1 **_Original Research Article_**

2

3 # *Cynara cardunculus L.* var *Cynara scolymus* L. extract reverse D-galactose-

4 induced skin aging changes in enzymatic antioxidant defense system in rats.

5
6

7 **ABSTRACT**

8 Skin aging is multitarget persistence processing that immediately involve hyperproduction of
9 free radicals under influence of intrinsic and extrinsic factors and deterioration in intimal
10 antioxidant defense system. The goal of the study was the evaluation of the anti-oxidant potential
11 of *Cynara cardunculus L.,* var *Cynara Scolymus* L. standartizated extracts, 2%, as a protective
12 strategy against skin age-associated oxidative damage caused by D-galactose in rats. 48 female
13 Wistar rats included in the experimental design. D-galactose induced aging was reproduced in 36
14 animals of main group, and 12 rats included in control group. All animals in main group were
15 randomized for 3 groups: I – animals with skin aging reproduced model receive saline, II –
16 animals with skin aging rats receive artichoke extracts (with content of chloroagenic acid 2.0%)
17 in a dose of intradermal injection 0.13 mg and main III group - animals with skin aging receive
18 1.3 mg artichoke extract twice at weeks during 4 weeks. Influence of artichoke extracts restores
19 skin relative weight and leads to decreasing the rate of generation of superoxide anion, hydrogen
20 peroxide and lipid peroxidation (LPx), increasing activity of superoxide dismutase (SOD),
21 glutathione peroxidase (GSH-Px) and reverse ratio SOD/(catalase+GPx) to the production of
22 H_2O_2 from superoxide dismutation coupling with the decrease ratio of generated O_2^-/H_2O_2.
23 Local prolonged treatment with artichoke extracts activated the enzymatic link in innate
24 antioxidant defense system in D-galactose induced skin aging model.

25 *Key words*: *skin, aging, Cynara cardunculus L., var Cynara Scolymus L. extract, oxidant*
26 *defense system, superoxide anion, glutathione system.*

UNDER PEER REVIEW

Abbreviation. GSH - Reduced glutathione, GSSG - oxidized glutathione, GSH-Px – glutathione peroxidase, lipid peroxidation MDA - Malone aldehyde, Mt –Mitochondrial, ROS - Reactive oxygen species, SOD – superoxide dismutase

1. INTRODUCTION

Despite a vast repertoire of ageing studies performed over the past century, the exact causes of ageing remain unknown. Skin changes with age mainly includes gloomy skin, relaxation, moisture reduction, thinning, is an inevitable spontaneous process and complex natural phenomenon characterized aging [1-4]. More popular hypothesis that at the molecular level aging is multifactorial gradual biological process associated with diminishes homeostasis, mitochondrial DNA (mtDNA) damage, and progressive decline of innate defense systems of the body, and endogenous antioxidant defense system and oxidative stress formation, particularly [4-5]. Free radical and mitochondrial theories of aging supported by estimation of positive relation between the sings of aging and progression of imbalance of free radical metabolism and oxidative damage affects replication and transcription of mtDNA, which closely accompanied the structure and function deterioration in energy supply systems of tissues and organs of the aging and age-related diseases. The decline or/and disturbances of energy supply system functioning leads to increased mitochondrial reactive oxygen species (ROS) generation, ROS-induced lipid peroxidation in mitochondrial membranes and release of cytochrom C. These together with antioxidant defense systems imbalance results in further greater overproduction of ROS and to a vicious cycle of premature cellular senescence, skin aging and aged related diseases [4-6]. To get a better understanding of skin aging and to prevent its effects on skin, chronic systemic administration of D-galactose, a sugar found abundantly in milk and to a lesser extent in fruits and vegetables, was established as a model for pharmacological studies of age-dependent alterations [7-12]. At high levels, D-galactose, an aldohexose, monosaccharide sugar, occurs naturally in the body in normal concentration and induced disruption in carbohydrate

UNDER PEER REVIEW

metabolism pathway and causes oxidative stress via stimulation of free radical production and accumulation, apoptosis and inflammation in beyond normal concentration [6-8]. In according to one of the hypothesis that expressive administration of D-galactose could induced damage associate with mitochondrial dysfunction caused by complex I deficiency [6-8, 12] and can accelerate ageing was suggested and then confirmed in experimental and clinical data. In order to evaluated the molecular mechanism involved in the controlling of oxidative stress formation we firstly investigated the formation of superoxide anion and hydrogen peroxide and activity of much important components of enzymatic part of antioxidant defense system in D-galactose induced skin aging model in experimental animals. For prevention of D-galactose induced skin aging damage we choice rich in natural antioxidants plant extract of artichoke (*Cynara scolymus* L. (Asteraceae), folium) [13-15]. Early in clinical practice [14-17] and in experimental studies it was shown antioxidant [18-24], antitoxic activities [25-26], glycemia-lowering effect [19-20, 22, 26-28], and etc., but therapeutic properties of artichoke leaves extract on the skin aging process practically have not been investigated. In this study, we examined the possible protective effect of artichoke leaf extract on deterioration in skin oxidant defense system in experimental animals with D-galactose induced skin aging.

2. MATERIALS AND METHODS.

2.1. Plant materials and Authentication

The fresh leaves of the artichoke Cynara cardunculus L. (Grosso Romanesco) var. Cynara scolymus L., femaly Aesreraceae, were collected at harvest maturity from the June to the middle of October during the 2016-17 years in Mtskhetis region (Rosenthal, Georgia, latitude 41° 56' 02" N and longitude 44° 34' 36" E), average minimum temperature -1°C and maximum 35°C. the plant was identified at the Pharmaceutical Natural Sciences Department of Institute of Pharmacy of Sechenov First Moscow State Medical University (Sechenov University)

UNDER PEER REVIEW

2.2. Preparation of plant extracts and its toxicity study

The leaves the artichoke Cynara cardunculus L. were separated, washed, cleaned, and drying in according with Eur Ph monograph 01/2008:1866 corrected 6.0. Extraction of dried leaves artichoke, separation and identification of volatiles Artichoke (Cynara cardunculus L.) was preparated in according with Eur Ph monograph 01/2009:2389 (content of chlorogenic acid <2,5%). Crude aqueous extracts of dried leaves (100 g) were prepared by infusion with distilled water (plant:solvent ratio of 5:1) at 96 °C to the homogenized leaves for 120 min, and extraction for four hours using bi-distillated water as a solvent. Prepared extracts filtered through a metallic mesh to remove any kind of solid particle, cooled at room temperature and centrifuged at 5000-6000 rpm (revolutions per minute) for 15 min. The obtained primary extract was filtered throughout closed sterile filtration systems with 0.45 μ and 0.2 μ. After sterile filtration extracts concentrated by lyophilization with a FTS Systems Lyostar II LYOACC3P1, USA lyophilizer (initial temperature of -30°C, the time of lyophilisation 24 h, additional drying at 32°C for 6 h), previous freezing at -55º C. The resulting yields were 14.1 g for dry leaf water extracts. The studying extracts of Cynara Cardunculus L., 2% in ampoule was characterized by the content of chloroagenic acid 1.95% (related to the requirements of assessment report on Cynara scolymus L., folium EMA/HMPC/150209/2009), total phenolic content equal 0,31±0.04 mg gallic acid equivalent/100 mg extract, total flavonoids 1.6% and total antioxidant activities determinate as 50% inhibition of 1,1-diphenyl-2-picrylhydrazyl (DDPH) 15.1±0.9% (methods of measurement of paramemeters were described early [29]). The toxicity of studding artichoke extracts under i.p. administration is very low, LD50 exceeds 1g/kg body weight and no rats exhibited visible signs of toxicity under 14 days of intradermal injection of extracts of Cynara Cardunculus L., 2% including absence of physiologically changes in skin and fur, eyes or mucous membranes. Moderately irritating reactions induced by extracts of Cynara Cardunculus L., observed at

UNDER PEER REVIEW

103 concentration more than 10% and extracts of Cynara Cardunculus L., 2% shows good skin
104 compatibility in patch test [29].

105 2.2. Animals and experimental study design.

106 2.2.1. Ethical statement

107 Animals received humane care in compliance with "Guide for the Care and Use of Laboratory
108 animals" (National Institutes of Health publication 86-23, Revised 1996) and performed with
109 approval of the local Interinstitutional (International Scientific Centre of Introduction of New
110 Biomedical Technology, Department of Medical Pharmacology and Pharmacotherapy, Tbilisi
111 State Medical University, Tbilisi) Animal Care and Use Committee. All animals secured under
112 specific pathogen free conditions according to the Federation of European Laboratory Animal
113 Science Associations guidelines in humidity- and temperature-controlled environment, with a
114 daylit environment for at least 1 week before the experiments. Animals were fed commercial
115 laboratory rat's food pellet and allowed drink tap water ad libitum before the experiments.

116 2.2.2. Study design

117 Experiments carried out in 58 female Wistar rats weighing 180-200 g. After 7 days of
118 adaptation, all animals randomized into two groups: control and main. Animals in main group
119 after randomization received injection with D-galactose (reducing sugar, is a naturally occurring
120 substance in the body, 100 mg/kg/day, i.p. [29-30]), while in control group received placebo
121 (0.9% saline, 0.5 ml/day, i.p.), for 8 weeks. At 21 days after injection with D-galactose the 3 cm
122 round tattoo area was prefabricated on each side of rats previously disinfected hip under sterile
123 condition and general anesthesia with pentobarbital (40 mg/kg). All animals in main group (36
124 animals) were secondly randomized into 3 groups in dependence to treatment (twice in week of
125 intradermal injection under general anesthesia) for 5 weeks: control III group animals treated
126 with microinjection of saline (n=12), main I group receive 0.13 mg of 2% lyophilized powder of
127 Artichoke extracts salivated in water for injection (equivalent of average intradermal dose for
128 patients 10 mg, n=12) and main II – animals receive 1.3 mg 2% lyophilized powder of

UNDER PEER REVIEW

129 Artichoke extracts (n=12). After the experiments, all the rats euthanized by pentobarbital (60
130 mg/kg intraperitoneally). Body weight and skin oedema evaluation was investigated as described
131 below [29].

132 2.3. Determination of activities of enzymatic part of endogenous antioxidant 133 defense system of skin of rats

134 Isolation of mitochondria and measured of velocity of superoxide anion generation, superoxide
135 dismutase (total), catalase, gluthatione peroxidase and malone aldehyde (MDA) were described
136 [31-32]. Rate of H_2O_2 production was determinate as described below [33-34]. Superoxide
137 anion generation in isolated rat skin mitochondria was determined immediately following the
138 isolation procedure. Briefly, mitochondria (0.5 mg/ml) were incubated with buffer (6 mM
139 succinate, 70 mM sucrose, 220 mM mannitol, 2 mM, Hepes, 25 mM KH_2PO_4, 2.5 mM $MgCl_2$,
140 0.5 mM EDTA, 5 µg/ml catalase, pH 7.4) at 37˚C. At the indicated time points, 40 mM
141 acetylated cytochrome c was added and the change in absorbance at 550 nm was measured for 1
142 min at 37˚C. The activity of glutathione redox system including determination of glutathione
143 peroxidase (GSH-Px) and glutathione reductase by velocity of redox $NADP^+$ formation, and
144 redox glutathione in homogenate of lyophilized in liquid nitrogen skin tissue in according to [32-
145 34]. The protein concentration was determined with BSA protein assay kit.

146 2.4. Statistical analysis

147 All variances in the measurement data expressed as mean ± standard deviation of mean (SD),
148 and statistical significance assessed using Student t-test for normally distributed variables and p
149 < 0.05 considered as a significant. All statistical calculations were performed using the Statistical
150 Sciences (SPSS, version 23.1).

151 3. RESULTS

152 The studying extracts of artichoke (Cynara cardunculus, cultivated in Georgia, Mtskhetis
153 region), 2% Artichoke extract related to water artichoke extracts with content of chloroagenic

154 acid <2.5% and about 10% of total phenolic acids in according with the Assessment report on
155 Cynara scolymus L., folium EMA/HMPC/150209/2009.

156 3.1. Changes in body weight and skin oedema during D-galactose induced 157 skin aging and influence of lyophilized artichoke extracts, 2%

158 Early was shown that animals with D-galactose induced skin aging during 12 weeks and
159 demonstrated evident symptoms of aging: a unique skin appearance, with wrinkling's and
160 furrows. Prior to euthanized, no morbidity/mortality and clinically relief differences in food
161 intake and water consumption in subgroups of main group were not observed. The relative
162 weight of skin markedly decrease in D-galactose model of aging. Artichoke at the doses of 0.13
163 and 1,3 mg/kg improved body weight of D-galactose induced aging rats (table). While the
164 administration of Artichoke extracts in normal rats for 8 weeks did not change, the body weight
165 compared to the control group. Thus, ttreatment with artichoke extracts, 2% restores the water
166 dysbalanced in the aging skin in both dosage.

167 3.2. D-galactose-induced aging changes in skin and activity of total SOD and 168 generation of superoxide anion.

169 D-galactose in dose 100 mg/kg i.p. during 8 weeks cause to significant decreased in total SOD
170 activity in skin in comparison with control I and control II, while differences in SOD activity
171 between control I and control II groups did not mentioned (table). At the same time, the velocity
172 of superoxide anion generation increased by 15% in control II group when comparing the rate of
173 O_2^- production in 240 days rats (table). Treatment with 2% artichoke extract from the 21 days
174 after D-galactose induced aging in rats leads to increase SOD activity by 50% and by 23% in
175 comparison with control III groups and this accompanied with markedly decreasing in velocity
176 of O_2^- generation by 27% and 25% in low and high doses of extracts, respectively. The velocity
177 of superoxide anion generation at the end of the treatment in both dosage of artichoke extracts
178 did not differences from the level in placebo (control II) group.

UNDER PEER REVIEW

Table. **Therapeutic efficacy of different doses of artichoke extracts for maintenance the activity of endogenous enzymatic antioxidant defense system D-galactose induced aging skin in experimental animals.**

Groups	**Control I, n=10**	**Control II, n=12**	**D-galactose agin sking rats, n=36**		
			Control III, n=12	**Liophylized extract artichoke, dose, mg/kg intradermally**	
				0.13, n=12	**1.3, n=12**
Body weight, g	187±22	312±23	245±25$^{*\#\#\#}$	278±24**	268±21*
Relative weight, mg dry/100 mg wet weight	31.5±2.1	32.8±1.4	23.5± 2.3$^{**\#\#}$	29.2± 1.8^{x}	29.7± 2.1^{x}
Velocity of O_2^- generation	0.27±0.02	0.31±0.03	0.48± 0.06$^{**\#\#}$	0.35± 0.04^{*x}	0.36± 0.05^{*x}
H_2O_2, µmol/L · min	1.59±0.14	1.80±0.14	5.15± 0.23$^{***\#\#\#}$	3.02± 0.32$^{***\#\#\#xxx}$	3.17± 0.21$^{***\#\#\#xxx}$
SOD, U/mg protein/min	0.33± 0.04	0.32± 0.03	0.26± 0.02$^{*\#}$	0.39± 0.03$^{\#xxx}$	0.32± 0.03$^{\#x\S}$
Catalase, nMol H_2O_2/mg protein/min	64±9	67±8	42±4$^{**\#\#}$	68±6	59±6$^{\#}$
Glutathione redox potential, GSH/GSSG	3.18± 0.38	2.90± 0.29	1.83± 0.23$^{***\#\#}$	2.41± 0.19^{**xx}	2.23± 0.15^{**x}
Glutathione peroxidase, nMol NADP/mg protein	2.44± 0.22	2.69± 0.33	1.73± 0.23$^{**\#\#}$	2.51± 0.20^{x}	1.97± 0.13$^{*\#}$
Gluthatione reductase,	0.10±	0.19±	0.29±	0.18±	0.11±

UNDER PEER REVIEW

μMol NADPH/g wet tissue	0.02	0.03*	0.04***##	0.04*x	0.03#xxx
MDA, μmol/mg protein	0.88± 0.08	0.92± 0.10	1.48± 0.16***##	0.96± 0.06xxx	1.09± 0.09 xx

Note: *- compared with control 1, # - with control 2 group, x - with control 3 and § - between artichoke extracts treatment groups; significance of difference of comparison: one symbol – $p<0.05$, two – $p<0.01$, three - $p<0.001$, absence of symbol indicated that differences is not significance ($p>0.05$).

3.3. D-galactose-induced aging changes in skin and activity of catalase and generation of hydrogen peroxide

There were no significant differences in catalase activity between control groups. Exposure to D-galactose did not induced changes in catalase activity in skin tissue (table). However, the production of H_2O_2 increased under treatment of D-galactose and exceeds control II level by 186%. Treatment with 2% artichoke leaf extract increased the level of catalase activity, and decrease the level of H_2O_2 production by 42% in dosage of 0.13 mg and by 25% under higher doses.

3.4. D-galactose-induced aging changes in skin and activity of glutathione redox system

Exposure to D-galactose reduced the GSH content in skin tissue from 1.20±0.13 nmol/mg/protein to 0.74±0.13 nmol/mg/protein ($p< 0.01$ *vs*. control III). Treatment with artichoke extract at doses 0.13 and 1.3 mg/kg significantly recovered the GSH content up to 0.98±0.09 and 0.89±0.09 nmol/mg/protein ($p<0.01$ and $p<0.05$, respectively) when compared to D-galactose-treated animals. Simultaneously the GSH/GSSG ratio is proportionately decreased in D-galactose skin aging model by 37%. Treatment with artichoke extracts in doses of 1.3 mg/kg restored the gluthatione redox and it has reached level in the same aging groups while at higher doses treatment the GSH/GSSG ratio increased only by 22% (table). Due to D-galactose

UNDER PEER REVIEW

205 treatment observed significantly decreasing of GSH-Px activity, withought any differences in
206 GR activity (table). Treatment with artichoke extracts in dose of 0.13 mg increased the level of
207 GSH-Px by 31% and only by 14% (NS) at doses of 13 mg/kg. Ratio of activities of
208 SOD/(Catalase + GSH-Px), which represents equilibrium between formation of hydrogen
209 peroxide from superoxide dismutation and its utilization by catalase and GSH-Px equal
210 $5.0\pm0.3x10^{-3}$ in rats at the beginning of the experiments and 4.6 ± 0.2 $x10^{-3}$ in control II group. In
211 D-galactose model of aging skin ratio SOD/(Catalase + GSH-Px) increased to 6.0 ± 0.2 $x10^{-3}$, and
212 decreased to 5.5a±0.2 and 5.2±0.2 after artichoke extracts treatments in low and high dosage,
213 respectively. Simultaneously, the redox potential, ratio of generation O^{2-}/H_2O_2 which equal in
214 intact group 0.17±0.04 decrease to 0.09±0.01 in D-galactose treated control III group and
215 increase to 0.12±0.2 ($p<0.01$) after artichoke treatment. There were no correlation between the
216 level of ratio SOD/(Catalase + GSH-Px) and MDA content in skin (r=0,37, NS).

217 3.5. D-galactose-induced aging changes in skin MDA content

218 Despite that level of MDA also determinated as a marker of lipid peroxidation in skin and other
219 tissues, MDA content, as a final product of lipid peroxidation, could not reflects the
220 disturbances in the sensitivity of lipid to oxidation [35]. In the model of D-galactose induced
221 aging levels of MDA in skin significant elevated, when compared to the control group ($p <$
222 0.001) following 42 days of exposure to D-galactose, but not in aging group without D-galactose
223 (table). Interestingly, treatment of rats with artichoke at doses of 0.13 and 1.3 mg/kg
224 significantly decreased the levels of MDA in skin in both cases.

225 4. DISCUSSION

226 D-galactose is pharmacological adaptive aging model, because D-galactose primary roles in
227 pathogenesis of aging. Skin aging is a complicated multitargets dysbalancing progression in the
228 epidermis and dermis which documented by rising in superoxide anion production in D-
229 galactose induced skin aging model in rats. Influence of artichoke extracts restored skin relative
230 weight and leads to an increase of solubility in neutral salt, acid, and decreased pepsin solubility

UNDER PEER REVIEW

231 collagen fraction, restored the hexosamine/collagen (hydroxyproline) ratio and decreased the
232 activity of nuclear transcription factor (NF-kB). Local prolonged treatment with artichoke
233 extracts improved collagen metabolism and attenuated the progression of inflammation in D-
234 galactose induced skin aging model [29]. Early it was shown, that chronic (6-8weeks)
235 administration of D-galactose induced blocking of glycometabolism (hyperproduction of
236 advanced glycation products), dysbalanced and loses of antioxidant activity of tissue (decreasing
237 the level of SOD and gluthatione peroxidase activity) and increased level of MDA in dose
238 dependent manner (50-500 mg/kg i.p. or subcutaneously) [8, 30, 36-38]. Rats in the model group
239 exhibited the typical changes of aging skin compared with the control group, rats in the model
240 group had significantly increased MDA content, and decreased serum SOD and GSH-Px
241 activities ($P<0.05$). The end product of free radicals oxidizing of unsaturated lipids of biological
242 membranes is MDA which can influence exchange of substances between cells, and finally lead
243 to rupture and death of cells. Extract of artichoke is rich in phenolic and flavonoids and gives a
244 powerful antioxidant activity [12-14, 38]. Pre-clinical and clinical investigations have suggested
245 that the artichoke leaf extract has potential lipid-lowering and hepatoprotective effects [14-17,
246 19-20, 22-23]. The beneficial effects of artichoke could mainly attributed to its antioxidant
247 components: the main substances are mono- and dicaffeoylquinic acid (cynarin and chlorogenic
248 acid), caffeic acid (1%) and volatile sesquiterpene and flavonoids (1%) that include the
249 glycosides luteolin-7-beta-rutinoside (scolymoside), luteolin-7-beta-D-glucoside and luteolin-4-
250 beta-D-glucoside [12-14, 37]. Several *in vitro* studies have shown that the antioxidant potential
251 of artichoke extracts is dependent on radical scavenging and metal ion chelating effect of its
252 constituents such ascynarin, chlorogenic acid and flavonoids. However, pure constituents of
253 artichoke extracts shown to produce less inhibitory activity on free radical production than the
254 extract itself [12-13]. Interestingly, that artichoke is favors that synthesis of coenzymes
255 NAD(($NADH_2$)) and NADP($NADPH_2$)) and mainly of the NADP($NADPH_2$) pair, which take
256 key plays in the regulation of antioxidant/prooxidant status of the cell and its including in the

UNDER PEER REVIEW

257 antioxidant properties of artichoke extracts could be included. Preincubation of HUVEC cells or
258 human leukocytes with the artichoke extract at concentrations of 25–100 µg/mL for 24 h
259 abolished ROS generation induced by lipopolysaccharide and oxidation of low density
260 lipoproteins [18, 38]. Early it was shown that artichoke (Cynara Scolymus L.) in dosage 20, 40
261 80 mg/kg daily per os in D-galactose (40 mg/kg body weight) daily for 36 days increase activity
262 of SOD in brain and liver, GSH-Px in brain, and catalase activity in liver [30]. In present article
263 for the first time was study influence of local intradermal action of Cynara Scolymus L. exstract
264 on restoration the ability of endogenous antioxidant defense system to prevent free radical injury
265 development in D-galactose (100 mg/kg daily for 8 weeks, i.p.) skin aging in
266 rats. D-galactose (100 mg/kg daily for 8 weeks, i.p.) skin aging in rats
267 characterized increasing in superoxide anion generation in and hydrogene
268 peroxide in widely applied to anti-aging pharmacology studies sub-acutely
269 aging models of rodents induced by chronic injection of D-galactose [37].
270 States of skin in this model accompanied with decrease in the activity of
271 SOD, catalase and GSH-Px, and increased production of superoxide anion and
272 hydroperoxide. Hyperproduction of hydrogen peroxide in aging occurs in
273 response disturbances in aerobic respiration and one molecule of catalase can
274 inactivate about 6 million hydrogen peroxide molecule per min by combined
275 them two a time. Thus, the less increased in catalase activity under
276 treatment of artichoke really could sufficient to neutralized produced
277 hydrogen peroxide under decreasing of superoxide anion generation and as a result its oxidation
278 to H_2O_2 by SOD. Oxidative damage was concomitant to an imbalance in the principal
279 antioxidant cytoplasmic agent - a significant reduction in cellular GSH, which exerts antioxidant
280 activity by acting as a free-radical scavenger during the reductive detoxification of hydrogen
281 peroxide and lipid peroxide is one of the important target of skin-whitening effect of aging.

UNDER PEER REVIEW

282 Exposure to D-galactose reduced the GSH content in skin tissue, while artichoke extract at doses
283 0.13 and 1.3 mg/kg significantly recovered the GSH content. Due to D-galactose treatment
284 observed significantly decreasing of GSH-Px activity, withought any differences in GR activity
285 (table). Treatment with artichoke extracts in dose of 0.13 mg markedly increased the level of
286 GSH-Px by 45% and 13% in dose of 13 mg/kg. The data suggest that oxidative stress reduces
287 gluthathione redox potential and that prevention disturbances in GSH redox cycle activity
288 appears to be an important component of the antiaging phenomenon.

289 ## 5. CONCLUSION

290 In conclusion the redox potential of the $O_2/2H_2O$ redox system could play a key role in the "Free
291 Radical Theory of Aging" , seems to address a key facet of intrinsic biological instability of
292 living systems throughout unavoidably formed ROS in the course of metabolism and arising due
293 to the action of various exogenous factors, damage biomolecules [1-5, 35-37]. Obtained data
294 indicate that the concomitant use of 2% artichoke extract improve reserve ability of antioxidant
295 defense system and exert antiaging action in this model of skin aging in experimental animals.
296 The increased reserve ability of intrinsic antioxidant defense system of skin after course of local
297 treatment with artichoke extracts emphasizes artichoke dry extract efficacy in cosmetic
298 formulation and its beneficial effects for anti-aging skin care.

299 CONSENT

300 Is not applicable

301 ETHICAL APPROVAL

302 All animals procedures and study protocols carried out in compliance with "Guide for the Care
303 and Use of Laboratory animals" (National Institutes of Health publication 86-23, Revised 1996)
304 and performed with approval of the local Interinstitutional (International Scientific Centre of
305 Introduction of New Biomedical Technology, Department of Medical Pharmacology and
306 Pharmacotherapy, Tbilisi State Medical University, Tbilisi) Animal Care and Use Committee.
307 All animals secured under specific pathogen free conditions according to the Federation of

UNDER PEER REVIEW

308 European Laboratory Animal Science Associations guidelines in humidity- and temperature-
309 controlled environment, with a daylit environment for at least 1 week before the experiments.

310 **ACKNOWLEDGMENTS**

311 Authors are grateful to the Chief animal's clinic Kamkamudze R. and his staff for animal
312 housing and Associate Professor Bobkova Natalia V. of the Pharmaceutical Natural Sciences
313 Department Institute of Pharmacy of Sechenov First Moscow State Medical University
314 (Sechenov University) for authentication of artichoke leaves used in this study and Tsivtsivadze
315 E, PhD as a director of "Biotechpharm GE" in part of artichoke extracts preparations.

316 COMPETING OF INTEREST

317 The authors declare that they have no conflict of interests regarding the publication of this paper.
318 The authors alone are responsible for the content and writing of this article.

319 **REFERENCES**

320 1. Ganceviecene R, Liakou AI, Theodoridis A, Mafrantonaki E, Zouboulis CC. Skin anti-aging
321 strategies. Dermato-Endocrinology. 2012; 3:308-319. http://doi.org/10.4161/derm.22804.
322 2. Gilchrest BA, Campisi J, Chang H, Fisher G, Kulesz-Martin M. Skin aging: molecular mechanisms
323 and tissue consequences. J Invest Dermatol. 2015; 135(4):950-953. doi.: 10.1038/jid.2014.546.
324 3. Gragnani A, MacCornic SM, Chominski V, Ribeiro de Noronha SM, Correa de Naronha SAA,
325 Ferreira LM. Review of major theories of skin aging. Advances in Aging Res. 2014; 3:265-284.
326 http://dx.doi.org/10.4236/aar.2014.34036.
327 4. eSilva SA, Leonardi GR, Michniak-Kohn B. An overview about oxidation in clinical practice of skin
328 aging. An Bras Dermatol. 2017; 92(3):367-374. http://dx.doi.org/10/1590/abd1806-4841.20175481.
329 5. Polisak B, Dahmane RG, Godic A. Intrinsic skin aging: The role of oxidative stress. Acta
330 Dermatovenerologica . 2012; 21:33-36. doi: 10.2478/v10162-012-0009-0
331 6. Rinnerthaler M, Bishof J, Streubel MK, Trost A, Richter K (2015). Oxidative stress in aging human
332 skin. Biomolecules. 5: 545-589. https://doi.org/10.3390/biom5020545.

FOR REVIEW

333 7. Chang L, Liu X, Li H, Liu J, Guo Z, Xiao K et al. D-galactose induces a mitochondrial complex 1
334 deficiency in mouse muscle: potential benefits of nutrient combination in ameliorating muscle
335 impairment. J Mol Food. 2014; 1-8. Doi:10.1989/jmf.2013.2830

336 8. Chen P, Chen F, Zhou B. Antioxidatve, anti-inflammatory and anti-apoptotic effects of ellagic acid in
337 liver and brain of rats treated by D-galactose Scientific Reports. 2018; 8:1465-
338 1474.doi:10.10.1038/s41598-018-19732-0

339 9. Ho SC, Liu JH, Wu RY. Establisment of the mimetic aging effect in mice caused by D-galactose.
340 Biogerontology. 2003; 4(1):15-18

341 10. Liu C, Hu J, Mao Z, Kang H, Liu H, Fu W, Lv Y, Zhou F. Acute kidney injury and
342 inflammatory response of sepsis following cecal ligation and puncture in d-galactose-induced
343 aging rats. Clin Interv Aging. 2017; 12: 593-602.

344 *11.* Liu S, Chen Z, Cai X, Sun Y, Zhao C, Liu F, Liu D. Effects of dimethylaminoethanol and
345 compound amino acid on D-galactose induced skin aging model of rat. Scientific World J.
346 2014; article ID 507351, 7 pages. hhtp://dx.doi.org/10.1155/2014/507351.

347 12. Shwe T, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. Role of D-galactose induced brain
348 aging and its potential used for therapeutic intervention. J Ex. Gerantology. 2018; 101:13-36.
349 https://doi.org/10.1016/j.exger.2017.10.029

350 13. Georgievs SS, Boyadzhieva SS, Angelov G. Intensification of extraction of bioactive substances from
351 artichoke wastes. Bulgarian Chemical Communication. 2016; 48 (special issue E): 451-455.

352 14. Ben Salem M, Affes H, Ksouda K, Dhouibi R, Sahnoun Z, et al. Pharmacological Studies of
353 Artichoke Leaf Extract and Their Health Benefits. Plant Foods Hum Nutr. 2015; 70(4):441-53. doi:
354 10.1007/s11130-015-0503-8.

355 15. Joy JF, Haber SL. Clinical uses of artichoke leaf extract. Am J Health Syst Pharm. 2007; 64: 904-909.
356 https://doi.org/10.2146/ajhp070013

357 16. El-Deen SAS. Artichoke, the ancient hepatic friend and promising multifunctional
358 therapeutic agent. J Pharmaciol Ther Res. 2018; 2(1):4-5.
359 http://www.alliedacademies.org/journal-pharmacology-therapeutic-research/

FOR PEER REVIEW

360 17. Rondanelli M, Opizzi A, Faliva M, Sala P, Perna S, et al. Metabolic management in
361 overweight subjects with naive impaired fasting glycaemia by means of a highly standardized
362 extract from Cynara scolymus: a double-blind, placebo-controlled, randomized clinical trial.
363 Phytother Res. 2014;28(1):33-41. doi: 10.1002/ptr.4950.
364 18. Juzyszyn Z., Czerny B., Pawlik A. Droździk M. The effect of artichoke (*Cynara scolymus*
365 L.) extract on ROS generation in HUVEC cells. Phytotherapy Res. 2008; 22(9): 1159-1161
366 https://doi.org/10.1002/ptr.2385.
367 19. Gebhardt R. Antioxidative and protective properties of extracts from artichoke (Cynara
368 scolymus L.) against hydroperoxide-induced oxidative stress in cultured rat hepathocytes.
369 Toxicol. Appl Pharmacol. 1997; 144(2): 279-286. https://doi.org/10.1006/taap.1997.8130
370 20. Brown JE, Rice-Evans CA. Luteolin rich artichoke extract protects low-density lipoprotein from
371 oxidation *in vitro*. Free Radical Res. 1998; 29:247–55.
372 21. Perez-Garcia F, Adzet T, Canigueral S. Activity of artichoke leaf extract on reactive oxygen
373 species in human leukocytes. Free Radical Res. 2000; 33:661–665.
374 https://doi.org/10.1080/10715760000301171.
375 22. Küskü-Kiraz Z, Mehmetçik G, Dogru-Abbasoglu S, Uysal M. Artichoke leaf extract reduces
376 oxidative stress and lipoprotein dyshomeostasis in rats fed on high cholesterol diet.
377 Phytotherapy Res. 2010; 24 (4): 565–570. https://doi.org/10.1002/ptr.2985.
378 23. Ibrahim GE, El-Raey MA, Eldahshan OA, Souleman AMA. Effect of extraction on phenolic
379 content, symymarin and antiradical activities of artichoke leaves and roots. Eur Scientific J.
380 2013; 9(6):100-110.
381 24. Zapolska-Downar D, Zapolska-Dowanar A, Naruszewicz M, Siennicka A, Krasnodebska B,
382 Koldxej B. Protective properties of artichoke (Cynara scolymus) against oxidative stress
383 induced in cultured endothelial cells and monocytes. Life Sci. 2002; 71:2897-2908.
384 https://doi.org/10.1016/S0024-3205(02)02136-7.

UNDER REVIEW

385 25. Heidarian E, Rafieian-Kopaei M. Protective effect of artichoke (Cynara scolymus) leaf
386 extract against lead toxicity in rat. Pharm Biol. 2013; 51(9): 1104-9.
387 https://doi.org/10.3109/13880209.2013.7779312013
388 26. El Morsy E M, Kamel R. Protective effect of artichoke leaf extract against paracetamol-
389 induced hepatotoxicity in rats, Pharmaceutical Biology. 2015; 53(2): 167-173. doi:
390 10.3109/13880209.2014.913066
391 27. Fantini N, Colombo G, Giori A, Riva A, Morazzoni P, et al. Evidence of glycemia-lowering
392 effect by a Cynarascolymus L. extract in normal and obese rats. Phytotherapy Res. 2011;
393 25:463-466. https://doi.org/10.1002/ptr.3285
394 28. Safaa M, Hanaa A, Abdel F, Nahila A, Abdelaaty S. Cynara scolymus for relieving on
395 nonalcoholic steatohepatitis induced in rat. Int. J. Pharmacy&Pharm. Sciences. 2013; 5(1):
396 57–66. http://dx.doi.org/10.1155/2016/4030476.
397 29. Sukoyan G. Tsivtsivadze E, Golovach V, Kezeli T, Demina N. Anti-aging effect of Cynara
398 Cardunculus L. var. Cynara Scolymus L. extract in D-galactose-induced skin aging model in
399 rats. Pharmacology&Pharmacy. 2018; 9: 428-439. https://doi.org/10.4236/pp.2018.910032.
400 30. Song S, Zhao L, Wang W, Cong T, He H (2012) Using aging rats model to investigate anti-
401 oxidative ability of artichoke (Cynara Scolymus L.) leaf extract.
402 https://doi.org/10.17660/ActaHortic.2012.944.15
403 31. Galenko-Yaroshevskii VP, Agadzanova AV, Lapina IV, Gaironovskaya TV, Popkova LV, et
404 al. Effectiveness of combined treatment of superoxide dismutase and reamberin in skin
405 ischemia. Bull Exp Biol Med. 2006; 142(6):707-709. https://doi.org/10.1007/s10517-006-
406 0457-9
407 32. Galenko-Yaroshevskii VP, Bagmetova EN, Fil'chukova IA, Sidel'nikov A, Lapina IV, et al.
408 Antihypoxic and antinecrotic effect of mexidol in skin ischemia. Bull Exp Biol Med. 2005;
409 139(2): 702-704. https://doi.org/10.1007/s10517-005-0248-8.

410 33. Karsanov NV, Sukoyan GV, Antelava NA, Varazanashvili NA. The effect of energostim on
411 myocardial damage during coronary artery occlusion. Exper. Clin Pharmacol. 2003; 66(5),
412 17-21. doi: https://doi.org/10.30906/0869-2092-2003-66-5-17-21
413 34. Sukoyan GV, Mumladze MR, Oboladze ED, Varazanashvili NA. *In vitro* effects of
414 gentamicin, ampicillin, and cefobid on energy supply and antioxidant protection systems of
415 venous blood erythrocytes in newborns. Bull Exp Biol Med. 2005; 139(6): 671-674. doi:
416 10.1007/s10517-005-0374-3.
417 35. Dzhanashia P, Vladitskaya OV, Kobalava MA, Sukoyan GV, Sherosia Ts, Andriadze NA.
418 Lipid hyperperoxidation in the impaired structural organization of thrombocytic membranes
419 in patients with chronic heart failure (communication 1). Russian Medical Journal. 2005:4:
420 35-50.
421 36. Lisanti MP, Martinez-Outschoorn UE, Lin Z, Pavlides S, Whitaker-Menezes D, et al.
422 Hydrogen peroxide fuels aging, inflammation, cancer metabolism and metastasis. Cell Cycle.
423 2011; 10(15):2440-2449. https://doi.org/10.4161/cc.10.15.16870
424 37. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources
425 against skin aging. Phytomedicine. 2011; 19:64-73.
426 https://doi.org/10.1016/j.phymed.2011.10.003.
427 38. Piston M, Machado I, Branco CS, Cesio V, Heinzen H et al. Infusion, decoction and
428 hydroalcoholic extracts of leaves from artichoke (Cynara cardunculus L. subsp. Cardunculus)
429 are effective scavengers of physiologically relevant ROS and RNS. Food Res. Intern. 2014;
430 64:150-156. hhttp://dx.doi.org/10.1016/j.foodres.2014.05.078

431

432