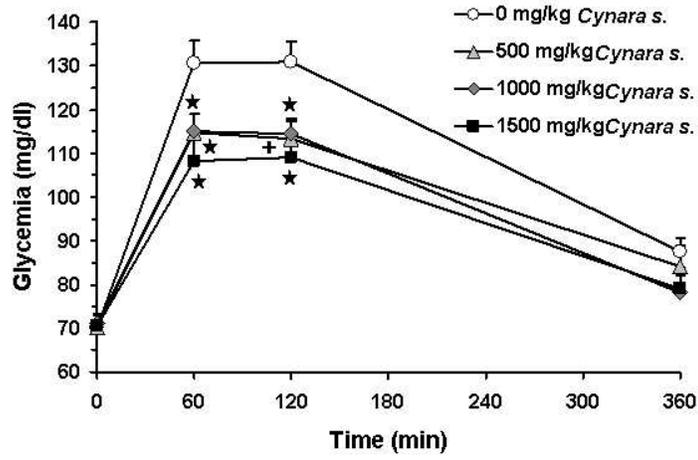
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Figure captions

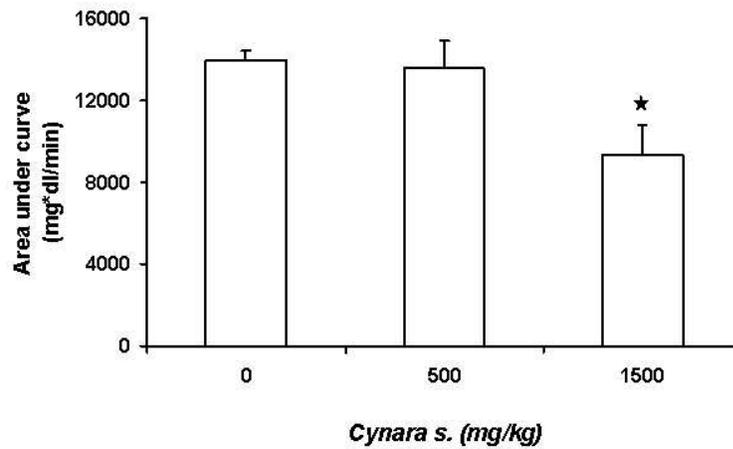
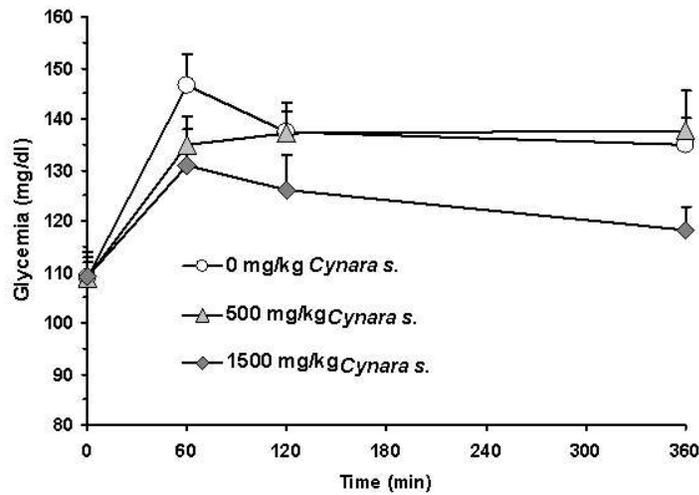
Figure 1 – Effect of the acute administration of a *Cynara s.* flowering head extract on time-course of glycemia (top panel) and area under the curve of the time-course of glycemia (bottom panel) in Wistar rats given a 1-hour (corresponding to the 0-60 min time interval) access to regular rat chow and 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)