



## Homeostatic changes of some trace elements in geriatric rats in the condition of oxidative stress induced by aluminum and the beneficial role of resveratrol



Florin Muselin<sup>a</sup>, Zeno Gârban<sup>b</sup>, Romeo T. Cristina<sup>a,\*</sup>, Alexandru O. Doma<sup>a</sup>, Eugenia Dumitrescu<sup>a</sup>, Alexandru B. Vițălaru<sup>c</sup>, Ioan Bănățean-Dunea<sup>a</sup>

<sup>a</sup> BUASMV "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, Romanian Society for Trace Elements in Medicine, Romania

<sup>b</sup> Working Group for Xenobiochemistry, Romanian Academy-Branch Timisoara, Romania

<sup>c</sup> University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Romania

### ARTICLE INFO

#### Keywords:

Aluminum  
Oxidative stress  
Resveratrol  
Homeostasis  
Geriatric rats

### ABSTRACT

**Background:** Elderly individuals are exposed to trace element imbalances due to the reduced capacity of their organism to utilize minerals in a direct relationship with many circumstances.

**Objectives and methods:** The aim of this study was to assess the protective role of resveratrol upon the homeostatic changes of some trace elements in geriatric rats in the condition of oxidative stress induced by aluminum exposure. Forty Wistar rats, 18–20 months old, were divided randomly into four groups (n = 10): control (C) - receiving 1 ml of physiologically saline (P.S.) via intraperitoneal (i.p) administration, E1 - 1 ml of P.S. and 1000 ppb aluminum sulphate (AS) in drinking water ad libitum, E2 - 20 mg/kg<sup>-1</sup> resveratrol, i.p. and 1000 ppb AS in drinking water, E3 - 20 mg/kg<sup>-1</sup> resveratrol i.p. The groups C and E3 received distilled water as drinking water ad libitum. The i.p administrations were once a week for four weeks period. The levels of oxidative stress marker's were analyzed (glutathione, glutathione' peroxidase, superoxide dismutase, and catalase) of the proteins' (total protein, albumin, and hemoglobin) in serum and also the levels of the main trace elements (copper, zinc, iron, selenium, manganese and magnesium) in blood, liver, kidney and spleen.

**Results:** Significant decrease (p < 0.05) of total protein (TP), albumin (ALB), catalase (CAT), increase significant (p < 0.05) of glutathione reductase (GSH-r), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in E1 groups, compared with control, E2, and E3 groups was ascertained. There were also observed significant (p < 0.05) decreases in Cu, Zn, Fe and Mg, not significant (p > 0.05) increase of Se and Mn in blood, significant (p < 0.01) increase of Cu, Zn, Mg, Se, Mn in kidney and liver and Fe, in spleen of geriatric rats from E1 group compared to the control group. Insignificant differences (p > 0.05) were recorded in groups which received resveratrol (E2 and E3) compared to the control group, but significant differences (p < 0.05), especially in blood and liver samples, compared to E1.

**Conclusions:** The results suggest that resveratrol can prevent the homeostatic imbalance of trace elements in geriatric rats in the condition of oxidative stress induced by aluminum exposure.

### 1. Introduction

Geriatric individuals are the most vulnerable to the trace elements' imbalance due to the reduced capacity of their organism to utilize minerals in direct correlation with pathophysiological and environmental factors [1].

The oxidative stress theory of "aging" appeared relatively recently in attention and it is based on the structural damage hypothesis. In this

assumption, the age-associated functional losses are a consequence of the oxidative damage accumulation to the macromolecules through different reactive oxygen species (ROS) or reactive nitrogen species (RON) [2,3].

Even so, the exact mechanism of oxidative stress-induced aging is still not clear and it is known that, probably, the increased ROS or RON levels may lead to cellular senescence, a physiological mechanism followed by the stop of cellular proliferation in response to damages that

\* Corresponding author at: BUASMV "King Michael I of Romania" from Timisoara, Faculty of Veterinary Medicine, Pharmacology and Pharmacy Depts., Romanian Society for Trace Elements in Medicine, 119 Calea Aradului, 300645, Timisoara, Romania.

E-mail address: [romeocristina@usab-tm.ro](mailto:romeocristina@usab-tm.ro) (R.T. Cristina).

<https://doi.org/10.1016/j.jtemb.2019.06.013>

Received 29 April 2019; Received in revised form 5 June 2019; Accepted 17 June 2019

0946-672X/ © 2019 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

occur during replication [3].

There is evidence which suggests that the trace elements are able to affect the mechanism of aging. However, a link between the aging process and trace elements remain unclear or indirect [1,4]. An imbalance and accumulation of trace elements and minerals in some organs could be a consequence of degenerative disease linked to aging [1,3].

The aluminum (Al) is a trace element that is ubiquitous in the environment, being the third most abundant element found in the earth crust [5,6]. It has the ability to induce oxidative damage through multiple mechanisms [7,8].

Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin isolated from grapes, peanuts, pines, etc., that have the capacity to affect several biological activities acting as an anti-inflammatory, anti-mutagenic and anti-oxidant [9,10]. Majority of the polyphenols from the plants, including here the resveratrol, has the capacity to interact with oxidative stress and the trace elements [7,9–12].

The aim of our study was to assess the protective role of resveratrol upon the homeostatic imbalance of copper (Cu), iron (Fe), selenium (Se), magnesium (Mg), manganese (Mn) and zinc (Zn) in geriatric rats in the condition of oxidative stress induced by aluminum exposure.

## 2. Materials and methods

### 2.1. Animals and experimental protocol

Forty healthy Wistar albino geriatric rats (16 males / 24 females) aged 18–20 months and  $400 \pm 20$  g, obtained from the authorized animal care unit of the University of Medicine and Pharmacy "Victor Babes" from Timisoara, Romania were housed in standard polycarbonate cages ( $l \times w \times h = 750 \times 720 \times 360$  mm) and fed *ad libitum* with a standard diet (Biovetimix, code 140-501, Romania) (Table 1).

The environmental conditions were maintained at  $22 \pm 2$  °C, the relative humidity of  $55 \pm 10\%$  and 12 h light / dark cycle. Before the start of the experiment, animals were kept in the same cages one week for acclimatization and were handled in accordance with Directive 2010/63/EU on the handling of animals used for scientific purposes [13] and guidelines of the National Research Council (NRC) [14].

The experiment was approved by the Ethical Committee of the Banat University of Agricultural Sciences and Veterinary Medicine from Timisoara (No. 120/2018).

The rats were randomly distributed in four experimental groups ( $n = 10$ ) as follows:

- Group C – Control; 1 ml of physiologically saline (P.S.) via i.p administration;

**Table 1**

Composition of the standard diet (Biovetimix) given to rats.

Composition	Unit	Content value
Protein	%	15
Fat	%	10
Cellulose	%	8
Ash	%	3
Ca	%	0.5
P	%	0.4
Vit. A	IU/kg	8500
Vit. D3	IU/kg	1500
Vit. E	mg/kg	20
Mn	mg/kg	52
Zn	mg/kg	30
Fe	mg/kg	40
Mg	mg/kg	55
Cu	mg/kg	8
I	mg/kg	1.5
Se	mg/kg	0.1

- Group E1 – 1 ml of P.S. and 1000 ppb aluminum sulfate (AS) in drinking water *ad libitum* [8];
- Group E2 – 20 mg/kg<sup>-1</sup> resveratrol, i.p. and 1000 ppb AS in drinking water;
- Group E3 – resveratrol control, 20 mg/kg<sup>-1</sup> resveratrol i.p. [15,16].

The groups C and E3 received distilled water as drinking water *ad libitum*. The resveratrol (Resveratrol 99% purity, 228.24 g mol<sup>-1</sup>, Sigma-Aldrich, Germany) was dissolved in a carrier-solution containing 5% ethanol and 95% physiological saline. The food intake, water consumption and behavioral changes were monitored daily during the experiment.

The i.p. administration was once a week for four weeks. At the end of the experiment all rats were euthanized by overdosing anesthetic agents using 300 mg kg bw<sup>-1</sup> of ketamine (Ketamine 10%, CP Pharma, Burgdorf, Germany) and 30 mg kg bw<sup>-1</sup> of xylazine (Narcoxyl, Intervet International, Boxmeer, the Netherlands), in accordance with Directive 2010/63/EU [13], and SVH AEC SOP.26 [17] and blood and organ samples were collected.

Blood samples were collected into heparinized BD Vacutainer (Ref. no. 367884) and centrifuged for 10 min at 3000 × g to separate the erythrocytes and plasma. Subsequently, the erythrocytes were processed according to kits manufacturer, were washed three times with saline solution and the separates were frozen and stored at  $-80$  °C until analyses were performed. The measured parameters were activity of catalase (CAT) glutathione reductase (GSH-r) and superoxide dismutase (SOD) in erythrocytes, the activity of glutathione peroxidase (GPx) in whole blood, the concentration of total protein (TP) and albumin (ALB) in plasma. The levels aluminum (Al), manganese (Mn), magnesium (Mg), zinc (Zn), iron (Fe), selenium (Se) and copper (Cu) in blood, liver, kidney, and spleen were also analyzed.

### 2.2. Samples analysis

The SOD, GSH-r and GPx activities were determined with a Randox RX-Daytona automated analyzer (Randox, Crumlin, UK) using commercially available kits (Ransod Cat. No. SD125, Cat. No. GR2368 and Ransel Cat. No. RS504, Randox, Crumlin UK).

The antioxidant enzyme activities were measured at 37 °C and expressed in U/g Hb.

The hemoglobin (Hb) was assessed using a standard cyano-methemoglobin method with a diagnostic kit (Cat. No. HG1539, Randox, Crumlin UK). The CAT activity was assessed by a method of Hadwan [18], briefly CAT activity is directly proportional to the rate of dissociation of hydrogen peroxide and in this case hydrogen peroxide acts to oxidize cobalt (II) to cobalt (III) in the presence of bicarbonate ions, this process ends with the production of a carbonate-cobaltate (III) complex with maximum absorbance at 440 nm. The measurements were made with a Shimadzu UV mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan).

Albumin and total protein levels were determined by spectrophotometry [19] according to appropriate standardized procedures by the reaction with copper sulfate ( $\lambda$  540 nm) for TP and by the reaction with bromocresol green ( $\lambda$  628 nm) for ALB, using commercially available kits from Chema Diagnostica (Italy, REF: albumin – BC0100CH, total protein – TP0100CH).

For determination of aluminum, copper, iron, manganese, magnesium, selenium and zinc, sample preparations were performed by microwave digestion. The samples (1 g) were deposited in the digestion tubes adding 10 mL of concentrated nitric acid and 2 mL of hydrogen peroxide. The flasks were covered with a lid, and inserted into the protective sleeve and then submitted to microwave digestion system (Multiwave GO, Anton Paar, GmbH, Austria), the working schedule being: 20 min, 120 °C and 800 W. After digestion, the samples were placed into flasks rated 25 mL and double-distilled water was added up to the mark.

**Table 2**  
The working parameters for trace elements analysis.

Parameter	Al	Se	Cu	Zn	Fe	Mn	Mg
Wavelength (nm)	309.3	196.0	324.8	213.9	248.3	279.5	285.2
Slit width (nm)	0.7	2.0	0.7	0.7	0.2	0.2	0.7
Lamp source	HCL	HCL	HCL	HCL	HCL	HCL	HCL
Lamp current (mA)	25	280	15	15	30	20	6
Method	furnace	furnace	flame	flame	flame	flame	flame
Gas flow	Argon (250 mL/ min)	Argon (250 mL/ min)	Air/Acetylene (17/ 2 L/min)				

HCL (Hollow Cathode Lamp).

Readings were made by graphite furnace AAS (GF-AAS), with a platform graphite tube for Se and Al and by flame absorption spectroscopy for Cu, Mg, Zn, Mn, and Fe, using a Perkin Elmer AA analyst 800 spectrophotometer (Perkin Elmer Inc. USA) as are presented in Table 2. All reagents used in this study were a high-purity grade (Suprapur, Merck, Germany). Working standards were prepared by serial dilutions of a CertiPur ICP (Merck) 1000 mg/L stock standard solution.

### 2.3. Statistical analysis

The obtained values were expressed as mean  $\pm$  SEM (mean's middle error) and for the estimation of the difference between groups, one-way ANOVA with the Tukey multiple comparison tests were used considering that the differences are statistically provided when  $p < 0.05$  or lower. The software used was GraphPad Prism 6.0 for Windows (GraphPad Software, San Diego, USA).

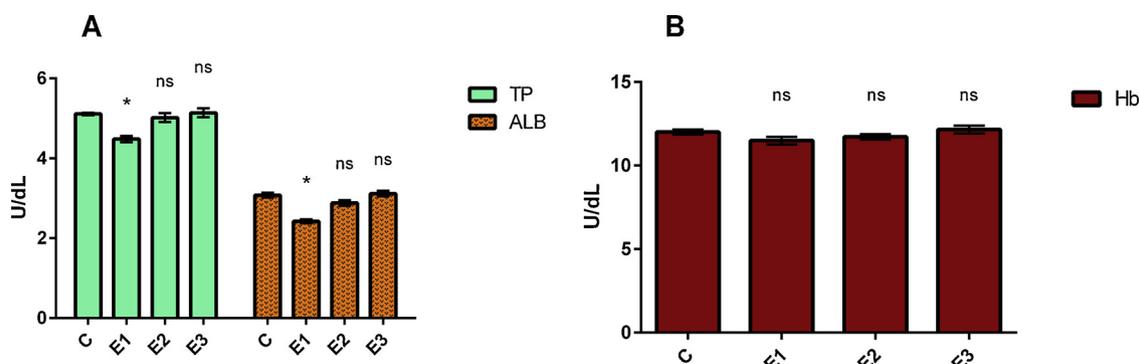
## 3. Results

### 3.1. General observations

There were no changes regarding food intake and behavior during the experiment in all groups. In what concerns water consumption, there were no significant changes observed, except the group receiving AS, where it was observed a minor, but not significant, decrease of water consumption during the last week of experiment, compared with the first week (-2.78%,  $p > 0.05$ ) this, possibly due to a palatability change.

### 3.2. Serum proteins

TP and ALB decreased significantly ( $p < 0.05$ ) in geriatric rats from the group exposed to AS (-12.33; -21.43%) but presented a not significant ( $p > 0.05$ ) decrease when resveratrol was administered (-1.76%; -6.49%). On the other side, Hb presented not significant ( $p > 0.05$ ) fluctuations, especially a slight decrease in the case of AS exposure (-4.32%) (Fig. 1).



**Fig. 1.** Levels of total proteins, albumin (A) and hemoglobin (B) in geriatric rats exposed to AS and resveratrol. Comparative to control group: \*  $p < 0.05$ , ns-not significant.

### 3.3. Oxidative stress enzymes

The level of SOD activity increased significantly (+35.34%,  $p < 0.001$ ) in groups exposed to AS compared to the control group and decreased significantly (-19.5%,  $p < 0.01$ ) in rats exposed to aluminum but treated with resveratrol, the level of SOD remaining to a level not significantly higher than in control (+8.95%,  $p > 0.05$ ). The same dynamic was noted in case of GPx activity, significantly increased (+23.59%,  $p < 0.01$ ) compared to the control, significantly decrease when resveratrol was administered (-17.96%,  $p < 0.01$ ) remained a level not significantly, higher than control (+1.39%,  $p > 0.05$ ). In turn, CAT activity significantly decreased (-32.96%,  $p < 0.001$ ) in geriatric rats from group exposed to AS. Administration of resveratrol to exposed rats significantly increased the CAT activity compared to groups that did not receive resveratrol (+20.45%,  $p < 0.001$ ) but the values remained to a significantly lower level than in the control (-19.24%,  $p < 0.05$ ).

The GSH-r level was also significantly higher in geriatric rats exposed to AS compared to control (+21.81%,  $p < 0.001$ ), decreasing significantly when resveratrol was administered to geriatric rats to a level significantly lower than in those exposed to AS (-11.30%,  $p < 0.05$ ) but still remained significantly higher than in the control (+14.25%,  $p < 0.05$ ).

There were not observed significant differences between the control and the resveratrol control group (E3/C:  $p > 0.05$ ) (Fig. 2).

### 3.4. Trace elements levels in blood and organs

The trace elements levels in geriatric rats exposed to AS and resveratrol are presented in Table 3.

### 3.5. Blood

The levels of Fe, Cu, Mg and Zn decreased significantly in the blood of the group that received AS compared to control (Fe: -21.03%,  $p < 0.001$ ; Cu: -30.11%,  $p < 0.05$ ; Mg: -28.66%,  $p < 0.05$ ; Zn: -45.67%,  $p < 0.05$ ) but, in this group, the levels of Al, Se and Mn

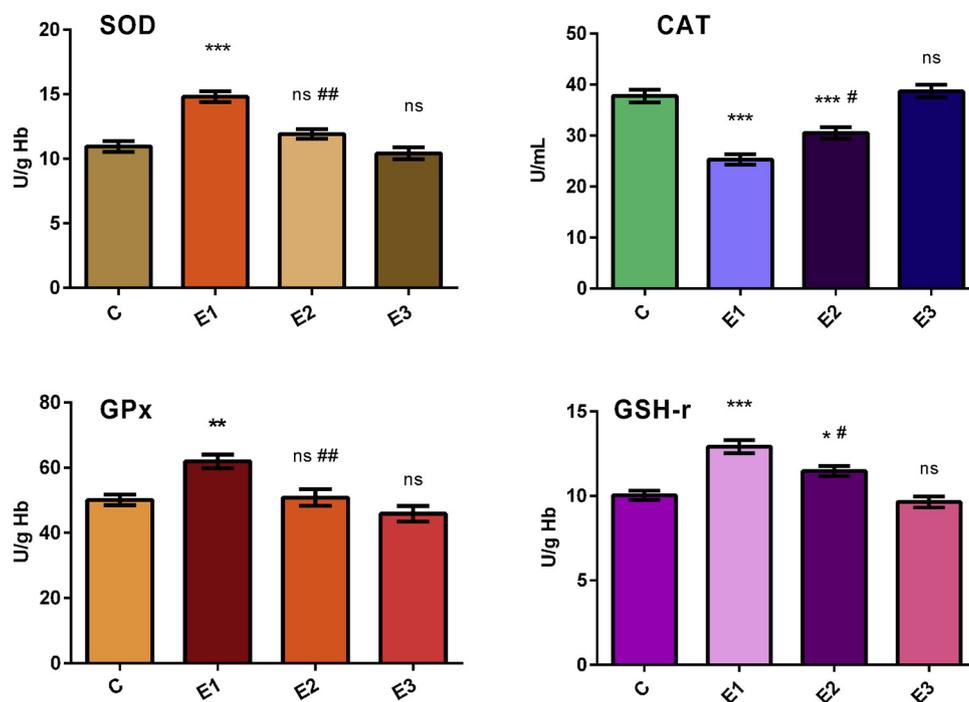


Fig. 2. Oxidative stress biomarkers in geriatric rats exposed to aluminum and resveratrol. Comparative to: C ns- not significant; \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001, Comparative to: E1 # P < 0.05, ## P < 0.01.

recorded an increase, this being significant only in case of Al level (Al: +160.67%, p < 0.001; Se: +5.12, p > 0.05; Mn: +19.5%, p > 0.05). In the group exposed to AS and injected i.p with resveratrol, the levels of Fe, Cu, Mg and Zn increased, this increase being statistically significant only in case of Zn level (Fe: +9.67%, p > 0.05; Cu:

+26.26%, p > 0.05; Mg: +18.13%, p > 0.05; Zn: +60.0%, p < 0.05).

Even with this tendency to reestablish the homeostasis of these trace elements, the levels did not reach the control group level, remaining different compared to the control, statistically significant only in the

Table 3 Trace elements levels in geriatric rats exposed to AS and resveratrol.

Group		C	E1 (Al)	E2 (Al + Res)	E3 (Res)
Parameter		X ± SEM			
Al	blood (ng/ml)	10.35 ± 0.54	26.98 ± 1.79***	20.12 ± 1.50** #	8.91 ± 0.65 ns
	liver (ng/g)	41.44 ± 2.56	60.17 ± 2.10***	52.84 ± 1.61*** #	45.87 ± 2.07 ns
	kidney (ng/g)	28.51 ± 1.84	36.55 ± 2.54**	33.65 ± 1.78 ns	28.11 ± 0.57 ns
	spleen (ng/g)	40.13 ± 1.89	49.71 ± 2.45**	45.22 ± 1.32 ns	39.91 ± 0.85 ns
Fe	blood (µg/ml)	408.20 ± 7.93	322.34 ± 12.97***	353.52 ± 10.90***	424.33 ± 11.71 ns
	liver (µg/g)	146.84 ± 10.74	128.26 ± 7.50 ns	127.57 ± 8.19 ns	154.05 ± 6.38 ns
	kidney (µg/g)	75.96 ± 2.45	64.72 ± 1.77 ns	68.37 ± 2.35 ns	75.88 ± 1.88 ns
	spleen (µg/g)	361.79 ± 9.06	419.50 ± 14.76***	398.17 ± 11.94*	361.93 ± 9.70 ns
Se	blood (ng/ml)	206.43 ± 5.11	217.01 ± 5.38 ns	210.44 ± 4.62 ns	199.53 ± 3.68 ns
	liver (ng/g)	350.90 ± 14.70	480.56 ± 15.32***	422.76 ± 12.21** #	352.31 ± 17.81 ns
	kidney (ng/g)	275.04 ± 8.95	515.93 ± 31.40***	326.20 ± 25.96 ns ###	253.43 ± 9.56 ns
	spleen (ng/g)	212.64 ± 10.02	212.44 ± 14.26 ns	209.33 ± 17.17 ns	206.88 ± 7.49 ns
Cu	blood (µg/ml)	4.28 ± 0.22	2.97 ± 0.25*	3.75 ± 0.11 ns	4.12 ± 0.08 ns
	liver (µg/g)	6.73 ± 0.17	8.71 ± 0.38***	8.34 ± 0.28**	6.26 ± 0.57 ns
	kidney (µg/g)	9.71 ± 0.64	11.74 ± 0.39***	10.50 ± 0.28 ns	8.82 ± 0.48 ns
	spleen (µg/g)	3.79 ± 0.24	4.15 ± 0.29 ns	3.91 ± 0.25 ns	3.72 ± 0.21 ns
Mg	blood (µg/ml)	33.46 ± 1.32	23.87 ± 1.51*	28.20 ± 1.35 ns #	33.48 ± 2.06 ns
	liver (µg/g)	64.83 ± 3.06	85.41 ± 3.31***	71.19 ± 3.35 ns ##	62.63 ± 1.96 ns
	kidney (µg/g)	91.81 ± 2.87	105.04 ± 3.27**	97.34 ± 2.23 ns	91.68 ± 2.31 ns
	spleen (µg/g)	77.01 ± 2.97	74.50 ± 4.33 ns	74.34 ± 4.01 ns	75.45 ± 3.42 ns
Mn	blood (µg/ml)	1.41 ± 0.11	1.68 ± 0.12 ns	1.52 ± 0.10 ns	1.32 ± 0.13 ns
	liver (µg/g)	2.66 ± 0.18	4.26 ± 0.44***	3.14 ± 0.08 ns ###	2.67 ± 0.18 ns
	kidney (µg/g)	1.54 ± 0.13	3.01 ± 0.23***	1.97 ± 0.09 ns ##	1.49 ± 0.15 ns
	spleen (µg/g)	1.23 ± 0.17	1.33 ± 0.25 ns	1.33 ± 0.20 ns	1.01 ± 0.12 ns
Zn	blood (µg/ml)	5.43 ± 0.41	2.95 ± 0.32*	4.72 ± 0.36 ns #	5.72 ± 0.48 ns
	liver (µg/g)	10.93 ± 0.76	14.54 ± 0.81**	13.06 ± 0.74 ns	10.74 ± 0.75 ns
	kidney (µg/g)	9.85 ± 0.87	13.11 ± 0.91**	10.86 ± 0.66 ns	9.01 ± 0.30 ns
	spleen (µg/g)	9.93 ± 0.75	9.20 ± 0.48 ns	9.35 ± 0.51 ns	10.40 ± 0.81 ns

Comparative to C: ns – not significant, \* p < .05, \*\* p < 0.01, \*\*\* p < 0.001. Comparative to E1: # p < 0.05, ## p < 0.01, ### p < 0.001.

case of Al and Fe levels (Al: +94.39%,  $p < 0.01$ ; Fe: -13.12%,  $p < 0.001$ ; Se: +1.94%,  $p > 0.05$ ; Cu: -12.38%,  $p > 0.05$ ; Mg: -15.72%,  $p > 0.05$ ; Mn: +7.80%,  $p > 0.05$ ; Zn: -13.07%,  $p > 0.05$ ). There weren't significant differences recorded regarding the levels of studied trace elements between control (C) and resveratrol control (E3) groups.

### 3.6. Liver

In the liver the levels of Al, Se, Cu, Mg, Mn and Zn significantly increased ( $p < 0.001$ ) in geriatric rats exposed to AS compared to control (Al: +45.19%,  $p < 0.001$ ; Se: +36.95%,  $p < 0.001$ ; Cu: +29.42%,  $p < 0.001$ ; Mg: +31.74%,  $p < 0.001$ ; Mn: +60.15%,  $p < 0.001$ ; Zn: +33.02%,  $p < 0.01$ ), but there was recorded a not significant decrease of Fe (-12.65%,  $p > 0.05$ ). In the group which received resveratrol (E2) the levels of studied trace elements decreased compared to the group which received AS alone, this decrease being significant only in the case of Al, Se, Mg and Mn (Al: -12.18%,  $p < 0.05$ ; Fe: -0.53%,  $p > 0.05$ ; Se: -12.03%; Cu: -4.25%,  $p > 0.05$ ; Mg: -16.65%,  $p < 0.01$ ; Mn: -26.29%,  $p < 0.001$ ; Zn: -10.17%,  $p > 0.05$ ) but, still remained different from those of the control group (Al: +27.51%,  $p < 0.001$ ; Fe: -13.12%,  $p > 0.05$ ; Se: +20.47%,  $p < 0.01$ ; Cu: +23.29%,  $p < 0.01$ ; Mg: +9.81%,  $p > 0.05$ ; Mn: +18.04%,  $p > 0.05$ ; Zn: +19.48%,  $p > 0.05$ ). Not significant ( $p > 0.05$ ) differences were noted between the two control groups.

### 3.7. Kidney

In the kidneys was observed the same dynamic as in the liver, significant increase of Al, Se, Cu, Mg, Mn and Zn levels (Al: +28.20%,  $p < 0.01$ ; Se: +87.58%,  $p < 0.001$ ; Cu: +20.91%,  $p < 0.001$ ; Mg: +14.41%,  $p < 0.01$ ; Mn: +95.45%,  $p < 0.001$ ; Zn: +33.09%,  $p < 0.01$ ) and a not significant ( $p > 0.05$ ) decrease of Fe level (-14.79%).

In the group that received resveratrol the levels of studied trace elements decreased (exception Fe, which increased not significant) compared with geriatric rats from the group exposed to AS (E1) but, the significance was assured only in the case of Se and Mn levels (Al: -7.93%,  $p > 0.05$ ; Fe: +5.64%,  $p > 0.05$ ; Se: -36.77%,  $p < 0.001$ ; Cu: -5.78%,  $p > 0.05$ ; Mg: -7.33%,  $p > 0.05$ ; Mn: -37.55%,  $p < 0.01$ ; Zn: -17.16%,  $p > 0.05$ ).

In the group that received resveratrol and AS the levels of studied trace elements remained higher than in control but the differences were not significant ( $p > 0.05$ ) from statistic point of view (Al: +18.02%; Fe: -9.99%; Se: +18.60%, Cu: +8.13%, Mg: +6.02%, Mn: +27.92%, Zn: +10.25%). Not significant ( $p > 0.05$ ) differences were noted between the two control groups.

### 3.8. Spleen

In the spleen were noted, generally, not significant ( $p > 0.05$ ) fluctuation of the studied trace elements, exception the Al and Fe levels which present a significant increase ( $p < 0.01$ ) in AS exposed geriatric rats. Exposure to AS was followed by the increase of Al, Fe, Cu, Mn and a decrease of Se, Mg and Zn comparative to control (Al: +23.87%,  $p < 0.01$ ; Fe: +15.95%,  $p < 0.001$ ; Se: -0.09%,  $p > 0.05$ ; Cu: +9.49%,  $p > 0.05$ ; Mg: -3.25%,  $p > 0.05$ ; Mn: +8.13%,  $p > 0.05$ ; Zn: -7.35%,  $p > 0.05$ ).

Administration of resveratrol was followed by the not significant ( $p > 0.05$ ) decrease of studied trace elements, except for the Mn level which remained at the same level and Zn which increase to not significant level compared to AS exposed group, these changes going until to a not significant ( $p > 0.05$ ) level compared to control (E2/E1 changes: Al: -9.03%, Fe: -5.08%, Se: -1.46%, Cu: -5.78%, Mg: -0.21%, Mn: 0%, Zn: +1.63% and E2/C changes: Al: +12.68%, Fe: +10.05%, Se: -1.55%, Cu: +3.16%, Mg: -3.46%, Mn: +8.13%, Zn: -5.84%). Not

significant ( $p > 0.05$ ) differences were noted between the two control groups.

## 4. Discussions and conclusions

Trace elements such as Cu, Zn, Fe, Mn, Mg, and Se have the capacity to reduce oxidative damage or enhance repair capacity by acting as essential co-factors for anti-oxidant enzymes such as superoxide dismutase (Cu, Zn, Mn), catalase (Cu, Fe), and glutathione peroxidases (Se), these enzymes being crucial to limit oxidation of nucleic acids, lipids or proteins occurring in chronic diseases and aging [4].

It is well known that Al can act as a prooxidant, and thus, can induce oxidative stress disturbing the activity of different antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase [8,20].

Total protein and albumin are important indicators of the hepatic protein synthesis [5].

In this present study, we observed a significant decrease of TP and ALB in the geriatric rats exposed to AS and the increase of these in the group which received resveratrol.

Fe, Cu, and Zn are also transported by ALB, and a direct correlation between serum ALB and Cu, Fe and Zn blood levels were ascertained. Similar findings were acquired by Wang et al., [5] in chickens exposed to aluminum chloride and by Belles et al., in pregnant rats exposed to aluminum hydroxide [21].

In the study, we observed a significant increase of Al in blood and organs of geriatric rats exposed to AS and decrease of Al in rats that received resveratrol. Aluminum in the blood circulates mainly bound to transferrin and some compounds with low molecular mass (e.g., citrate) [22,23], in this way, Al is able to interfere with Fe homeostasis by displacing it from transferrin, and as a result of this interference, Fe is released into the bloodstream. Being a redox-active metal, Fe can interact with molecular oxygen and generate the superoxide anion, which generates highly reactive hydroxyl radical [24].

However, data from our experiment did not prove the way of Al action, which was also proven by Staneviciene et al., [25], in agreement to our findings.

Iron is an indispensable trace element necessary for a proper CAT activity [26]. We have observed the direct correlated decreased activity of this enzyme in the geriatric rats exposed to AS, thus concluding that it may be a reason for the significant decrease of Fe level in the blood.

GSH-r is important to maintain the reduced GSH levels and therefore, it plays a major role in the GPx reaction, as an adjunct, in the control of peroxides and free radicals. The decrease of the CAT's level is followed by the activation of the glutathione-dependent enzyme [27], as it was also observed in the present study. It is clear that iron metabolism changes with age, and there are specific groups of elderly subjects that may suffer from iron deficiency, while others, could accumulate this metal in excess [1].

In this study we ascertained a decrease of Fe in blood, liver, and kidney of geriatric rats exposed to AS and a significant accumulation in spleen. This condition tended to normalize in the group which received resveratrol. There are studies which demonstrated that Al ions can interfere with Se metabolism and may directly or, indirectly, affect the Se homeostasis in animals [28]. GPx is closely connected with GSH-r, which has a role in maintain an adequate level of reduced glutathione (GSH) [29]. The increased activity of GSH-r may be due, in this case, to the production of the reduced form of glutathione.

It is well known that the redox system of GSH is Se-dependent [30], Se being an important cofactor of the GPx enzyme, that acts together with GSH-r, and by this, Se is an important element in the antioxidative system that protects against metal-induced ROS [25,31].

We observed a significant increase of GPx activity in geriatric rats exposed to AS and also an increase of Se in a direct correlation, and a decrease of this in the geriatric rats that received resveratrol. Being a Se dependent protein, the circulating GPx activities are directly responsive to the Se status in the biological systems [32]. GPx activity levels could

be used as a functional marker of selenium status because GPx requires selenium for its activity, and different studies have shown the positive association with the plasma selenium [31,33]. We observed also this direct, positive, correlation between GPx activity and Se blood level. Organs, especially kidneys can accumulate high amounts of Se representing the major source of plasma GPx [34], this also being observed in our study.

Aging is very often associated with Mg insufficiency and chronic Mg deficiency may result in excessive production of oxygen-derived free radicals [35]. In this respect we observed the significant decrease of blood Mg and the increase of liver and kidney Mg in the geriatric rats exposed to AS, with a clear tendency to recover when resveratrol was administered. The bone metabolism could be altered with age, and the capacity of the skeleton to store and release of Mg decreases markedly [1]. The inadequate availability of Mg could lead to a reduced mitochondrial efficiency and an increased production of reactive oxygen species (ROS), and as a consequence, the structural and functional impairment of proteins may lead to the mitochondrial function decline in the skeletal muscle which is often associated with aging, fact demonstrated so far in humans [36].

Depletion of Mg in rats is associated with structural damage to muscle cells, mitochondrial swelling, and the altered ultra structure it was associated with the increased ROS production [35]. In the condition of the present study, Mn levels increased in all studied organs and blood of geriatric rats exposed to AS and reduced in rats which received resveratrol. The elevated levels of blood Mn versus the decrease of Cu and Zn blood levels and also the decrease of Cu / Zn ratio could be explained as a result of the SOD activity increase [10]. The augmentation of SOD and GPx activity occurs as a result of a compensatory mechanism in response to the oxidative stress [31].

In our study, we have identified the significant decrease of blood Cu level and a significant increase of Cu levels in the liver and kidney. The excessive accumulation of Cu in the situation of Fe decrease from tissues may be a result of abnormal sequestering of Cu and failure of the occurrence of normal mobilization of this element as was reported by Sherman and Tissue [37].

The possible factor known to affect the sequestering of Cu during Fe decrease is the reduction in the synthesis of ceruloplasmin which is required to accomplish the ferroxidase needs, thereby promoting the rate of incorporation of Fe into transferrin. If the ceruloplasmin is less synthesized, more Cu will be stored in the tissues. It is also possible that in the time of Fe decrease, the Cu binding proteins could have a greater affinity causing a slow release of Cu followed by increased tissue concentration of this trace element [38].

Phenolic compounds can suppress free radical reactions via chelation of some catalytic metal ions as Fe and Cu, resveratrol is known to have the ability to chelate especially Cu [10–12], this being a potential explanation of our findings.

The protective effect of resveratrol against aluminum toxicity was pointed out by Hammoud and Shalabi [39] and Al Dera [40] in rats exposed to aluminum chloride. Al Dera [40] for example pointed out that resveratrol protects against aluminum nephrotoxicity and this could also be a possible explanation for the imbalance of some trace elements which could be eliminated through the kidney.

The significant increase of Cu, Mg and Zn levels and decrease of Fe levels from liver and kidneys was reported by Olajji [38] in iron-deficient rats.

Decrease of iron content and the increase of Cu content, a significant decrease of Mg content and a considerable decrease of Zn content were observed in the serum of rats with induced mammary carcinoma and treated with copper and resveratrol as were noted by Skrajnowska et al [26] in accordance with our study.

In a rat model, Khanna and Nehru [41], noted that the interaction between Al and Zn interfered with SOD activities, and by this, higher plasma Al concentrations may interfere with both Zn and Se homeostasis, which consequently leads to low plasma Zn and Se

concentrations and high oxidative stress, this fact being also observed by Sadauskienė et al., in mice models [42].

In our study, we observed that a high aluminum concentration in blood was followed by a decrease of Zn and a not significant increase of Se without affecting the SOD activity.

We observed also a significant decrease of Zn in blood and a significant increase in liver and kidney of geriatric rats exposed to AS, and a tendency to re-establish the imbalance of this trace element in rats which received resveratrol. Even when, the blood Zn level decreased, the activity of SOD was not modified as it was expected. The increase of Zn levels in liver and kidney could be due to the fact that transferrin is also essential for Zn transportation and transferrin will be available during Fe decreased levels [38].

In agreement with our study, Singla and Dhawan [43] in their research observed the significant increase of CAT, SOD and GSH-r activities in rats exposed to aluminum chloride orally and the protective effect of Zn administration against the deleterious activity of Al on rat's brain [44,45].

Excessive Zn level in some organs of geriatric people may be harmful to the normal metabolism of cells because Zn enhances the activity of telomerase, an enzyme which seems to be responsible for unlimited cell proliferation [46].

In a study, Drobyshev et al., [47] ascertained a significant increase of blood Al level followed by a not significant increase of Mg, Mn, Fe, Cu and Se and a not significant decrease of Zn blood level confirming partially our findings.

In the blood and all studied organs of the group which received resveratrol the imbalance of studied trace elements tended to normalize, proving the beneficial role of resveratrol.

The most important outcomes of resveratrol were observed upon the levels of Se and Mn in liver and kidney, Mg in liver and Zn in blood. To have a better overview of these aspects, additional studies are needed, for assessing the specific proteins such as: ceruloplasmin, ferritin, and transferrin.

## Acknowledgments

The work was supported by the project "Ensuring excellence in RDI activity within USAMVBT" code 35PFE, submitted in competition Program 1 - Development of the national research and development system, Subprogram 1.2 - Institutional performance, Institutional development projects - Projects for financing excellence in CDI. Special thanks for their support to Mr. Giorgio Gherardi and Mrs. Daniela Csoric from GG Diagnostica, Romania.

## References

- [1] M.P. Vaquero, Magnesium and trace elements in the elderly: intake, status, and recommendations, *J. Nutr. Health Aging* 6 (2002) 147–153 PMID:12166371.
- [2] K.B. Beckman, B.N. Ames, The free radical theory of aging matures, *Physiol. Rev.* 78 (1998) 547–581, <https://doi.org/10.1152/physrev.1998.78.2.547>.
- [3] I. Liguori, G. Russo, F. Curcio, e G. Bulli, L. Aran, D. Della-Morte, G. Gargiulo, G. Testa, F. Cacciatore, D. Bonaduce, P. Abete, Oxidative stress, aging, and diseases, *Clin. Interv. Aging* 13 (2018) 757–772, <https://doi.org/10.2147/CIA.S158513>.
- [4] C. Méplan, Trace elements and ageing, a genomic perspective using selenium as an example, *J. Trace Elem. Res. Med. Biol.* 25S (2011) S11–S16, <https://doi.org/10.1016/j.jtemb.2010.10.002>.
- [5] B. Wang, Y. Zhu, H. Zhang, L. Liu, G. Li, Y. Song, Y. Li, Effects of aluminium chloride on the serum protein, bilirubin and hepatic trace elements in chickens, *Toxicol. Ind. Health* 32 (2016) 1693–1699, <https://doi.org/10.1177/0748233715578035>.
- [6] F. Muselin, R.T. Cristina, V. Îgna, E. Dumitrescu, D. Brezovan, A. Trif, The consequences of aluminium intake on reproductive function in male rats: a three-generation study, *Turk. J. Med. Sci.* 46 (2016) 1240–1248, <https://doi.org/10.3906/sag-1501-101>.
- [7] M.M.H. Zakaria, B. Hajipour, R. Estakhri, B.M. Saleh, Anti-oxidative effect of resveratrol on aluminium induced toxicity in rat cerebral tissue, *Bratisl. Med. J.* 118 (2017) 269–272 doi: 10.4149/BLL.2017.053.
- [8] F. Muselin, A. Trif, G.L. Stana, R.T. Cristina, C. Grăvilă, I. Măcinic, E. Dumitrescu, Protective effects of aqueous extract of *Sempervivum tectorum* L. (Crassulaceae) on aluminium-induced oxidative stress in rat blood, *Trop. J. Pharm. Res.* 13 (2014)

- 179–184, <https://doi.org/10.4314/tjpr.v13i2.2>.
- [9] S.M. Hadi, M.F. Ulah, A.S. Azmi, A. Ahmad, U. Shamin, H. Zubair, H.Y. Khan, Resveratrol mobilizes endogenous copper in human peripheral lymphocytes leading to oxidative DNA breakage: a putative mechanism for chemoprevention of cancer, *Pharm. Res.* 27 (2010) 979–988, <https://doi.org/10.1007/s11095-010-0055-4>.
- [10] S. Asadi, M.N. Moradi, N. Khyripour, M.T. Goodarzi, M. Mahmoodi, Resveratrol attenuates copper and zinc homeostasis and ameliorates oxidative stress in type 2 diabetic rats, *Boil. Trace. Elem. Res.* 177 (2017) 132–138, <https://doi.org/10.1007/s12011-016-0861-6>.
- [11] J.E. Brown, H. Khodr, R.C. Hider, C.A. Rice-Evans, Structural dependence of flavonoid interaction with Cu<sup>2+</sup> ions: implications for their antioxidant properties, *Biochem. J.* 330 (1998) 1173–1178 PMID: 9494082.
- [12] J.J. Zhang, M. Wu, N.W. Schoene, W.H. Cheng, T.T.Y. Wang, A.A. Alshatwi, M. Alsaif, K.Y. Lei, Effect of resveratrol and zinc on intracellular zinc status in normal human prostate epithelial cells, *Am. J. Physiol. Cell. Physiol.* 297 (2009) C632–C644, <https://doi.org/10.1152/ajpcell.00139.2009>.
- [13] Directive 2010/63/EU of the European Parliament and of the Council of 22, September 2010 on the Protection of Animals Used for Scientific Purposes, (2018) (Accessed 22 May 2017), <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF>.
- [14] National Research Council, Institute of Laboratory Animal Research (NRC), Guide for the Care and Use of Laboratory Animals, eighth edition, The National Academies Press, Washington, DC, USA, 2011.
- [15] Z. Gárban, F. Muselin, C. Baltá, A. Avacovici, E. Simiz, O.M. Boldura, R. Ujhelyi, Peculiarities of the resveratrol action on some biochemical and hematological parameters preliminary data, The 20<sup>th</sup> Int. Symp. on Analytical and Environmental Problems (2014) 10–13.
- [16] Y. Yazir, T. Utkan, N. Gacar, F. Aricioglu, Resveratrol exerts anti-inflammatory and neuroprotective effects to prevent memory deficits in rats exposed to chronic unpredictable mild stress, *Physiol. Behav.* 138 (2015) 297–304, <https://doi.org/10.1016/j.physbeh.2014.10.010>.
- [17] S. Peirce, SOP. 26 Euthanasia in Mice and Rats, (2006) Available: [www.pcarp.usp.br](http://www.pcarp.usp.br) (Accessed 22 May 2017).
- [18] M.H. Hadwan, Simple spectrophotometric assay for measuring catalase activity in biological tissues, *BMC Biochem.* 19 (2018) 7, <https://doi.org/10.1186/s12858-018-0097-5>.
- [19] V.A. Buzanovskii, Determination of proteins in blood. Part 1: determination of total protein and albumin, *Ref. J. Chem.* 7 (2017) 79–124, <https://doi.org/10.1134/S2079978017010010>.
- [20] M.I. Yousef, Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid, *Toxicol.* 199 (2004) 47–57, <https://doi.org/10.1016/j.tox.2004.02.014>.
- [21] M. Belles, M.L. Albina, D. Sanchez, J. Corbella, J.L. Domingo, Effects of oral aluminium on essential trace elements metabolism during pregnancy, *Biol. Trace Elem. Res.* 79 (2001) 67–81 doi: 0163-4984/01/7901-0067.
- [22] R.A. Yokel, Brain uptake, retention, and efflux of aluminum and manganese, *Environ. Health Perspect.* 110 (2002) 699–704, <https://doi.org/10.1289/ehp.02110s5699>.
- [23] R.A. Yokel, The toxicology of aluminum in the brain: a review, *Neurotoxicology* 21 (2000) 813–828 PMID: 11130287.
- [24] J.M. Gutteridge, A. Smith, Antioxidant protection by haemopexin of haem-stimulated lipid peroxidation, *Biochem. J.* 256 (1988) 861–865 PMID: 3223958.
- [25] I. Staneviciene, L. Ivanov, L. Kursvietiene, D. Viezeliene, Short-term effects of aluminum and selenium on redox status in mice brain and blood, *Trace Elem. Electrolytes* 34 (2017) 74–80, <https://doi.org/10.5414/TEX01472>.
- [26] D. Skrajnowska, B. Bobrowska-Korczak, A. Tokarz, S. Bialek, E. Jezierska, J. Makowska, Copper and resveratrol attenuates serum catalase, glutathione peroxidase, and element values in rats with DMBA-induced mammary carcinogenesis, *Biol. Trace Elem. Res.* 156 (2013) 271–278, <https://doi.org/10.1007/s12011-013-9854-x>.
- [27] G.F. Gaetani, H.N. Kirkman, R. Mangerini, A.M. Ferraris, Importance of catalase in the disposal of hydrogen peroxide within human erythrocytes, *Blood* 84 (1994) 325–330 PMID: 8018928.
- [28] C.H. Guo, G.S. Hsu, C.J. Chuang, P.C. Chen, Aluminum accumulation induced testicular oxidative stress and altered selenium metabolism in mice, *Environ. Toxicol. Pharmacol.* 27 (2009) 176–181, <https://doi.org/10.1016/j.etap.2008.10.001>.
- [29] A. Zablocka, M. Janusz, The two faces of reactive oxygen species, *Post. Hig. Med. Dosw.* 62 (2008) 118–124 PMID: 18388851.
- [30] A. Mohammadir, M. Abdollahi, A systematic review on oxidant/antioxidant imbalance in aluminium toxicity, *Int. J. Pharmacol.* 7 (2011) 12–21, <https://doi.org/10.3923/ijp.2011.12.21>.
- [31] Q. Shazia, Z.H. Mohammad, T. Rahman, H.U. Shekhar, Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in beta thalassemia major patients: a review of the literature, *Anemia* 270923 (2012) 7, <https://doi.org/10.1155/2012/270923>.
- [32] L. Patrick, Selenium biochemistry and cancer: a review of the literature, *Altern. Med. Rev.* 9 (2004) 239–258 PMID: 15387717.
- [33] C.B. Stephensen, G.S. Marquis, S.D. Douglas, L.A. Kruzich, C.M. Wilson, Glutathione, glutathione peroxidase, and selenium status in HIV-positive and HIV-negative adolescents and young adults, *Am. J. Clin. Nutr.* 85 (2007) 173–181, <https://doi.org/10.1093/ajcn/85.1.173>.
- [34] N. Avisar, D.B. Ornt, Y. Yagil, S. Horowitz, R.H. Watkins, E.A. Kerl, K. Takahashi, I.S. Palmer, H.J. Cohen, Human kidney proximal tubules are the main source of plasma glutathione peroxidase, *Am. J. Physiol.* 266 (1994) C367–C375, <https://doi.org/10.1152/ajpcell.1994.266.2.C367>.
- [35] M. Barbagallo, L.J. Dominguez, Chapter 16. Magnesium, oxidative stress, and aging muscle, in: V.R. Preedy (Ed.), *Aging: Oxidative Stress and Dietary Antioxidants*, Elsevier Inc., 2014, pp. 157–166.
- [36] K.R. Short, M.L. Bigelow, J. Kahl, et al., Decline in skeletal muscle mitochondrial function with aging in humans, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2005) 5618–5623, <https://doi.org/10.1073/pnas.0501559102>.
- [37] A.R. Sherman, N.T. Tissues, Tissue iron, copper and zinc levels in offspring of iron-sufficient and iron deficient rats, *J. Nutr.* 111 (1987) 266–275, <https://doi.org/10.1093/jn/111.2.266>.
- [38] T.A. Oladiji, Tissue levels of iron, copper, zinc and magnesium in iron deficient rats, *Biokemistri* 14 (2003) 75–81.
- [39] G.M. Hammoud, R.A. Shalaby, Experimental evaluation of protective action of resveratrol against aluminium-induced toxicity in male rats, *Int. J. Adv. Res. Biol. Sci.* 6 (2019) 11–24, <https://doi.org/10.22192/ijarbs.2019.06.01-002>.
- [40] H.S. Al Dera, Protective effect of resveratrol against aluminium chloride induced nephrotoxicity in rats, *Saudi Med. J.* 37 (2016) 369–378, <https://doi.org/10.15537/smj.2016.4.13611>.
- [41] P. Khanna, B. Nehru, Antioxidant enzymatic system in neuronal and glial cells enriched fractions of rat brain after aluminum exposure, *Cell. Mol. Neurobiol.* 27 (2007) 959–969, <https://doi.org/10.1007/s10571-007-9233-2>.
- [42] I. Sadauskienė, A. Liekis, I. Staneviciene, D. Viezeliene, G. Zekonis, V. Simakauskienė, D. Baranauskienė, R. Naginiene, Post-exposure distribution of selenium and aluminum ions and their effects on superoxide dismutase activity in mouse brain, *Mol. Biol. Rep.* 45 (2018) 2421–2427, <https://doi.org/10.1007/s11033-018-4408-0>.
- [43] N. Singla, D.K. Dhawan, Zinc modulates aluminium-induced oxidative stress and cellular injury in rat brain, *Metallomics* 6 (2014) 1941–1950.
- [44] N. Singla, D.K. Dhawan, Zinc, a neuroprotective agent against aluminum-induced oxidative DNA injury, *Mol. Neurobiol.* 48 (2013) 1–12, <https://doi.org/10.1007/s12035-013-8417-7>.
- [45] N. Singla, D.K. Dhawan, Influence of zinc on the biokinetics of (65) Zn in brain and whole body and its bio-distribution in aluminium-intoxicated rats, *Cell. Mol. Neurobiol.* 34 (2014) 269–276, <https://doi.org/10.1007/s10571-013-0010-0>.
- [46] V. Zaichick, S. Zaichick, Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive x-ray fluorescent analysis, *MOJ Gerontol. Ger.* 1 (2017) 133–140, <https://doi.org/10.15406/mojgg.2017.01.00028>.
- [47] E.J. Drobyshev, N.D. Solovyev, B.M. Gorokhovskiy, V.A. Kashuro, Accumulation patterns of sub-chronic aluminum toxicity model after gastrointestinal administration in rats, *Biol. Trace Elem. Res.* 185 (2018) 384–394, <https://doi.org/10.1007/s12011-018-1247-8>.