Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

The effect of lipoic acid on acrylamide-induced neuropathy in rats with reference to biochemical, hematological, and behavioral alterations

Mohamed A. Lebda, Shereen B. Gad & Rashed R. Rashed

To cite this article: Mohamed A. Lebda, Shereen B. Gad & Rashed R. Rashed (2015) The effect of lipoic acid on acrylamide-induced neuropathy in rats with reference to biochemical, hematological, and behavioral alterations, Pharmaceutical Biology, 53:8, 1207-1213, DOI: 10.3109/13880209.2014.970288

To link to this article: https://doi.org/10.3109/13880209.2014.970288

Pharmaceutical Biology

http://informahealthcare.com/phb
ISSN 1388-0209 print/ISSN 1744-5116 online
Editor-in-Chief: John M. Pezzuto
Pharm Biol, 2015; 53(8): 1207–1213
 DOI: 10.3109/13880209.2014.970288

informa
healthcare

ORIGINAL ARTICLE

The effect of lipoic acid on acrylamide-induced neuropathy in rats with reference to biochemical, hematological, and behavioral alterations

Mohamed A. Lebda[1], Shereen B. Gad[2], and Rashed R. Rashed[3]

[1]Department of Biochemistry, [2]Department of Physiology, and [3]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

Abstract

Context: Acrylamide (ACR) is a well-known neurotoxicant and carcinogenic agent which poses a greater risk for human and animal health.
Objective: The present study evaluates the beneficial effects of α-lipoic acid (LA) on ACR-induced neuropathy.
Materials and methods: A total of 40 male rats were divided into four groups: a placebo group; LA-treated group, administered orally 1% (w/w) LA mixed with diet; ACR-treated group, given 0.05% (w/v) ACR dissolved in drinking water; and LA + ACR-treated group, given LA 1% 7 d before and along with ACR 0.05% for 21 d. After 28 d, blood samples were collected, the rats were decapitated, and the tissues were excised for the measurement of brain biomarkers, antioxidant status, and hematological analysis. Also, the gait score of rats was evaluated.
Results: ACR-exposed rats exhibited abnormal gait deficits with significant ($p < 0.05$) decline in acetylcholine esterase (AChE) and creatine kinase in serum and brain tissues, respectively. However, the lactate dehydrogenase activity was increased in serum by 123%, although it decreased in brain tissues by −74%. ACR significantly ($p < 0.05$) increased the malondialdehyde level by 273% with subsequent depletion of glutathione *S*-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) activities and reduced the glutathione (GSH) level in brain tissue. Interestingly, LA significantly ($p < 0.05$) improved brain enzymatic biomarkers, attenuated lipid peroxidation (LPO), and increased antioxidant activities compared with the ACR-treated group.
Discussion and conclusion: These results suggested that LA may have a role in the management of ACR-induced oxidative stress in brain tissues through its antioxidant activity, attenuation of LPO, and improvement of brain biomarkers.

Keywords

Acetylcholinesterase, gait score, glutathione, malondialdehyde

History

Received 27 March 2014
Revised 22 September 2014
Accepted 23 September 2014
Published online 8 April 2015

Introduction

Acrylamide (ACR) is a water-soluble vinyl monomer primarily used for the production of polymers that have broad applications in various chemical industries, e.g., water and waste-water management, ore processing, and dye synthesis. It is used extensively in the molecular laboratories for gel chromatography (Friedman, 2003) and is present in certain foods prepared at very high temperatures (Tareke et al., 2000). Although the polymer is non-toxic, occupational exposure of humans and experimental exposure of laboratory animals with the monomeric form produced a neurotoxic syndrome characterized by ataxia, skeletal muscle weakness, and weight loss (LoPachin et al., 2004). ACR is a well-documented neurotoxicant in humans (Hagmar et al., 2001) and in laboratory animals (LoPachin et al., 2003) that produces central and peripheral distal axonopathies (Odland et al., 1994). Peripheral neuropathy and hemoglobin adduct formation had been noticed in humans who are occupationally exposed to ACR and in experimental animals (Konings et al., 2003).

Administration of ACR forms adduct with the reduced glutathione (GSH) and increases the production of the hydrogen peroxide, leading to increased levels of the lipid peroxidation (LPO) and the carbonyl content, and reduced enzymatic and non-enzymatic antioxidants with a decrease in the acetylcholine esterase (AChE) activity in the brain tissue (Allam et al., 2011). The imbalance between the production and the removal of free radicals increased the oxidative stress and was related to the neurodegenerative diseases (Gopinath et al., 2011).

Extensive studies in rodents and other laboratory animals provided evidence that exposure to ACR causes genotoxicity (Friedman et al., 1995), cellular damage in both nervous and reproductive systems (Tyl et al., 2000), and produces tumors in certain hormonally responsive tissues (Hamdy et al., 2011). ACR neurotoxicity had been known to affect the nerve terminal and ACR could adduct the cysteine residues in

Correspondence: Mohamed A. Lebda, Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt. Tel: +20 10 08479197. Fax: +20 45 2960450. E-mail: biochemistry232@yahoo.com

functionally important presynaptic proteins, resulting in neurotransmitter release inhibition and eventual process degeneration (Lopachin et al., 2006).

Lipoic acid (LA) is a naturally occurring compound synthesized in small quantities by most plants and animals. In humans, it is synthesized from octanoic acid in the mitochondria (Jordan & Cronan, 1997). LA functions as an important cofactor for several important enzymes like pyruvate and α-ketoglutarate dehydrogenase complexes; it acts as a cofactor that bridges glycolysis and Kreb's cycle and it is able to cross the blood–brain barrier and acts as a redox couple with very low reduction potentials (Lodge et al., 1997). Due to these properties, LA is capable of regenerating other important antioxidants such as glutathione, vitamin C, and vitamin E (May et al., 1998). LA is frequently referred as a universal antioxidant due to its action in both the membrane and aqueous phases, and it has specificity for free radical quenching and metal-chelating apart from regeneration of other cellular antioxidants (Perera et al., 2011).

LA plays a principle role in antioxidant defense of the brain and has neuroprotective effects in experimental brain injury caused by trauma and subarachnoid hemorrhage (Ersahin et al., 2010; Toklu et al., 2009). Some reports support this speculation that LA may have a neuroprotective role by reducing apoptosis in both caspase-dependent and caspase-independent manner (Santos et al., 2011). Additionally, it could reduce spinal cord injury induced by oxidative stress and exerts neuroprotection by inhibiting the LPO, GSH depletion, and DNA fragmentation (Toklu et al., 2010).

A number of studies have found LA to be useful in the treatment of nerve problems in diabetics and that it can improve insulin sensitivity in people with Type II diabetes (Singh & Jialal, 2008). Other studies have suggested that it might be useful in ameliorating certain pathophysiologies of many chronic diseases (Smith et al., 2004). Laboratory and animal studies have also suggested that LA (50–100 mg/kg) may be helpful in treating cerebral ischemia (Connell et al., 2011), cataract formation (Davis et al., 2010), some nervous system diseases such as Alzheimer disease (Holmquist et al., 2007), radiation injury (Manda et al., 2007), and cisplatin-induced nephrotoxicity (Somani et al., 2000). Pretreatment with LA protects the structural integrity of the erythrocyte cell membrane components as a result of oxidative damage due to gamma radiation (Desouky et al., 2011).

Accordingly, this study was designed to determine the possible protective effect of LA against ACR-induced oxidative stress in rat brain by determining some biochemical and hematological parameters, and behavioral examination.

Materials and methods

Chemicals and reagents

ACR, α-LA, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma-Aldrich Co. (St. Louis, MO). GSH, thiobarbituric acid (TBA), and butylated hydroxytoluene (BHT) were purchased from Fluka (Buchs, Switzerland). All the other reagents were of analytical, high-performance liquid chromatography (HPLC), or the best available pharmaceutical grade.

Animals and experimental design

Forty adult male Sprague–Dawley rats, weighing 170 ± 10 g and aged 7–8 weeks, were purchased from the Medical Research Institute, Alexandria University, Egypt. All animals were housed in gang cages maintained in a room with controlled environmental conditions and a 12 h light–dark cycle. The animal experiments were carried out in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the local authorities (Faculty of Science, Alexandria University, Egypt). All efforts were made to minimize the number of animals used and their suffering. After 2 weeks of acclimatization, all animals were randomly divided into four experimental groups of 10 rats in each group. Rats in the control or vehicles-received group were orally given normal experimental diet and tap water; those in the ACR-treated group were orally given ACR at a daily dosage of 0.05% in drinking water for 21 d; rats in the α-LA group were given α-LA at dosage of 1% mixed with rat diet for 28 d; and those in the α-LA + ACR-treated group were given α-LA 7 d before and along with ACR treatment (the dose was same as that in the α-LA and ACR-treated groups).

Twenty-four hours after the last administration of α-LA or ACR, the rats were anesthetized with ketamine/xylazine (7.5:10 mg/kg, 1 mg/kg i.p). Then, blood samples were collected from the inner canthus of the eye by the heparinized capillary tube and divided into two types of 2-ml clean test tubes. One contains heparin as an anticoagulant for hematological analysis and the other without an anticoagulant. Following standing at room temperature for at least 30 min, the blood was centrifuged at $3400 \times g$ for 10 min; at that point the serum was separated, transferred to Eppendorf tubes, and stored at $-20\,^{\circ}C$ prior to measure the activities of brain enzymes, and to determine other biochemical parameters. Immediately after the collection of blood samples, the animals were euthanized, and their brains were quickly excised, rinsed in ice-cold saline, and used immediately or stored frozen at $-80\,^{\circ}C$ until analysis.

The behavioral index (gait scores) examination

The gait scores were examined weekly according to the methods described by LoPachin (2005). Rats were placed in a clear Plexiglas box and observed for 3 min. Following observation, a gait score was assigned from 1 to 4, where 1 = a normal, unaffected gait, 2 = a slightly affected gait (foot splays, slight hind limb weakness, and spread), 3 = a moderately affected gait (foot splay, moderate hind limb weakness, and moderate limb spread during ambulation), and 4 = a severely affected gait (foot splay, severe hind limb weakness, dragging hind limbs, and inability to rear).

Estimation of blood parameters

Hemoglobin (Hb) concentration, red blood cell (RBC) count, packed cell volume (PCV), white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) values were estimated using a semiautomatic hematology analyzer (SWELAB IEO Model, Sony Corp, Tokyo, Japan). The auto-counter utilized 20 µl of blood in 16 ml of commercially prepared diluents. The machine's ability to count cells was based on the principle of electronic impedance.

Biochemical blood serum analysis

Using commercially available diagnostic kits (Vitro Scient Co., Hannover, Germany), the activities of the serum and brain creatine kinase (CK; EC: 2.7.3.2), lactate dehydrogenase (LDH; EC: 1.1.1.27), and acetylcholinesterase (AChE; EC: 3.1.1.7), were spectrophotometrically determined following the manufacturer's instructions.

Indices of antioxidant and oxidative stress in brain tissues

Tissue homogenates were separately prepared from frozen brain samples in 10 volumes of 0.1 M Tris-EDTA buffer (pH 7.4) and centrifuged at $8000 \times g$ for 30 min at 4 °C. Aliquots of the supernatant were utilized for the spectrophotometrical assessment of the levels of the following: LPO, as the production of the TBA reactive substances (TBARS) in the presence of BHT (Beuge & Aust, 1978); GSH, by using Ellman's reagent (Sedlak & Lindsay, 1968); glutathione *S*-transferase (GST, EC 2.5.1.18) activity, as the rate of GSH conjugation of CDNB (Habig et al., 1974); the glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined using GSH and cummene hydroperxide as a substrate by the modified method of Paglia and Valentine (1967), and the glutathione reductase (GR, EC 1.8.1.7) activity was measured according to the method of Horn (1965).

Statistical analysis

Data were analyzed using the SPSS package (SPSS Inc., Chicago, IL). Results are expressed as mean ± SE with the experiment repeated at least three times. Statistical evaluations were done using one-way analysis of variance (ANOVA). A p value of < 0.05 was considered significant.

Results

Body weight and neurological evaluation

Rats exposed to ACR developed changes in body weight and classic signs of ACR behavioral neurotoxicity. Control rats had a starting mean body weight of 169 ± 18 g, which increased steadily to 236 ± 28 g at the endpoint (28 d). This represents a 40% increase in the body weight during the experimental period. Rats in the ACR-treated groups had a similar starting weight (161 ± 14 g), but gained only 11% of their original weight, i.e., at the 28-d endpoint (179 ± 16 g). Rats treated with LA had gained a 40% increase in the body weight as control rats. ACR/LA-treated rats had improved body gain of 16% when compared with ACR-treated rats (Table 1). Exposure of rats to ACR caused progressive development of gait abnormalities, an unsteady walking pattern with abduction and external rotation of hind limbs, and the rats dragged their feet as they walked. Finally, the weakness of the most severely ACR-exposed rats included the forelimbs (the rat's gait score ranged from grades 2 to 4). Treatment with LA improved the gait score of rats (Table 2).

Table 1. Effect of acrylamide and pretreatment with lipoic acid on the body weight (g) of exposed rats.

	1 week	2nd week	3rd week	4th week
I	169.2 ± 8.6[a]	186.5 ± 8.7[a]	209.8 ± 9.2[a]	236.5 ± 8.9[a]
II	161.4 ± 4.8[a]	166.5 ± 5.2[c]	170.3 ± 6.4[c]	179.6 ± 6.5[c]
III	164.3 ± 8.9[a]	176.4 ± 9.3[b]	181.5 ± 4.7[b]	191.6 ± 7.7[b]
IV	164.1 ± 6.4[a]	188.2 ± 5.3[a]	207.6 ± 7.4[a]	228.1 ± 7.2[a]

Values are expressed as mean ± SE.
The values with different superscript letters within the same column significantly differ at $p < 0.05$. I, control group; II, acrylamide-treated group; III, acrylamide + lipoic acid-treated group; IV, lipoic acid-treated group.

Table 2. Time course of change in gait scores for acrylamide-treated rats with and without lipoic acid.

	Number of rats with each grade (grade 1/grade 2/grade 3/grade 4)[a]			
	1st week	2nd week	3rd week	4th week
I	5/0/0/0	5/0/0/0	5/0/0/0	5/0/0/0
II	4/1/0/0	0/4/1/0	0/2/1/2	0/0/1/4
III	5/0/0/0	0/4/1/0	0/4/1/0	0/0/3/2
IV	5/0/0/0	5/0/0/0	5/0/0/0	5/0/0/0

The number of animals: five per each group.
[a]The degree of abnormalities: grade 1, normal gait; grade 2, slightly abnormal gait with slight degree of ataxia and foot splay; grade 3, moderately abnormal gait with moderate degree of ataxia, foot splay, and limb abduction; grade 4, severely affected gait with hind limb paralysis including inability to support the body weight as well as foot splay. I, control group; II, acrylamide-treated group; III, acrylamide + lipoic acid-treated group; IV, lipoic acid-treated group.

Hematological findings

The data presented in Table 3 reveals that ACR administration caused a significant ($p < 0.05$) reduction in RBC and WBC counts, hemoglobin level, and PCV value without any changes in blood indices (MCV, MCH, and MCHC) when compared with the control group (Table 3). LA treatment insignificantly increased RBC count, WBC count, hemoglobin (Hb) g%, and PCV value without changes in blood indices when compared with the control group. Administration of LA together with ACR significantly ($p < 0.05$) increased RBC count, WBC count, Hb g%, and PCV value when compared with the ACR-treated group (Table 3).

Enzymatic biomarkers of brain functions

As compared with control rats, ACR-treated rats exhibited a significant decrease in both serum and brain AChE activities, increase in the serum LDH activity while decrease in its activity in the brain tissue was observed; however, a non-significant decrease in serum CK activity and a significant decrease in brain CK activity were observed ($p < 0.05$; Table 4). Administration of α-LA alone did not alter serum and brain AChE and CK activities, while decreasing serum LDH activity that was increased in the brain tissue. α-LA-

Table 3. Effect of acrylamide and pretreatment with lipoic acid on hematological parameters of rats.

	RBCs (10^6/μl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)	WBCs (10^3/μl)
I	7.18 ± 0.21^{b}	13.74 ± 0.37^{a}	41.61 ± 0.75^{a}	59.64 ± 0.21^{a}	19.15 ± 0.34^{ab}	33.03 ± 0.73^{ab}	8.56 ± 0.39^{bc}
II	3.75 ± 0.21^{d}	7.78 ± 0.55^{c}	20.81 ± 1.02^{c}	55.61 ± 1.83^{ab}	20.84 ± 1.43^{a}	35.98 ± 1.45^{a}	5.36 ± 0.62^{c}
III	5.63 ± 0.21^{c}	11.12 ± 0.29^{b}	33.41 ± 0.75^{b}	58.33 ± 0.93^{ab}	19.78 ± 0.44^{ab}	33.32 ± 0.81^{ab}	7.52 ± 0.31^{b}
IV	7.94 ± 0.21^{a}	13.88 ± 0.34^{a}	43.21 ± 0.81^{a}	54.55 ± 1.49^{b}	17.52 ± 0.51^{b}	30.02 ± 2.46^{b}	9.48 ± 0.31^{a}

Values are expressed as mean ± SE.
The values with different superscript letters within the same row significantly differ at $p < 0.05$. I, control group; II, acrylamide-treated group; III, acrylamide + lipoic acid-treated group; IV, lipoic acid-treated group.

Table 4. Effect of acrylamide and pretreatment with lipoic acid on enzymatic biomarkers of brain functions in serum and brain tissues of rats.

	Control	Acrylamide	Acrylamide + lipoic acid	Lipoic acid
Serum				
CK (U/l)	316.21 ± 26.12^{a}	227.31 ± 20.69^{a}	258.71 ± 37.97^{a}	326.42 ± 36.11^{a}
LDH (U/l)	550.89 ± 14.64^{b}	681.03 ± 19.08^{a}	554.41 ± 11.61^{b}	442.81 ± 13.13^{c}
AChE (U/l)	158.68 ± 3.32^{a}	124.82 ± 2.72^{c}	145.42 ± 2.31^{b}	160.95 ± 2.41^{a}
Brain				
CK (U/g tissue)	16.34 ± 1.57^{a}	1.49 ± 0.16^{c}	8.01 ± 1.13^{b}	16.74 ± 1.55^{a}
LDH (U/g tissue)	245.95 ± 10.78^{b}	64.91 ± 2.55^{c}	$247.57 \pm 1\ 1.51^{b}$	666.31 ± 30.11^{a}
AChE (U/g tissue)	216.61 ± 4.78^{a}	106.04 ± 6.71^{c}	147.09 ± 9.29^{b}	231.54 ± 4.52^{a}

Values are expressed as mean ± SE.
The values with different superscript letters within the same row significantly differ at $p < 0.05$.

Table 5. Effect of acrylamide and pretreatment with lipoic acid on oxidative stress and antioxidant status of rats.

	Control	Acrylamide	Acrylamide + lipoic acid	Lipoic acid
MDA (nmol/g tissue)	90.27 ± 3.05^{c}	246.62 ± 4.37^{a}	185.91 ± 6.04^{b}	78.93 ± 7.11^{c}
GSH (μmol/g tissue)	37.74 ± 3.06^{b}	13.52 ± 1.56^{d}	23.91 ± 1.43^{c}	46.35 ± 2.21^{a}
GST (U/g tissue)	355.34 ± 12.39^{b}	187.56 ± 4.17^{d}	261.08 ± 6.91^{c}	422.06 ± 12.03^{a}
GPX (U/g tissue)	125.29 ± 5.11^{b}	72.17 ± 3.34^{c}	81.41 ± 17.56^{c}	157.88 ± 2.53^{a}
GR (U/g tissue)	51.91 ± 2.13^{a}	16.71 ± 2.67^{c}	27.81 ± 2.56^{b}	55.47 ± 2.61^{a}

Values are expressed as mean ± SE.
The values with different superscript letters within the same row significantly differ at $p < 0.05$.

pretreated ACR-challenged rats exhibited an improvement in these biochemical parameters, which were near the range observed in the control group.

LPO and antioxidant indices finding

ACR-treated rats showed a significant increase in the level of brain MDA with a concurrent decrease in the activities of antioxidant enzymes; GST, GPX, and GR and the level of GSH in brain tissue when compared with control ones ($p < 0.05$; Table 5). Administration of LA significantly decreased the MDA level and increased GST, GPX, and GR enzymatic activities and the GSH level. Pretreatment of LA in ACR-challenged rats significantly decreased the MDA level and increased the activities of antioxidant enzymes and the GSH level near the controls.

Discussion

The present study aims to clarify the effect of ACR on some hematological and biochemical parameters, as well as neurological behavior. In a well-described rodent model, ACR exposure at 15–50 mg/kg/d produced several neurological deficits, including hind-limb foot splay, decreased fore- and hind-limb grip strength, ataxia, and skeletal muscle weakness (LoPachin et al., 2002). Moreover, ACR exposure resulted in the central–peripheral neuropathy in humans (LoPachin, 2004) and in laboratory animals, including rats and monkeys (Seale et al., 2012).

Shukla et al. (2002) found hind limb paralysis in 58% of the rats treated with ACR for 10 d, which attributed to ACR neurotoxicity. El-Yamany and Bayomy (2007) concluded that the abnormal body posture, muscle weakness, and legs play accompanying ACR-intoxication in rats may be related to the degeneration of the brain monoaminergic system. Also, Rawi et al. (2012) indicated that the administration of ACR induced alterations in the whole brain monoamines concentration and histopathological changes varying from focal gliosis in the cerebral cortex and the cerebrum, focal hemorrhage in the meninges, and vacuolization in the cerebral cortex, cerebrum, cerebellum, and medulla oblongata.

Treatment with LA significantly improved the neurological deficits; this result was in accordance with Bhadri et al. (2013) who reported that LA significantly alleviated the cisplatin-induced peripheral neuropathy in rats, which is evident by improved body weight, decreased thermal hyperalgesia, improved motor coordination, increased grip strength, improved nerve conduction velocity, and higher antioxidant enzymatic activities compared with cisplatin-treated rats.

Body weight is frequently the most sensitive indicator of adverse effects of toxicants. The present study revealed a decrease in the body weight following ACR administration, which may be attributed to the direct effect of ACR on growth (Wang et al., 2005), excessive breakdown of the tissue proteins (Chatterjea & Shinde, 2002), or decreased both plasma and tissue proteins (Yousef & El-Demerdash, 2006). In the current study, ACR affects the hematological parameters, ACR is not only neurotoxic and carcinogenic but also damages the erythrocyte membrane and generates the micronucleated erythrocytes, as well as alters the blood viscosity parameters (Arihan et al., 2011).

Tarskikh (2006) observed some changes in physiochemical characteristics of biological membranes, decrease in erythrocyte acid resistance and activation of LPO at the early stage after ACR administration accompanied by a decrease in erythrocyte count. The present study revealed that Hb decreased significantly after oral administration of ACR as reported by Barber et al. (2001) in which the ACR covalently binds to the cysteine residues and forms adducts with sulfhydryl groups on hemoglobin, resulting in the loss of heme part of hemoglobin molecules, thereby reducing the amount of Hb in the blood, which in turn may also be responsible for the anemic conditions as evident by the low level of RBCs observed in the present investigation. Similarly, some previous investigators indicated that the PCV value of rats was found to be decreased due to ACR treatment (Sharma & Jain, 2008).

The present study also showed a significant reduction in the total WBC count following ACR treatment. This decrease in the total WBC count suggests that ACR may be immunosuppressive, which could be due to their diminished production, redistribution of peripheral blood into the tissues, or rapid destruction of WBC (Debaun, 2005). The protective effect of LA on hematological parameters may be attributed to its antioxidant activity.

Our data showed that CK, AChE, and LDH enzymatic activities were significantly decreased in the serum and brain of ACR-treated rats, with exception for LDH that was increased in the serum. These results were in agreement with Lakshmi et al. (2012) who reported that the activity of AChE was decreased in the ACR-induced rats. Free radical formation by ACR may upset the prooxidant/antioxidant balance within the brain, which could be one of the reasons for the decrease in the AChE activity (Tsakiris et al., 2000). Furthermore, Yousef and El-Demerdash (2006) mentioned that ACR at different doses caused a significant decrease in the activities of AChE and CK with a non-significant change in the LDH activity in both serum and brain tissue of rats. Also, the inhibition of AChE was explained by LoPachin et al. (2004), who suggested that ACR induced synaptic dysfunction, which involved in the adduction of presynaptic protein thiol groups and subsequent reduction in neurotransmitter release. Moreover, Barber and LoPachin (2004) reported that the neurological defects associated with ACR exposure are mediated by impaired neurotransmission at the central and peripheral synapses.

Creatine kinase (adenosine triphosphate: creatine, *N*-phosphoryl transferase) catalyzes the reversible transfer of the phosphoryl group from magnesium adenosine triphosphate to creatine, leading to phospho-creatine and adenine triphosphate, so it plays an important role in the cellular energy metabolism in vertebrates (Saksa et al., 1996). Matosoka et al. (1996) reported that CK activity in the brain and the blood seems to be the most sensitive indicators of ACR exposure. Meng et al. (2001) found that ACR at a concentration range of 0–1 M markedly inhibited CK and depleted the protein thiols in which thiol alkylation is a critical event in the inactivation of CK induced by ACR. Furthermore, ACR binds to CK and changing its surface charge may have the same effect as the toxicity of ACR towards other proteins.

LA treatment significantly increased the activities of brain biomarker enzymes depleted by ACR. This result was in harmony with de Souza et al. (2010) who showed that administration of LA alone did not alter the AChE activity in rat's hippocampus which was killed an hour after Pilocarpine administration. However, a single injection of LA, 30 min before administration of Pilocarpine, produced an increased AChE activity. The authors attributed the increment in AChE to the compensatory mechanism of long-term administration with LA and the up-regulation of AChE activity.

The level of MDA in the brain was significantly increased in the ACR-treated rats. In contrast, the brain antioxidant enzymes; GST, GPx, and GR activities and GSH content significantly decreased in the ACR-treated rats when compared with the control group (Table 4). These results are inconsistent with those of Lakshmi et al. (2012) who reported that the ACR-intoxicated rats exhibited an increase in the LPO, protein carbonyl, hydroxyl radical, and hydroperoxide levels with subsequent decrease in the activities of enzymatic antioxidants, and the level of GSH. ACR promotes the generation of reactive oxygen species (ROS) and the depletion of antioxidants, leading to neurodegeneration (Zhu et al., 2008). Decreased level of GSH increases the LPO and cellular degradation in ACR toxicity (Fennell & Friedman, 2005).

Enhancement of LPO is a consequence of depletion of GSH to certain critical levels. ACR is oxidized to glycidamide, a reactive epoxide, and undergoes conjugation with GSH. DNA adducts from glycidamide had been reported following the administration of ACR (Yousef & El-Demerdash, 2006). In the present study, decreased GSH content and increased LPO in the brain can be explained by the reaction of ACR with GSH, which in turn causes the depletion of GSH and the enhancement of LPO (Alturfan et al., 2012). Free radical scavenging enzymes such as GST, GPX, and GR protect the biological systems from oxidative stress. The current study showed a significant decrease in these enzymatic activities in rats treated with ACR, which may be related to the impairment of GSH metabolism and increased oxidative damage (Pradeep et al., 2007). Another possible explanation might be that, with less GSH available to conjugate lipid peroxidative products, the latter becomes elevated to the point that it provides negative feedback on antioxidant enzymes (Pigeolet et al., 1990).

Supplementation of antioxidant LA alone or before the administration of ACR significantly decreased the LPO with subsequent increase in the antioxidant enzymes. These results were confirmed previously by the results of Pirlich et al.

(2002) who reported that the free radical scavenging properties of LA were effective in ameliorating the neurodegenerative disorders induced by ethanol. Also, El-Halwagy and Hassanin (2006) found a marked significant reduction in the MDA level in almost all profenofos-treated tissues after supplementation of LA that reflected the role of LA to overcome the oxidative stress induced by profenofos insecticide. Additionally, administration of LA has considerable effects on the tissue thiol status, raising the glutathione level by reducing the extracellular cystine to cysteine, which bypasses the cystine transporter (Han, 1997). In addition, Shila et al. (2005) concluded that LA acting as an alternative sulfhydryl nucleophile to GSH prevents its oxidation to GSH disulfide (GSSG) in detoxifying reaction against ROS and consequently increases the activity of GSH-related enzymes. Moreover, Arivazhagan et al. (2002) reported that LA-administered aged rats showed a duration-dependent reduction in the level of LPO and elevation in the activities of the antioxidant enzymes. Kütter et al. (2013) recorded that the intraperitoneal injection of LA in fish increased GST activity with decreased LPO in the brain tissue. Also, Ozturk et al. (2014) mentioned that LA treatment prevented the increase in the MDA level and the protein carbonyl content in the brain tissue of the rats exposed to ifosfamide toxicity.

In a more recent study, α-LA attenuated the oxidative stress and augmented the antioxidant activity in the 3-nitropropionic acid-induced experimental model of Huntington's disease (Mehrotra & Sandhir, 2014). Furthermore, Moraes et al. (2014) reported that LA could prevent the reduction in GPx, GR activities, and the reduced GSH level without any changes in the GST activity in hyerphenylalaninemic rats.

Conclusion

α-LA is a powerful and unique antioxidant in the brain, preventing the LPO, augmenting the enzymatic and the non-enzymatic antioxidant, improving the hematological parameters and correcting the neurological deficits, thus restoring the neurological activity after brain damage induced by ACR exposure.

Declaration of interest

The authors report that there are no declarations of interest.

References

Alturfan EI, Beceren A, Şehirli AO, et al. (2012). Protective effect of *N*-acetyl-L-cysteine against acrylamide-induced oxidative stress in rats. *Turk J Vet Anim Sci* 36:438–45.

Allam A, El-Ghareeb AA, Abdul-Hamid M, et al. (2011). Prenatal and perinatal acrylamide disrupts the development of cerebellum in rat: Biochemical and morphological studies. *Toxicol Ind Health* 27: 291–306.

Arihan O, Seringec NB, Gurel EI, Dikmenoglu NH. (2011). Effects of oral acrylamide intake on blood viscosity parameters in rats. *Clin Hemorheol Microcirc* 47:45–52.

Arivazhagan P, Shila S, Kumaran S, Panneerselvam C. (2002). Effect of DL-alpha-lipoic acid on the status of lipid peroxidation and antioxidant enzymes in various brain regions of aged rats. *Exp Gerontol* 37:803–11.

Barber DS, LoPachin RM. (2004). Proteomic analysis of acrylamide protein adduct formation in rat brain synaptosomes. *Toxicol Appl Pharmacol* 201:120–36.

Barber DS, Hunt JR, Ehrich MF, et al. (2001). Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. *Neurotoxicology* 22: 341–53.

Beuge JA, Aust SD. (1978). Microsomal lipid peroxidation. *Methods Enzymol* 52:302–11.

Bhadri N, Sanji T, Madakasira-Guggilla H, Razdan R. (2013). Amelioration of behavioural, biochemical, and neurophysiological deficits by combination of monosodium glutamate with resveratrol/alpha-lipoic acid/coenzyme Q10 in rat model of cisplatin-induced peripheral neuropathy. Sci World J 2013:565813.

Connell BJ, Saleh M, Khan BV, Saleh TM. (2011). Lipoic acid protects against reperfusion injury in the early stages of cerebral ischemia. *Brain Res* 1375:128–36.

Chatterjea MN, Shinde R. (2002). *Text Book of Medical Biochemistry.* 5th ed. New Delhi: Jaypee Brothers, Medical Publishers Ltd.

Davis JG, Wan XS, Ware JH, Kennedy AR. (2010). Dietary supplements reduce the cataractogenic potential of proton and HZE-particle radiation in mice. *Radiat Res* 1733:353–61.

Debaun MR. (2005). Hydroxyurea as secondary prevention for stroke in children with sickle cell anaemia. *J Pediatr* 147:560–1.

de Souza GF, Saldanha GB, de Freitas RM. (2010). Lipoic acid increases glutathione peroxidase, Na^+, k^+-ATPase and acetylcholinesterase activities in rat hippocampus after pilocarpine-induced seizures? *Arq Neuropsiquiatr* 68:586–91.

Desouky OS, Selim NS, Elbakrawy EM, Rezk RA. (2011). Impact evaluation of alpha-lipoic acid in gamma-irradiated erythrocytes. *Radiat Phys Chem* 803:446–52.

El-Halwagy M, Hassanin L. (2006). Alpha lipoic acid ameliorate changes occur in neurotransmitters and antioxidant enzymes that influenced by profenofos insecticide. *J Egypt Soc Toxicol* 34:55–62.

EL-Yamany NA, Bayomy AA. (2007). Vitamin E alleviates the disturbances in monoamine levels in different brain regions of acrylamide-intoxicated rats. *Egypt J Zool* 48:93–111.

Ersahin M, Toklu HZ, Cetinel S, et al. (2010). Alpha lipoic acid alleviates oxidative stress and preserves blood brain permeability in rats with subarachnoid hemorrhage. *Neurochem Res* 35: 418–28.

Fennell TR, Friedman MA. (2005). Comparison of acrylamide metabolism in humans and rodents. *Adv Exp Med Biol* 561:109–16.

Friedman M. (2003). Chemistry, biochemistry, and safety of acrylamide: A review. *J Agric Food Chem* 51:4504–26.

Friedman MA, Dulak LH, Stedham MA. (1995). A lifetime oncogenicity study in rats with acrylamide. *Fundam Appl Toxicol* 27: 95–105.

Gopinath K, Prakash D, Sudhandiran G. (2011). Neuroprotective effect of naringin, a dietary flavonoid against 3-nitropropionic acid-induced neuronal apoptosis. *Neurochem Int* 59:1066–73.

Habig WH, Pabst MJ, Jakoby WB. (1974). Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249:7130–9.

Hagmar L, Tornqvist M, Nordander C, et al. (2001). Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. *Scand J Work Environ Health* 27: 219–26.

Hamdy SM, Bakeer HM, Eskande EF, Sayed ON. (2011). Effect of acrylamide on some hormones and endocrine tissues in male rats. *Hum Exp Toxicol* 5:1–9.

Han D, Handelman G, Marcocci L, et al. (1997). Lipoic acid increases *de novo* synthesis of cellular glutathione by improving cysteine utilization. *Biofactors* 6:321–38.

Holmquist L, Stuchbury G, Berbaum K, et al. (2007). Lipoic acid as a novel treatment for Alzheimer's disease and related dementias. *Pharmacol Ther* 1131:154–64.

Horn D. (1965). Glutathione reductase. In: Bergmayer H, ed. *Methods in Enzymatic Analysis*. New York: Academic Press, 875–9.

Jordan SW, Cronan JE. (1997). A new metabolic link. The acyl carrier protein of lipid synthesis donates lipoic acid to pyruvate dehydrogenase complex in *Escherichia coli* and mitochondria. *J Biol Chem* 272:17903–6.

Konings EJM, Baarsb AJ, van Klaveren JD, et al. (2003). Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem Toxicol* 41:1569–79.

Kütter MT, Monserrat JM, Santos RA, Tesser MB. (2013). Dose-response effects of the antioxidant α-lipoic acid in the liver and brain

of pompano *Trachinotus marginatus* (Pisces, Carangidae). *J Appl Ichthyol* 29:1123–8.
Lakshmi D, Gopinath K, Jayanthy G, et al. (2012). Ameliorating effect of fish oil on acrylamide induced oxidative stress and neuronal apoptosis in cerebral cortex. *Neurochem Res* 37:1859–67.
Lodge JK, Youn HD, Handelman GJ, et al. (1997). Natural sources of lipoic acid: Determination of lipoyllysine released from protease-digested tissues by high performance liquid chromatography incorporating electrochemical detection. *J Appl Nutr* 49:3–11.
LoPachin RM. (2004). The changing view of acrylamide neurotoxicity. *NeuroToxicology* 25:617–30.
LoPachin RM. (2005). Acrylamide neurotoxicity: Neurological, morhological and molecular endpoints in animal models. *Adv Exp Med Biol* 561:21–37.
LoPachin RM, Balaban CD, Ross JF. (2003). Acrylamide axonopathy revisited. *Toxicol Appl Pharmacol* 188:135–53.
LoPachin RM, Barber DS, He D, Das S. (2006). Acrylamide inhibits dopamine uptake in rat striatal synaptic vesicles. *Toxicol Sci* 89: 224–34.
LoPachin RM, Ross JF, Lehning EJ. (2002). Nerve terminals as the primary site of acrylamide action: A hypothesis. *NeuroToxicology* 23: 43–59.
LoPachin RM, Schwarcz AI, Gaughan CL, et al. (2004). *In vivo* and *in vitro* effects of acrylamide on synaptosomal neurotransmitter uptake and release. *NeuroToxicology* 25:349–63.
Manda K, Ueno M, Moritake T, Anzai K. (2007). Radiation-induced cognitive dysfunction and cerebellar oxidative stress in mice: Protective effect of alpha-lipoic acid. *Behav Brain Res* 177:7–14.
Matosoka M, Matosumura H, Igisu H. (1996). Creatinine kinase activities in brain and blood: Possible neurotoxic indicator of acrylamide intoxication. *Ocup Environ Med* 53:468–71.
May JM, Qu ZC, Mendiratta S. (1998). Protection and recycling of alpha tocopherol in human erythrocytes by intracellular ascorbic acid. *Arch Biochem Biophys* 349:281–9.
Mehrotra A, Sandhir R. (2014). Mitochondrial cofactors in experimental Huntington's disease: Behavioral, biochemical and histological evaluation. *Behav Brain Res* 261:345–55.
Meng FG, Zhou HW, Zhou HM. (2001). Effects of acrylamide on creatine kinase from rabbit muscle. *Int J Biochem Cell Biol* 33: 1064–70.
Moraes TB, Dalazen GR, Jacques CE, et al. (2014). Glutathione metabolism enzymes in brain and liver of hyperphenylalaninemic rats and the effect of lipoic acid treatment. *Metab Brain Dis* 29:609–15.
Odland L, Romert L, Clemedson C, Walum E. (1994). Glutathione content, glutathione transferase activity and lipid peroxidation in acrylamide-treated neuroblastoma N1E 115 cells. *Toxicol In Vitro* 8:263–7.
Ozturk G, Ginis Z, Nurkurt S, et al. (2014). Effect of alpha lipoic acid on ifosfamide induced central neurotoxicity in rats. *Int J Neurosci* 124: 110–16.
Paglia DE, Valentine WN. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158–70.
Perera J, Tan JH, Jeevathayaparan S, et al. (2011). Neuroprotective effects of alpha lipoic acid on haloperidol-induced oxidative stress in the rat brain. *Cell Biosci* 1:12–17.
Pigeolet E, Corbisier P, Houbion A, et al. (1990). GPX, SOD and catalase inactivation by peroxides and oxygen derived free radicals. *Mech Aging Dev* 51:383–97.
Pirlich M, Kiok K, Sandig G, et al. (2002). Alpha-lipoic acid prevents ethanol-induced protein oxidation in mouse hippocampal HT22 cells. *Neurosci Lett* 328:93–6.
Rawi SM, Marie MS, Fahmy SR, El-Abied SA. (2012). Hazardous effects of acrylamide on immature male and female rats. *Afr J Pharm Pharmacol* 6:1367–86.
Pradeep K, Mohan CV, Gobianand K. (2007). Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. *Eur J Pharmacol* 560:110–16.
Saksa VA, Ventura-Clapierc R, Aliev ML. (1996). Metabolic control and metabolic capacity: Two aspects of creatine kinase functioning in the cells. *Biochim Biophys Acta* 1274:81–8.
Santos PS, Feitosa CM, Saldanha GB, et al. (2011). Lipoic acid inhibits caspase-dependent and -independent cell death pathways and is neuroprotective against hippocampal damage after pilocarpine-induced seizures. *Pharmacol Biochem Behav* 97:531–6.
Seale SM, Feng Q, Agarwal AK, El-Alfy AT. (2012). Neurobehavioral and transcriptional effects of acrylamide in juvenile rats. *Pharmacol Biochem Behav* 101:77–84.
Sedlak J, Lindsay RH. (1968). Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25:192–205.
Shila S, Subathra M, Devi MA, Panneerselvam C. (2005). Arsenic intoxication-induced reduction of glutathione level and the activity of related enzymes in rat brain regions: Reversal by DL-alpha-lipoic acid. *Arch Toxicol* 79:140–6.
Sharma A, Jain J. (2008). Effects of oral exposure of acrylamide on plasma levels of thyroid hormones and haematological parameters in the Swiss albino mice. *Asian J Exp Sci* 22:317–24.
Shukla PK, Khanna VK, Ali MM, et al. (2002). Protective effect of *Acorus calamus* against acrylamide induced neurotoxicity. *Phytother Res* 16:256–60.
Singh U, Jialal I. (2008). Alpha-lipoic acid supplementation and diabetes. *Nutr Rev* 6611:646–57.
Smith S, Shenvi SV, Widlansky M, et al. (2004). Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Curr Med Chem* 119:1135–46.
Somani SM, Husain K, Whitworth C, et al. (2000). Dose-dependent protection by lipoic acid against cisplatin-induced nephrotoxicity in rats: Antioxidant defense system. *Pharmacol Toxicol* 865:234–41.
Tareke E, Rydberg P, Karlsson P, et al. (2000). Acrylamide: A cooking carcinogen. *Chem Res Toxicol* 13:517–22.
Tarskikh MM. (2006). Damage to erythrocyte membranes as the mechanism for acrylate toxicity. *Bull Exp Biol Med* 142:690–2.
Toklu HZ, Hakan T, Biber N, et al. (2009). The protective effect of alpha lipoic acid against traumatic brain injury in rats. *Free Radic Res* 43: 658–67.
Toklu HZ, Hakan T, Celik H, et al. (2010). Neuroprotective effects of alpha-lipoic acid in experimental spinal cord injury in rats. *J Spinal Med* 33:401–9.
Tsakiris S, Angelogianni P, Schulpis KH, Stavridis JC. (2000). Protective effect of L-phenylalanine on rat brain acetylcholinesterase inhibition induced by free radicals. *Clin Biochem* 33:103–6.
Tyl RW, Marr MC, Myers CB, et al. (2000). Relationship between acrylamide reproductive and neurotoxicity in male rats. *Reprod Toxicol* 14:147–57.
Wang QS, Xie KQ, Zhang CL, et al. (2005). Allyl chloride-induced time dependent changes of lipid peroxidation in rat nerve tissue. *Neurochem Res* 30:1387–95.
Yousef MI, El-Demerdash FM. (2006). Acrylamide-induced oxidative stress and biochemical perturbations in rats. *Toxicolology* 219:133–41.
Zhu YJ, Zeng T, Zhu YB, et al. (2008). Effects of acrylamide on the nervous tissue antioxidant system and sciatic nerve electrophysiology in the rat. *Neurochem Res* 33:2310–17.