Research Article

Effect of artichoke extract (*Cynara scolymus* L.) on palmitic-1-^{14}C acid oxidation in rats

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Studies on the effect of the artichoke extract (AE) on oxidation of palmitic-1-^{14}C acid administered intravenously to rats at a dose 25 and 50 mg/kg bw demonstrated marked enhancement of both $^{14}CO_2$ expiration rate and $^{14}CO_2$ recovery in the expired air. The extract suppressed accumulation of palmitic-1-^{14}C acid in serum lipids and epididymal fat pad tissue as well. The effects of the extract on $^{14}CO_2$ expiration rate, $^{14}CO_2$ recovery, as well as accumulation of palmitic-1-^{14}C acid were dose dependent. Total $^{14}CO_2$ recovery in expired air during 60 min was elevated by 17.3% ($p < 0.05$) and 52.1% ($p < 0.001$) in rats administered the extract at a dose of 25 and 50 mg/kg, respectively. The rats supplemented with the AE at a dose of 25 and 50 mg/kg bw were characterized by 10.0% (not significant) and 19% ($p < 0.05$) decrease in ^{14}C radioactivity of serum lipids as well as reduction of epididymal fat tissue ^{14}C radioactivity by 8.7 and 17.5% ($p < 0.05$), respectively, in comparison with the control rats. Thus, the results demonstrate that the AE possess stimulatory properties with respect to oxidation of palmitic acid administered to rats, and provide new information on the mechanism of antilipemic activity of the extract associated with activation of lipid oxidation in the organism.

Keywords: Artichoke extract / Hypolipemic activity / Palmitic-1-^{14}C acid oxidation / Phytotherapy / Plant food

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1 Introduction

Artichoke leaf extracts are traditionally applied in disease states associated with impaired gall production and flow, cholecystitis, liver failure, and lipid metabolism disturbances [1, 2]. Hypolipemic properties of artichoke extracts (AEs) were revealed in a series of experimental and clinical studies [3–5]. The extract not only promotes hepatic degradation of cholesterol and bile flow but also inhibits endogenous cholesterol synthesis. Bioactive compounds of the artichoke such as luteolin and chlorogenic acid are effective inhibitors of HMG-CoA reductase, a critical enzyme in a cholesterol synthesis metabolic pathway [6]. Experimental data demonstrate an influence of antioxidant compounds on energy homeostasis regulation, *e.g.*, effects on insulin-dependent metabolic processes [5]. Proven antioxidant properties of AEs [6–8] may suggest involvement of the artichoke active compounds in regulation of energy metabolism, *e.g.*, lipid oxidation.

The present study was aimed at evaluation of the AE hypolipemic activity mechanisms related to lipid metabolism. The effects of the extract on palmitic-1-^{14}C acid oxidation and accumulation of palmitic acid in serum and fat tissue in rats were determined.

2 Materials and methods

2.1 Plant material

The artichoke preparation studied, *i.e.*, aqueous extract (prepared by dissolution in distilled water – 1 g extract/100 mL water) "Extractum Cynarae aq. siccum" (2.9 = 1) (batch number 0061741), was supplied by (Phytopharm Kleka S.A., Poland), and contained: 0.49% cynarin, 5.4% polyphenolic compounds/chlorogenic acid; 5.0%, caffeoylquinic acids derivatives (CCS) 5.0%; luteolin-7-*O*-glucoside; and luteolin-7-*O*-rutinoside 0.72%.

The HPLC system consisted of a Merck Hitachi (L7100 pump) chromatograph, equipped with a photodiode array

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detector (L7200), an autosampler (L7200), an interface (D7000), and operated by HSM software for the extract analysis was used. LiChrospher RP-18e (250 × 4.6 mm id, particle size 5 µm, Merck) column with a guard column (LIChroCART, 4 × 4 mm^2, LiChrospher RP-18e, 5 µm, Merck) were used. The mobile phase was acidified with water and ACN. The flow rate was 1.5 mL/min, and detection was performed at 330 nm (scanning between 220 and 450 nm). Retention times and spectra were compared to pure standards of chlorogenic acid, luteolin-7-*O*-glucoside and cynarin.

2.2 Chemicals

Radiolabeled [1-^{14}C]-palmitic-acid (specific radioactivity 675.4 MBq/mmol) and $NaH^{14}CO_3$ (radioactivity concentration 370 MBq/mL) were obtained from Institute of Nuclear Studies (Swierk, Poland). All other chemicals of analytical grade and purchased from (Sigma Chemicals, USA).

2.3 Animals

Male Wistar albino rats (215.0 ± 6.2 g) were used in this experiment. They were allowed to acclimatize for a minimum of 10 days before the study. The rats were housed in a room maintained at 21 ± 1°C with a 12-h light–dark cycle, with the light beginning at 6:00 AM with free access to feed and water. Experimental protocol was approved by the local ethics committee for animal studies.

For the purpose of the study rats were randomly subdivided into six experimental groups of ten animals each. Twenty-four hours before the experiments the animals were starved but with free access to water.

2.4 Biochemical procedures

2.4.1 Oxidation of palmitic-1-^{14}C acid and $^{14}CO_2$ whole body production

Rats from group I (being control for groups II and III) were administered 1 mL H_2O intragastrically, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Animals from groups II and III were given the AE at a dose of 25 and 50 mg/kg bw, respectively, resuspended in H_2O (0.5 mL/100 g bw) intragastrically, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Rats from group IV (being control for groups V and VI) were administered 1 mL H_2O intragastrically, and 30 min later $NaH^{14}CO_3$ at a dose of 148 kBq/100 g bw was injected intraperitoneally. The animals from groups V and VI, similarly to groups II and III, the AE at a dose of 25 and 50 mg/kg bw, respectively, was given, and $NaH^{14}CO_3$ at a dose of 148 kBq/100 g bw was injected intraperitoneally after 30 min.

Immediately after palmitic-1-^{14}C acid or $NaH^{14}CO_3$ administration each rat was placed in a tight 750 mL chamber with a constant air supply of 300 cm^3/min. Determination of air flow and composition was carried out during 1–60 min for animals administered palmitic-1-^{14}C acid (groups I, II, III), and during 1–40 min for rats given $NaH^{14}CO_3$ (groups IV, V, VI). The measurements of $^{14}CO_2$ expiration rate and $^{14}CO_2$ recovery in the expired air were performed using a measuring device consisted of a glass chamber for rat; CO_2 analyzer Infralyt 2T (VEB Junkalor, Germany); CO_2 registrator K-200 (Carl-Zeiss-Jena, Germany); CO_2 registrator A-21 Scaler/Timer P-7B (Polon, Poland); $^{14}CO_2$ counter Scaler/Timer P-44 (Polon); total ^{14}C counter Scaler/Timer PT-72 (Polon); flow rate analyzer; and $^{14}CO_2$ absorber (Polon).

2.4.2 Serum lipid and tissue accumulation of palmitic-1-^{14}C acid

Blood and epididymal fat pad tissue were sampled under pentobarbitone anesthesia (50 mg/kg bw) immediately after measurement of expired air composition, *i.e.*, after 60 min from palmitic-1-^{14}C acid administration (groups I, II, III). Blood was sampled by puncture of the cardiac apex, then epididymal fat pad tissue was excised and weighted. Serum and epididymal fat pad lipids were extracted according to Folch method [9]. The extracts were evaporated in scintillation vials, the pellets were dissolved in ethyl alcohol, and radioactivity was measured after addition of scintillation liquid (toluene 1000 mL, 2,5-diphenyloxazole (PPO) – 4 g, 1,4-bis(5-phenyl-2-oxazolyl)benzene (POPOP) – 0.1 g and ethyl alcohol 95% – 500 mL). The $^{14}CO_2$ radioactivity in the collected samples was measured by liquid scintillation spectrometer (Packard TriCarb, model 3385). The results were corrected for counting efficiency and autoabsorption.

2.5 Statistical analysis

Statistical significance of differences between the groups was assessed with the use of one-way ANOVA followed by Dunnett's test taking the level of significance at $p < 0.05$.

3 Results

The present study on palmitic-1-^{14}C acid oxidation revealed that the evaluated AE increased both $^{14}CO_2$ expiration rate and $^{14}CO_2$ recovery in the expired air in rats. The extract suppressed in a dose dependent manner accumulation of palmitic-1-^{14}C acid in serum lipids and epididymal fat pad tissue as well. The expiration rate of $^{14}CO_2$ at 10 min of the study was higher by 20.2 and 41.8% in rats administered the AE at a dose of 25 and 50 mg/kg bw, respectively, in comparison with the control (Fig. 1). The 52, 61, and 79.5% of the administered dose of 74 kBq/100 g bw were recovered within 60 min in the expired air in the control rats as well as from animals administered the AE at a dose of 25 and 50 mg/kg bw, respectively. Total $^{14}CO_2$ recovery in

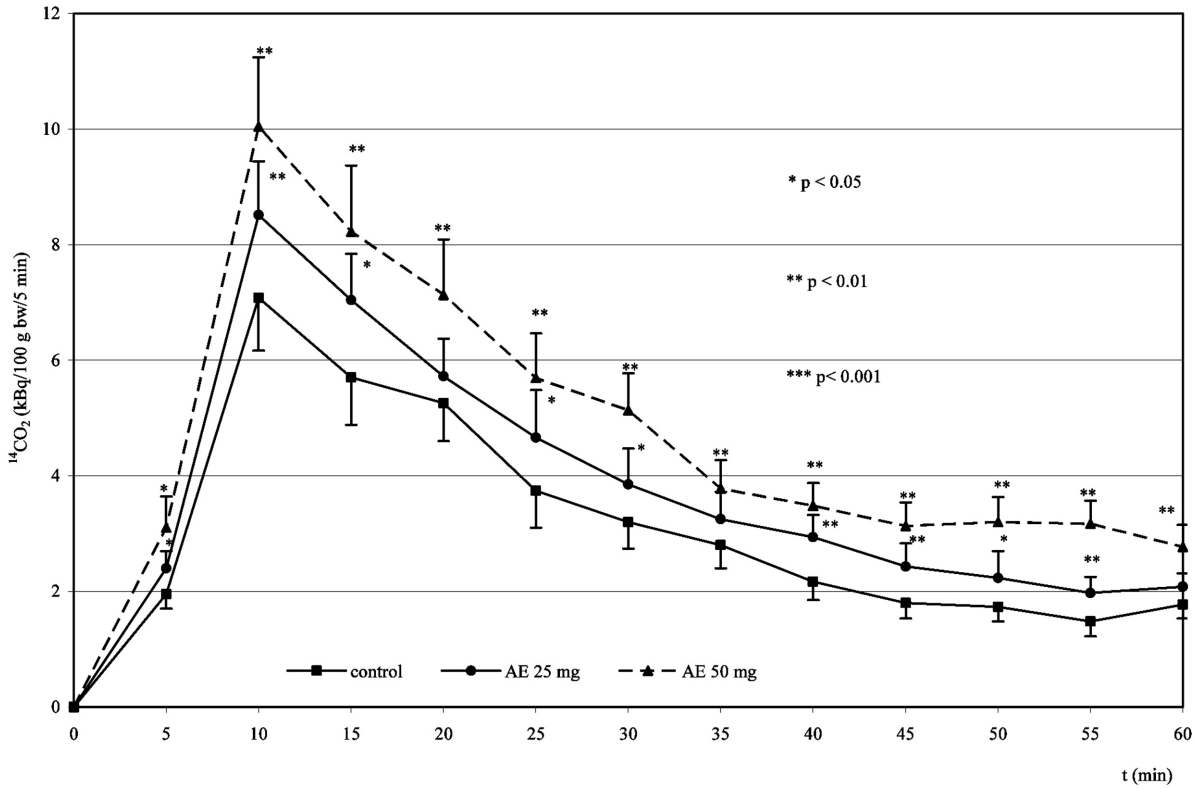

Figure 1. The effect of AE on the rate of $^{14}CO_2$ expiration by rats administered palmitic acid-1-^{14}C. Control rats (group I) were given 1 mL H_2O intragastrically, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Animals from groups II and III were given the AE at a dose of 25 and 50 mg/kg bw, respectively, resuspended in H_2O, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Determination of air flow and composition was carried out during 1 – 60 min (see Section 2.4). The results are shown as mean ± SD.

expired air during 60 min was elevated in animals given 25 and 50 mg/kg bw of the extract by 17.3% ($p < 0.05$) and 52.1% ($p < 0.001$) in comparison with the control group, respectively (Fig. 2). The AE did not affect $^{14}CO_2$ expiration rate and $^{14}CO_2$ recovery in the expired air after $NaH^{14}CO_3$ administration (Table 1). The rats supplemented with the AE at a dose of 25 and 50 mg/kg bw were characterized by 10.0% (not significant) and 19% ($p < 0.05$) decrease in ^{14}C radioactivity of serum lipids as well as reduction of epididymal fat tissue ^{14}C radioactivity by 8.7 and 17.5% ($p < 0.05$), respectively, in comparison with the control rats (Table 2). Mean weight of the epididymal fat pad tissue of the control animals as well as from animals given the AE at a dose of 25 and 50 mg/kg bw before palmitic-1-^{14}C acid administration did not differ significantly, and were 0.947 ± 0.120, 0.975 ± 0.201, and 0.954 ± 0.152 g, respectively. Based on the average plasma volume (54 – 70 mL/kg bw; mean 62.0 mL/kg bw) [10] and average epididymal fat pad weight of the studied rats (0.947 – 0.975 g) palmitic-1-^{14}C acid accumulation in these compartments was estimated. Plasma accumulation of palmitic-1-^{14}C acid in the control animals and these administered 25 and 50 mg/kg bw of the AE was estimated to be 7.52, 6.76, and 6.08% after 60 min from the acid administration. As for epididymal fat pad accumulation, the respective values (for the controls and groups II and III) were 0.13, 0.12, and 0.11% of the administered dose. In the remaining compartment of the body (muscles, connective tissue, nervous system, extracellular space) of the control rats and in the animals administered 25 and 50 mg/kg bw of the AE – 40.35, 32.12, and 14.31% of the administered dose of palmitic-1-^{14}C acid were determined 60 min from administration, respectively.

4 Discussion

The present study was aimed at evaluation of the AE hypolipemic activity mechanisms related to lipid metabolism. Before the experiment the animals were deprived of food in order to stimulate lipid utilization. In the present study, $^{14}CO_2$ recovered from palmitic acid metabolism as well as CO_2 from total body metabolism during 60 min after palmitic-1-^{14}C acid administration were measured. Recovery rate of $^{14}CO_2$ in the expired air after palmitic-1-^{14}C acid administration is considered to be a marker of the acid oxidation rate [11]. Palmitic-1-^{14}C acid administered was rap-

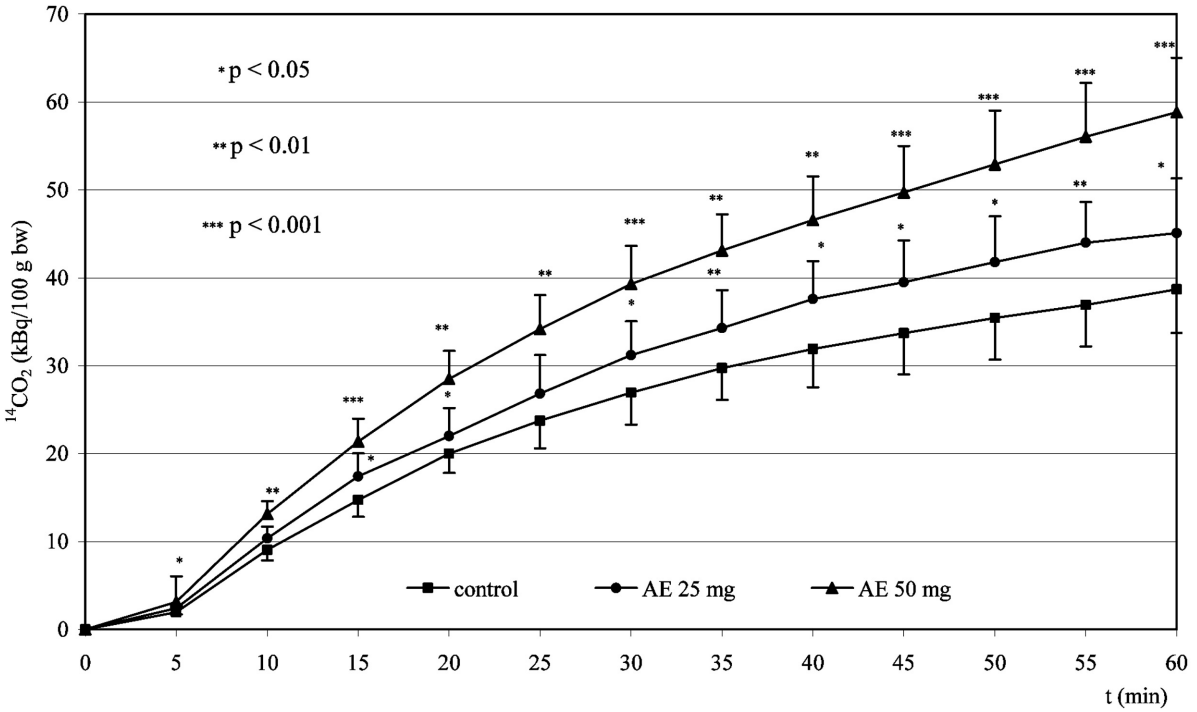

Figure 2. The effect of AE on total $^{14}CO_2$ expiration by rats given palmitic acid-1-^{14}C. Control rats (group I) were administered 1 mL H_2O intragastrically, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Animals from groups II and III were given the AE at a dose of 25 and 50 mg/kg bw, respectively, resuspended in H_2O, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Determination of air flow and composition was carried out during 1 – 60 min (see Section 2.4). The results are shown as mean ± SD.

idly incorporated in metabolic pathways as evidenced by fast onset of $^{14}CO_2$ detection in expired air, *i.e.*, within minutes from the acid injection (Fig. 1). The $^{14}CO_2$ recovery rate was the highest at 10 min of the experiment, and than gradually declined along with the acid oxidation process yielding its plasma concentrations. In animals administered the AE 30 min before palmitic-1-^{14}C acid injection a significant increase in the $^{14}CO_2$ expiration rate (Fig. 1) and $^{14}CO_2$ total recovery in expired air was revealed (Fig. 2). The $^{14}CO_2$ expiration rate and $^{14}CO_2$ total recovery were dose dependent (for the studied doses of 25 and 50 mg/kg bw). The 52, 61, and 79.5% of the administered dose of 74 kBq/100 g bw were recovered within 60 min in the expired air in the control rats as well as from animals administered the AE at a dose of 25 and 50 mg/kg bw, respectively.

In two groups (groups V and VI) of animals palmitic-1-^{14}C acid was substituted with $NaH^{14}CO_3$ in order to exclude a possibility of the AE influence CO_2 generation and transport. The AE did not affect total CO_2 and $^{14}CO_2$ expiration after $NaH^{14}CO_3$ administration (Table 1). The observations from the present study indicate that increased $^{14}CO_2$ in expired air of animals administered the AE resulted from stimulatory effects of the extract on palmitic acid oxidation without influence on carbonate elimination.

Analysis of the AE mechanisms involved in generation of $^{14}CO_2$ during palmitic acid metabolism prompted to study the extract effects on processes preceding the acid oxidation. In order to address the aforementioned aim, deposition of palmitic-1-^{14}C acid in serum lipids and epididymal fat tissue was evaluated. A reduced accumulation of ^{14}C in the studied tissues was revealed (Table 2). After 60 min from palmitic-1-^{14}C acid administration to the controls as well as to animals given the extract at a dose of 25 or 50 mg/kg bw – 48, 39, and 20.5% of the administered dose of 74 kBq palmitic-1-^{14}C acid was accumulated, respectively. The rate of ^{14}C tissue accumulation and its expiration reveals extensive metabolism of palmitic-1-^{14}C acid. The present results are in keeping with other reports on rate of lipid acid metabolism in rats [12].

There is a lack of published data on antioxidants' effects on lipid oxidation through mitochondrial respiratory chain, as known, being a source of reactive oxygen species. Fatty acids are also oxidized in peroxysomal β-oxidation pathway with associated production of hydrogen peroxide (H_2O_2) [13 – 15]. However, reports on insulin-like action of H_2O_2 in isolated adipocytes as well as effects of antioxidants on insulin-dependent metabolism of fat tissue in rats may undirectly point out some possible mechanisms implicated

Table 1. Total CO_2 and $^{14}CO_2$ (mean ± SD) expired after injection of $NaH^{14}CO_2$ preceded by administration of the AE

Time (min)	Control ($NaH^{14}CO_3$ 148 kBq/100 g bw)		AE + $NaH^{14}CO_3$ 148 kBq/100 g bw			
			AE 25 mg/kg bw		AE 50 mg/kg bw	
	Total $^{14}CO_2$ (kBq/100 g bw)	Total CO_2 (mmol/100 g bw)	Total $^{14}CO_2$ (kBq/100 g bw)	Total CO_2 (mmol/100 g bw)	Total $^{14}CO_2$ (kBq/100 g bw)	Total CO_2 (mmol/100 g bw)
5	18.2 ± 2.30	0.51 ± 0.06	21.6 ± 0.53	0.61 ± 0.07	18.4 ± 0.51	0.54 ± 0.06
10	45.3 ± 5.12	1.13 ± 0.16	49.4 ± 1.03	1.20 ± 0.17	47.2 ± 1.13	1.23 ± 0.17
15	62.7 ± 7.12	1.65 ± 0.20	66.0 ± 1.72	1.82 ± 0.25	63.7 ± 1.65	1.80 ± 0.22
20	72.7 ± 8.11	2.14 ± 0.24	75.6 ± 2.00	2.35 ± 0.33	76.7 ± 2.14	2.34 ± 0.28
25	77.4 ± 9.12	2.60 ± 0.29	82.6 ± 2.46	2.86 ± 0.33	83.3 ± 2.60	2.81 ± 0.35
30	81.0 ± 9.92	3.07 ± 0.36	86.4 ± 3.07	3.25 ± 0.38	89.0 ± 3.07	3.27 ± 0.38
35	83.2 ± 10.00	3.51 ± 0.40	89.7 ± 3.71	3.67 ± 0.40	92.4 ± 3.51	3.72 ± 0.38
40	85.2 ± 10.02	3.93 ± 0.49	91.8 ± 4.01	4.12 ± 0.52	94.6 ± 3.93	4.18 ± 0.49

Control rats were administered 1 mL H_2O intragastrically, and 30 min later $NaH^{14}CO_3$ at a dose of 148 kBq/100 g bw was injected intraperitoneally. The animals from groups V and VI were administered the AE at a dose of 25 and 50 mg/kg bw, and $NaH^{14}CO_3$ at a dose of 148 kBq/100 g bw was injected intraperitoneally after 30 min. Determination of air flow and composition was carried out during 1 – 40 min (see Section 2.4).

Table 2. The ^{14}C radioactivity in serum lipids and epididymal fat pad of rats (mean ± SD) after injection of palmitic-l-^{14}C acid preceded by administration of the AE

Groups	^{14}C-radioactivity (kBq/1g of wet tissue)	
	Serum lipids	Epididymal fat pad
Control		
Palmitic-1-^{14}C acid 74 kBq/100 g bw (n = 10)	0.089 ± 0.009	0.103 ± 0.07
AE 25 mg/kg bw + palmitic-1-^{14}C acid 74 kBq/100 g bw (n = 10)	0.080 ± 0.021	0.094 ± 0.08*
AE 50 mg/kg bw + palmitic-1-^{14}C acid 74 kBq/100 g bw (n = 10)	0.072 ± 0.008*	0.085 ± 0.06*

Control rats were administered 1 mL H_2O intragastrically, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Animals from groups II and III were given the AE at a dose of 25 and 50 mg/kg bw resuspended in H_2O intragastrically, and 30 min later palmitic-1-^{14}C acid at a dose of 74 kBq/100 g bw was injected into the tail vein. Blood and epididymal fat pad tissue were sampled under pentobarbitone anesthesia (50 mg/kg bw) immediately after measurement of expired air composition, *i.e.*, after 60 min from palmitic-1-^{14}C acid administration. * $p < 0.05$ *versus* control group.

in artichoke action. It was demonstrated that insulin stimulates intracellular generation of H_2O_2 as well as negative correlation between lipolysis and H_2O_2 generation (H_2O_2 in turn stimulates lipid synthesis in adipocytes) [16 – 18]. It is well established that insulin inhibits both mitochondrial transport of lipid acids and their oxidative metabolism [19, 20]. In the light of the aforementioned facts it is possible that antioxidants through inhibition of reactive oxygen species formation may stimulate lipid acid oxidation. The AE evaluated in the present study contains several compounds of well established, potent antioxidant properties, *e.g.*, chlorogenic acid, cynarin, luteolin [21], which suggest biochemical mechanisms underlying stimulatory effects of the extract on oxidation of palmitic acid administered to rats in the present study. It may be speculated that not only native compounds of the extract but also their active metabolites formed *in vivo* could affect metabolism of palmitic-1-^{14}C acid administered to rats.

In conclusion, it can be stated that the results of the present study revealed stimulatory effects of the AE on palmitic-1-^{14}C acid oxidation, which complement the current knowledge on hypolipemic properties of AEs. The extract influences the acid distribution, reducing its serum and fat tissue accumulation as well as stimulate its oxidation.

The authors have declared no conflict of interst.

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