Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 83 -89) 2019

Antioxidant and neuroprotective effects of caffeoylquinic acids from *Geigeria alata*

B. Dimitrova[1,2*], V. B. Vitcheva[1], D. Zheleva-Dimitrova[2], R. L. Simeonova[1], R. Gevrenova[2]

[1]*Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav St., 1000 Sofia, Bulgaria*

[2]*Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav St., 1000 Sofia, Bulgaria*

Received February 22, 2019; Revised April 24, 2019

Geigeria alata Benth. & Hook.f. ex Oliv. & Hiern is a traditional plant used in Sudanese folk medicine for treatment of diabetes, cough, epilepsy and intestinal complaints. The aim of the study was to evaluate *in vitro* the antioxidant potential of 3,5-dicaffeoylquinic acid (DCQA) and 3,4,5-tricaffeoylquinic acid (TCQA) isolated from *G. alata* roots and to assess *in vivo* the neuroprotective effects against ethanol-induced brain injury in rats of the more effectivecompound. For the *in vitro* study a non-enzyme induced lipid peroxidation (LPO) in brain microsomes from New Zealand Rabbit was used. TCQA showed more pronounced effect against LPO and was used for the *in vivo* experiment. Brain injury in male Wistar rats was induced by 5-day oral administration of 50 % ethanol (1 mL/100 g body weight). TCQA and 5-caffeoylquinic acid (chlorogenic acid, CGA) as a positive control, were administered for 14 days alone and in combination with ethanol. Five-day oral administration of ethanol resulted in a statistically significant ($p < 0.05$) oxidative stress, discerned by increased malondialdehide (MDA) quantity, decreased glutathione (GSH) content and activity of the antioxidant enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). Nine-day pre-treatment of the animals with TCQA and subsequent five-day co-administration with ethanol ameliorated above-mentioned parameters. Neuroprotective effects of TCQA in ethanol-induced brain damage in rats are most likely due to its antioxidant action.

Keywords: Caffeoylquinic acids, *Geigeria alata*, Oxidative stress, Antioxidant enzymes, Lipid peroxidation

INTRODUCTION

Oxidative stress and lipid peroxidation are involved in many pathological conditions, such as hypertension, diabetes [1], inflammation [2], neurodegenerative diseases [3]. Brain tissue is very sensitive to oxidative stress due to high oxygen consumption, iron availability, high content of polyunsaturated fatty acids, and low capacity of antioxidant enzymes [4].

Experimental toxicology uses various models of brain injury, mainly by inducing oxidative stress. Alcohol-induced brain injury is a widely used model [5]. The neurotoxic effects of alcohol are manifested by: severe depression, cognitive changes, impaired coordination and behavioral changes [6]. The pathophysiological mechanism underlying these disorders is the alcohol-induced change in gamma aminobutyric acid (GABA) and glutamate mediation, which in turn leads to secondary induction of oxidative stress. In recent years, the scientific community's attention has been focused on studying the neuroprotective effects of plants, foods and beverages of plant origin [4]. In a number of studies, the protective and antioxidant effects of coffee have been demonstrated, particularly in regards to the central nervous system (CNS). It has been found that these neuroprotective effects of coffee are due to its caffeoylquinic acid derivatives: 5-O-caffeoylquinic (chlorogenic) acid, 1,3-dicaffeoylquinic acid, 3,4,5-tricaffeoylquiinic acid. In the recent years, scientific information has been accumulated about the potential neuroprotective effects of caffeoylquinic acids, demonstrated in both *in vitro* [7] and *in vivo* models [8]. *In vitro* studies showed the neuroprotective and antioxidant effects of chlorogenic acid in neuronal cells [9,10] and its preventive action against neurodegenerative diseases [11]. Oboh et al. [11] found that CGA prevented the formation of senile plaques associated with Alzheimer`s disease (AD), inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), prolonging the effects of ACh and BCh as well as reduced inflammation brought on by primary microglia from brain tissue.

Caffeoylquinic acid derivatives are found in a large number of Asteraceae plants including the Sudanese plant *Geigeria alata* [12] Ethno-botanical data showed that in Sudanese traditional medicine, *G. alata* was used against epilepsy, as spasmolytic agent [13,14] and for cough and intestinal complaints [15]. Recently it was proved that water-alcoholic extracts from roots have *in vitro* α-glucosidase inhibitory activity and increase serum insulin levels *in vivo*, improving beta-cell function and antioxidant status [16]. Phytochemical investigations on the studied species led to the identification of a variety of acylquinic acids [12] . Quantitative HPLC-UV analysis showed the highest content of 3,5-dicaffeoylquinic acid in the roots

* To whom all correspondence should be sent:
E-mail: bojana_ruseva@yahoo.com

(25.96 ± 2.08 mg / g dw), while 4,5-dicaffeoylquinic acid was the major component in the leaves. [12].3,5-dicaffeoylquinic acid revealed a higher radical-scavenging activity and a reducing ability compared to the crude extract and the chlorogenic acid. 3,4,5-tricaffeoylquinic acid had a higher antimicrobial potential against susceptible and resistant to penicillin *S. aureus* strains of as well as against Methicillin Resistant *Staphylococcus aureus* (MRSA) [12].

Based on the data presented, we investigated the ability of two caffeoylquinic acids (CQA), 3,5-di-CQA (DCQA) and 3,4,5-tri-CQA (TCQA), isolated from *Geigeria alata* roots, to attenuate non-enzyme-induced lipid peroxidation in brain microsomes, isolated from New Zealand rabbits. Further, the purpose of this study was to investigate the neuroprotective and antioxidant effects of TCQA in a model of alcohol-induced oxidative stress in rats.

EXPERIMENTAL

Plant material

Geigeria alata roots were harvested in July 2011 from El-obeid (Latitude: 13° 09' 7.20" N; Longitude: 30° 13' 34.80" E), west Kordofan (Sudan). Botanical identification was performed by Dr. Wail El Sadig, and a voucher specimen № 41935/HNC was deposited in herbarium of Botany Department, Faculty of Sciences, University of Khartoum, Sudan. 3,5-dicaffeoylquinic acid and 3,4,5-tricaffeoylquinic acid were purified from *Geigeria alata* roots as described previously and a purity 96% was obtained as determined by ultra-high-performance liquid chromatography – high resolution mass spectrometry [17].

Chemicals

All the reagents used were of analytical grade. Bovine serum albumin (fraction V), beta–nicotinamide adenine dinucleotide 2`-phosphate reduced tetrasodium salt (NADPH), chlorogenic acid (CGA), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR) enzyme, and cumene hydroperoxide were purchased from Sigma Chemical Co. (Taufkirchen, Germany). 2,2- Dinitro-5,5 dithiodibenzoic acid (DTNB) was obtained from Merck (Darmstadt, Germany).

Preparation of brain microsomes

Brain microsomes were isolated from untreated rabbit, according to the procedure, described by Voirol et al.[17]. Immediately after decapitation, brains were excised, rinsed in cold buffer containing 0.32 M sucrose, 50 mM KH_2PO_4, 1 mM EDTA, 0.1 mM dithiothreitol, pH 7.4. All procedures were done on ice and all the centrifugations were carried out at 4ºC. The samples of 1 g were homogenized in 9 mL of cold 1 mM Tris HC1 buffer (pH 7.4) containing 154 mM NaCl using a glass homogenizer with a Teflon pestle [18]. The prepared brain homogenate was centrifuged at 17,000 x g for 25 min and then pelleted by centrifugation at 105,000 x g for 1 h. The microsomal pellets were resuspended in 0.1M potassium phosphate buffer, pH 7.4, containing 20% glycerol. The content of microsomal protein was determined by the method of Lowry [19] using bovine serum albumin as a standard and adjusted to 0.1 mg protein/ 0.5 mL.

Iron-ascorbate induced lipid peroxidation (Fe^{2+}/AA)

The isolated microsomes were pre-incubated with DCQA, TCQA and CGA, as a positive control at three consequently decreased equimolar concentrations: 100 μmol, 10 μmol, and 1μmol. The pre-incubation was performed at 37°C for 15 min. Lipid peroxidation was induced by incubating microsomes (0.1 mg protein/ 0.5 ml) with 84 μM $FeSO_4$ and 400 μM ascorbic acid in 1 mM Tris HC1, 154 mM NaCl, 0.1 mM EDTA, pH 7.4. Samples were incubated in a water bath, at 37°C, for 40 min. [20].The reaction was stopped with mixture of trichloracetic acid (TCA) 25% and thiobarbituric acid (TBA) 0.67% at 20 min after LPO initiation and MDA quantity was assessed [21].

Animals

Experiments were performed in 36 male Wistar rats (initial body weight 190-220 g). The animals were housed in Plexiglas cages (3 per cage) at 20 ± 2 °C and 12/12 h light/dark cycle. Food and water were provided ad libitum. All procedures were approved by the Bulgarian Agency of Food Safety (№ of permission 190) and performed strictly following the principles stated in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123).

Design of the in vivo experiment

TCQA was found to be more effective in the *in vitro* study (see Results) and was therefore used in the *in vivo* experiment.

The rats were randomly divided into six groups (n=6) as follows:

Group 1: control rats, treated with the saline solution, administered by gavage at 5 mL/kg bw/day, 14 days.

Group 2: rats treated with TCQA alone at 5 mg/kg bw/day, 14 days [22].

Group 3: rats, treated with the saline vehicle, administered by gavage at 5 mL/kg bw/day, 9 days, and ethanol 50% (10 mL/ kg/ day) for five consecutive days up to day 14.

Group 4: rats treated with TCQA at 5 mg/kg bw/day, 14 days. From day 10 to day 14, 45 minutes after administration of the tested compound 50 % ethanol was given orally (10 mL/ kg/ day).

Group 5: rats treated with CGA at a dose of 5 mg / kg p.o. for 14 days [23].

Group 6: rats treated with CGA at a dose of 5 mg / kg p.o. for 14 days. From day 10 to day 14, 45 minutes after administration of the CGA 50 % ethanol was given orally (10 mL/ kg/ day).

Urine analysis

For the 24-hours urine collection, animals were placed in metabolic cages (Ugo Basile, Italy). Urine was collected on the 14th day of the experiment. The following parameters were determined: pH, proteins, bilirubin, urobilinogen, ketones. Standard urine reagent strips were used to measure these parameters (Condor - Teco, Beijing, China)

The animals in all groups were sacrificed on the 15th day from the beginning of the experiment. Brains were taken for assessment of biochemical parameters. For all following experiments the excised brains were perfused with saline solution (0.9% NaCl, 4^{o}C), blotted dry, weighed, and homogenized with corresponding buffers (see Markers of oxidative stress).

Markers of oxidative stress

Reduced glutathione (GSH) was assessed by measuring non-protein sulfhydryls after precipitation of proteins with 5% trichloroacetic acid (TCA), using the method described by Bump [24]. A total of 10% homogenates were prepared in 0.05M phosphate buffer (pH 7.4) and centrifuged at 7 000 × g and the supernatant was used for antioxidant enzymes assay. Glutathione peroxidase (GPx) was measured by NADPH oxidation, using a coupled reaction system consisting of GSH, GR, and cumene hydroperoxide [25]. Catalase (CAT) activity was determined by measuring the decrease in absorbance at 240 nm of a reaction mixture consisting of H_2O_2 in phosphate buffer, pH 7.0, and requisite volume of supernatant sample. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine catalase activity. The specific activity was calculated and was expressed as μmol/min/mg of total protein [26]. Superoxide dismutase activity (SOD) was measured according to the method of Misra and Fridovich [27], following spectrophotometrically the autoxidation of epinephrine at pH 10.4, 30 ^{o}C, using the molar extinction coefficient of 4.02 mM^{-1} cm^{-1}.

Statistical analysis

Statistical analysis for the *in vitro* study was performed by ANOVA, followed by the Student's t-test. Three parallel samples were used.

Statistical programme 'MEDCALC' was used for the *in vivo* study. The results were expressed as mean ± SEM for six rats in each group. The significance of the data was assessed using the nonparametric Mann–Whitney *U* test. For both statistical methods, values of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In vitro iron-ascorbate induced lipid peroxidation

The results from *in vitro* LPO are presented on Figure 1 and Figure 2. In pure microsomes CGA and DCQA decreased MDA level by 19 % ($p < 0.05$) only at the highest concentration, compared to control microsomes. TCQA decreased MDA level in statistically significant and concentration dependent manner by 40 %, by 31% and by 24% respectively. The more pronounced effect of TCQA (100 μmol) against LPO in comparison with both DCQA and CGA was discerned by the decrease of the MDA level by 42% ($p < 0.05$).

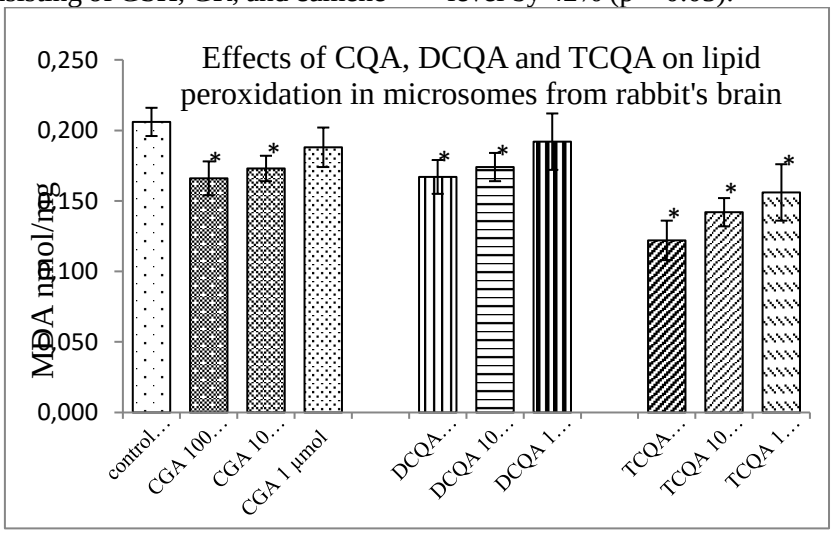

Figure 1. MDA quantity in microsomes incubated with the tested compounds.Data are presented as the mean ± SEM of 3 parallel samples. For comparison between groups, a Student's t-test test was used $^{a}p<0.05$ vs. control

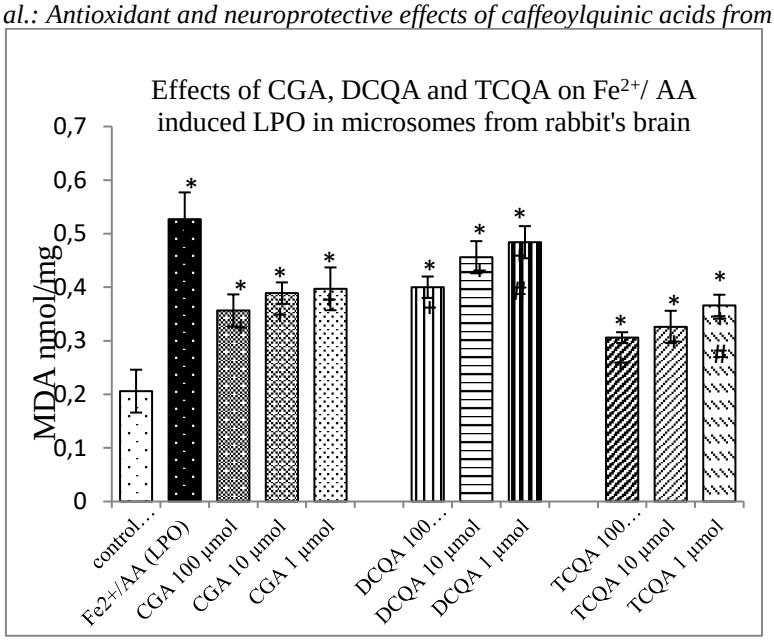

Figure 2. MDA quantity after induction of LPO. Data are presented as the mean ± SEM of 3 parallel samples. For comparison between groups, a Student's t-test test was used. $^{*}p<0.05$ vs. control, $^{+}p<0.05$ vs Fe^{2+}/ AA induced LPO, $^{\#}p<0.05$ vs concentration of 100 µmol

Changes in body weight and urine parameters

No death was observed in the experiment. Animal weight changes during the experiment are presented in Table 2. On the seventh day of the experiment, no differences in body weight were established. At the end of the experiment, the body weight gain of the animals treated with ethanol (10-14 days) was statistically significantly lower by 38% compared to the body weight gain in the control animals. In the groups treated initially with TCQA or CGA and then with ethanol, the final body weight is commensurable with the controls.

Table 1. Changes in the average body weight

Group	Average body weight (g)			
	Day 1	Day 7	Day 14	Change (day 1-14)
Control	205 ± 3	235 ± 5	270 ± 5	65
TCQA	190 ± 3	238 ± 4	268 ± 4	78
CGA	210 ± 4	242± 4	278 ± 4	68
EtOH	215 ± 3	245 ± 3	255 ± 3*	40*
TCQA + EtOH	205 ± 3	242 ± 6	272 ± 6^{+}	67^{+}
CGA + EtOH	212 ± 3	248 ± 4	268 ± 4^{+}	56^{+}

Data are presented as the mean ± SEM of six animals (n = 6). For comparison between groups, a Mann-Whitney *U* test was used. *p<0.05 vs. control, $^{+}p<0.05$ vs EtOH

Urine analysis

Changes in urine parameters: pH, proteins, bilirubin, urobilinogen, ketones are presented in Table 3. The five-day administration of ethanol leads to acidification of the urine (pH 4.5). Significant proteinuria, bilirubinuria, urobilinogenuria, and elevated levels of ketone bodies were observed. TCQA and CGA alone did not affect the urine parameters. When administered simultaneously with ethanol, they antagonized the effects of ethanol.

Table 2. Changes in urine parameters measured on day 14 of the experiment

Group	pH	Protein (mg/dL)	Bilirubin (mg/dL)	Urobilinogen (mg/dL)	Ketones (mg/dL)
Control	6.0	5.0 ± 0.5	0.4 ± 0.1	0.2 ± 0.1	3.0 ± 0.1
TCQA	6.0	4.0 ± 0.4	0.6 ± 0.2	0.3 ± 0.1	2.0 ± 0.1
CGA	6.5	5.0 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	3.0 ± 0.1
EtOH	4.5^{*}	$30.0 \pm 4.31^{*}$	$2.0 \pm 0.2^{*}$	$1.5 \pm 0.5^{*}$	$7.0 \pm 0.1^{*}$
TCQA + EtOH	6.0^{+}	$15.0 \pm 3.2^{+}$	$0.7 \pm 0.3^{+}$	$0.2 \pm 0.1^{+}$	$4.0 \pm 0.1^{+}$
CGA + EtOH	6.0^{+}	$14.0 \pm 4.3^{+}$	$0.8 \pm 0.3^{+}$	$0.3 \pm 0.1^{+}$	$\pm 0.1^{+}$

Data are presented as the mean ± SEM of six animals (n = 6). For comparison between groups, a Mann-Whitney *U* test was used. *p<0.05 vs. control, +p<0.05 vs EtOH

Changes in the levels of oxidative stress markers

The level of MDA and GSH, measured after 14 days administration of TCQA, alone and in combination with ethanol are shown in Table 4.

Alone, five-day administration of ethanol resulted in a statistically significant ($p < 0.05$) increased production of MDA by 48% and a reduced level of GSH by 81%. Application of TCQA alone has no effect on the tested parameters. It should be noted, however, that in the combined group, the nine-day pretreatment of the animals with TCQA substantially alleviated the effects of the subsequent five-day ethanol application. When compared to the pure ethanol group, MDA production was reduced by 32% ($p< 0, 05$) and the GSH level increased nearly fourfold. The effect of TCQA on these parameters is commensurable with that of chlorogenic acid (CGA) used as a positive control.

Table 3. Changes in MDA production and GSH levels in rat brain treated with TCQA, alone and in combination with ethanol

Group	MDA (nmol/g)	GSH (nmol/g)
Control	3.34 ± 0.17	1.65 ± 0.28
TCQA	3.29 ± 0.11	1.62 ± 0.25
CGA	3.41 ± 0.13	1.55 ± 0.26
EtOH	4.93 ± 0.18*	0.30 ± 0.09*
TCQA + EtOH	3.36 ± 0.39+	1.47 ± 0.16+
CGA + EtOH	3.47 ± 0.34+	1.42 ± 0.13+

Data are presented as the mean ± SEM of six animals (n = 6). For comparison between groups, a Mann-Whitney U test was used. *p<0.05 vs. control, +p<0.05 vs EtOH

Changes in the activity of the antioxidant enzymes

The activity of antioxidant enzymes: GPx, SOD and CAT, influenced by the administration of ethanol alone and in combination with TCQA and CGA, is presented in Table 5. The administration of ethanol alone leads to a statistically significant ($p < 0.05$) reduced activity of the enzymes as follows: GPx with 42%, SOD with 16% and CAT with 25%. Administration of TCQA alone did not affect the activity of the enzymes, whereas in the combined group, pre-treatment of animals with TCQA did not allow ethanol to exhibit its pro-oxidant effect

Table 4. Changes in the activity of antioxidant enzymes GPx, SOD and CAT after administration of the tested compounds and ethanol.

Group	GPx (μmol/mg/min)	SOD (nmol/mg/min)	CAT (nmol/mg/min)
Control	0.81 ± 0.07	25.25 ± 2.79	126.00 ± 6.81
TCQA	0.84 ± 0.05	25.33 ± 1.43	121.00 ± 4.29
CGA	0.77 ± 0.02	23.23 ± 0.83	123.17 ± 2.78
EtOH	0.47 ± 0.02*	21.07 ± 0.59*	94.33 ± 4.13*
TCQA + EtOH	0.69 ± 0.05*+	24.00 ± 0.16+	122.00 ±4.56+
CGA + EtOH	0.74 ± 0.04+	24.25 ± 0.57+	125.67 ± 1.63+

Data are presented as the mean ± SEM of six animals (n = 6). For comparison between groups, a Mann-Whitney U test was used. *p<0.05 vs. control, +p<0.05 vs EtOH

DISCUSSION

Oxidative stress is considered as one of the main mechanisms underlying a large number of human diseases such as neurological, endocrine and many others. The interest in biologically active substances from plants with antioxidant activity, has grown popularity as these are used both in the prevention and treatment of various diseases. Among the promising bioactive substances with antioxidant activity are phenolic acids, flavonoids, carotenoids, phenolic diterpenes and others. [28]

Acylquinic acids, also known as chlorogenic acids, are a group of esters that are formed between the hydroxycinnamic and quinic acids. They exist in the form of isomers in a large number of plants, especially in the Asteraceae family [29]. They are characterized by a variety of biological activities - they increase the accumulation of bile and stimulate the secretion of pancreatic enzymes; slow down aging; regulate lipid metabolism; exhibit anti-inflammatory and antioxidant activity [30].

Caffeoylquinic acid derivatives are found in Sudanese plant *Geigeria alata*. In our previous studies, the antioxidant effect of DCQA was evidenced by DPPH, ABTS and FRAP methods [12]. In addition, DCQA demonstrated antioxidant and antidiabetic effects in a model of streptozotocin-induced diabetes in Wistar rats [31].

This study aims to broaden research on caffeoylquinic acids isolated from *Geigeria alata* roots on the potential antioxidant and neuroprotective effects of TCQA in a model of alcohol-induced oxidative stress in a rat. The effect of TCQA is compared with the effect of CGA for

which neuroprotective activity has been demonstrated [32].

Various models of ethanol-induced brain toxicity in rodents exist in the scientific literature, one of the most cited being the result of a single oral administration of 50% ethanol [5,33]. Therefore, a brain neurotoxicity model was developed for the purpose of the study by a five-day oral administration of a 50% ethanol solution to male Wistar rats, resulting in a statistically significant ($p < 0.05$) increase in production of MDA by 48%, reduced GSH by 81% and decreased activity of the antioxidant enzymes GPx, SOD and CAT (Table 4 and Table 5). Our results correlate with the other investigations [5,33] and could explain the mechanism of damage caused by ethanol.

In a number of studies, it has been shown that increased production of free radicals as a result of acute alcohol consumption leads to decreased levels of endogenous antioxidants in both experimental animals and humans [33–35].

Here, we show for the first time that DCQA and TCQA, isolated from *G. alata*, revealed antioxidant effect, similar (DCQA) or more pronounced (TCQA) to that of the positive control CGA in the *in vitro* model of non-enzyme-induced LPO in isolated microsomes. TCQA had a discernible effect on the markers of oxidative stress MDA and GSH in the rat brain in a model of ethanol-induced injury. This is consistent with the previously published results on the beneficial effects of CGA. Thus, Wu et al. [36] reported CGA positive effect against nitroprusside-induced damage associated with excessive NO production in primary rat cerebellum cultures. In cortical slices of mouse brain, CGA reduces the production of MDA and free radicals after inducing oxidative stress with H_2O_2 [37] which support our results on the Fe/AA induced LPO. Our results are consistent with the earlier investigation where diabetic rat model showed that feeding with CGA effectively reduced lipid hydroperoxide production and increased the level of non-enzymatic antioxidants such as GSH and Vitamins C and E [38]. It has been shown that CGA prevents alcohol-induced brain damage in neonatal rats [39]. CGA attenuated the neuronal damage induced in alcohol exposed neonatal rats by decreasing the apoptosis of neuronal cells. Apoptosis was decreased based on its anti-inflammatory and antioxidant property. Moreover, CGA significantly alleviated the increased oxidative stress and concentration of inflammatory mediators in the brain tissues.

Koriem et al. [40] examined the antioxidant effect of CGA in rats with liver toxicity induced by methamphetamine. The effect was released by restoring liver SOD and GPx activities and preventing the accumulation of MDA. In keeping with aforementioned CGA effects, our results indicated that TCQA also exerted an antioxidant activity, discerned by normalizing the antioxidant enzymes activity of GPx, SOD and CAT to the control levels. In addition, TCQA administration was beneficial for attenuating urine parameters protein, bilirubin, urobilinogen and ketone bodies associated with the liver and kidney functions.

It is wort noting that compound lipophilicity is a key factor associated with the blood-brain barrier penetration. Due to the higher number of ester-bonded caffeoyl residues, TCQA has improved lipophilic properties compared to CGA and DCQA [41]. Accordingly, TCQA has more pronounced antioxidant capacity in lipophilic systems and acts as potential radical scavenging agent for protection against oxidative stress in the CNS.

CONCLUSION

Under the condition of this study we could conclude that TCQA, the main acylquinic acid in the *Geigeria alata* roots is more potent antioxidant than CQA and DQCA probably due to the higher number of caffeoyl residues. In ethanol-induced brain injury in Wistar rats TCQA showed pronounced antioxidant effect which protect neuronal cells from the deleterious impact of alcohol.

REFERENCES

1. B.K. Tiwari, K.B. Pandey, A.B. Abidi, S.I. Rizvi, *J. Biomarkers*. p.1–8, 2013
2. T. Farooqui, A.A. Farooqui, *Parkinsons. Dis.* p.247–467, 2011
3. L.M. Sayre, G. Perry, M.A. Smith, *Chem. Res. Toxicol.* **21**(1) p.172–88, 2008
4. C. Gemma, J. Vila, A. Bachstetter, P.C. Bickford, Brain Aging Model. Methods, Mech. *CRC Press/Taylor & Francis*, 2007
5. O.R. Oyenihi, B.A. Afolabi, A.B. Oyenihi, O.J. Ogunmokun, O.O. Oguntibeju, *Toxicol. Reports*. **3** p.288–94, 2016
6. J.M. Gohlke, W.C. Griffith, E.M. Faustman, *Birth Defects Res. Part B Dev. Reprod. Toxicol.* **83**(1) p.1–11, 2008
7. Y. Soh, J.-A. Kim, N.W. Sohn, K.R. Lee, S.Y. Kim, *Biol. Pharm. Bull.* **26**(6) p.803–7, 2003
8. J. Han, H. Isoda, *BMC Proc.* **5 Suppl 8**(Suppl 8) p.P20, 2011
9. W. Shen, R. Qi, J. Zhang, Z. Wang, H. Wang, C. Hu, Y. Zhao, M. Bie, Y. Wang, Y. Fu, M. Chen, D. Lu, *Brain Res. Bull.* **88**(5) p.487–94, 2012
10. J. Kim, S. Lee, J. Shim, H.W. Kim, J. Kim, Y.J. Jang, H. Yang, J. Park, S.H. Choi, J.H. Yoon, K.W. Lee, H.J. Lee, *Neurochem. Int.* **60**(5) p.466–74, 2012
11. G. Oboh, O.M. Agunloye, A.J. Akinyemi, A.O.

Ademiluyi, S.A. Adefegha, *Neurochem. Res.* **38**(2) p.413–9, 2013
12. D. Zheleva-Dimitrova, R. Gevrenova, M.M. Zaharieva, H. Najdenski, S. Ruseva, V. Lozanov, V. Balabanova, S. Yagi, G. Momekov, V. Mitev, *Phytochem. Anal.* **28**(3) p.176–84, 2017
13. G. El-Ghazali, Mahgoub S. El-Tohami, Awatif A. B. El-Egami, *National Center for Research, Medicinal & Aromatic Plants Research Institute, Khartoum, Sudan*, 1994
14. G.E.B. El-Ghazali, M.S. El-Tohami, A.A.B. El-Agami, *Khartoum Univ. Press*., 1997
15. H. Hashim EL-Kamali, M. Yagoub EL-amir, *Curr. Res. J. Biol. Sci.* **2**(2) p.143–6, 2010
16. R.M. Hafizur, R. Babiker, S. Yagi, S. Chishti, N. Kabir, M.I. Choudhary, *J. Endocrinol.* **214**(3) p.329–35, 2012
17. P. Voirol, M. Jonzier-Perey, F. Porchet, M.J. Reymond, R.C. Janzer, C. Bouras, H.W. Strobel, M. Kosel, C.B. Eap, P. Baumann, *Brain Res.* **855**(2) p.235–43, 2000
18. I.G. Morgan, L.S. Wolfe, P. Mandel, G. Gombos, *Biochim. Biophys. Acta - Biomembr.* **241**(3) p.737–51, 1971
19. O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, *J. Biol. Chem.* **193**(1) p.265–75, 1951
20. D. Mansuy, A. Sassi, P.M. Dansette, M. Plat, *Biochem. Biophys. Res. Commun.* **135**(3) p.1015–21, 1986
21. C. Deby, R. Goutier, *Biochem. Pharmacol.* **39**(3) p.399–405, 1990
22. J. Choi, J.-K. Park, K.-T. Lee, K.-K. Park, W.-B. Kim, J.-H. Lee, H.-J. Jung, H.-J. Park, *J. Med. Food*. **8**(3) p.348–52, 2005
23. N. Stefanello, R. Schmatz, L.B. Pereira, M.A. Rubin, J.B.T. da Rocha, G. Facco, M.E. Pereira, C.M. de A. Mazzanti, S. Passamonti, M.V. Rodrigues, F.B. Carvalho, M.M. da Rosa, J.M. Gutierres, A.M. Cardoso, V.M. Morsch, M.R.C. Schetinger, *Mol. Cell. Biochem.* **388**(1–2) p.277–86, 2014
24. E.A. Bump, Y.C. Taylor, J.M. Brown, *Cancer Res.* **43**(3) p.997–1002, 1983
25. A.L. Tappel, *Methods Enzymol.* **52** p.506–13, 1978
26. H. Aebi, In*Methods Enzym. Anal. Elsevier* p.673–84, 1974
27. H.P. Misra, I. Fridovich, *J. Biol. Chem.* **247**(10) p.3170–5, 1972
28. P. Pietta, P. Simonetti, P. Mauri, *J. Agric. Food Chem.* **46**(11) p.4487–90, 1998
29. A. Plazonić, F. Bucar, Ž. Maleš, A. Mornar, B. Nigović, N. Kujundžić, *Molecules*. **14**(7) p.2466–90, 2009
30. H. Morishita, M. Ohnishi, *Stud. Nat. Prod. Chem.* **25** p.919–53, 2001
31. V. Vitcheva, R. Simeonova, M. Kondeva-Burdina, D. Zh Zheleva-Dimitrova, V.I. Balabanova, S. Yagi, R.S. Gevrenova, Vol. 50, Bulg. Chem. Commun., 2018
32. S.-H. Kwon, H.-K. Lee, J.-A. Kim, S.-I. Hong, H.-C. Kim, T.-H. Jo, Y.-I. Park, C.-K. Lee, Y.-B. Kim, S.-Y. Lee, C.-G. Jang, *Eur. J. Pharmacol.* **649**(1–3) p.210–7, 2010
33. Y. Han, Q. Xu, J. Hu, X. Han, W. Li, L. Zhao, *Nutrients*. **7**(1) p.682–96, 2015
34. S.K. Reddy, K. Husain, E.C. Schlorff, R.B. Scott, S.M. Somani, *Neurotoxicology*. **20**(6) p.977–87, 1999
35. C. Pinto, J.J. Cestero, B. Rodríguez-Galdón, P. Macías, *Toxicol. reports*. **1** p.726–33, 2014
36. J. Wu, H. Chen, H. Li, Y. Tang, L. Yang, S. Cao, D. Qin, *Molecules*. **21**(3) p.260, 2016
37. Z. Gul, C. Demircan, D. Bagdas, R.L. Buyukuysal, *Neurochem. Res.* **41**(8) p.2075–85, 2016
38. K. Karthikesan, L. Pari, V.P. Menon, *J. Funct. Foods*. **2**(2) p.134–42, 2010
39. Z. Guo, J. Li, *Transl. Neurosci*. **8** p.176–81, 2017
40. K.M.M. Koriem, R.E. Soliman, *J. Toxicol.* **2014** p.583494, 2014
41. N. Razzaghi-Asl, J. Garrido, H. Khazraei, F. Borges, O. Firuzi, *Curr. Med. Chem.* **20**(36) p.4436–50, 2013