

Original Research Article

Protective Effects of Aqueous Extract of *Sempervivum tectorum* L (*Crassulaceae*) on Aluminium-Induced Oxidative Stress in Rat Blood

Muselin Florin¹, Trif Alexandra^{1*}, Stana Loredana Gabriela², Cristina Teodor Romeo³, Grăvilă Corina⁴, Măcinic Ioan¹ and Dumitrescu Eugenia³

¹USAMVB Faculty of Veterinary Medicine, Department of Toxicology, Vegetal Biology and Medicinal Plants, ²University of Medicine and Pharmacy "Victor Babeş", ³USAMVB Faculty of Veterinary Medicine, Department of Pharmacology, ⁴USAMVB Faculty of Zootehny and Biotechnology, Department of Biochemistry, Timișoara, România

*For correspondence: **Email:** al_trif@yahoo.com; **Tel:** +40256277076,

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Abstract

Purpose: To examine the effect of *Sempervivum tectorum* aqueous extract, as infusion, on some biomarkers of oxidative stress induced by aluminium exposure in Wistar albino rat.

Methods: The rats were randomly divided into five groups and treated daily for 3 months as follows: control received tap water; the 2nd group was given 1 mg/L aluminium as aluminium sulphate (AS) in drinking water daily; group 3, a combination of 1 mg/L AS and *Sempervivum tectorum* extract daily; the fourth group, *Sempervivum tectorum* extract daily; and fifth group 1 mg/L AS for three months and thereafter *Sempervivum tectorum* extract for one month.

Results: Aluminium (Al) exposure increased, compared to control group, the level of blood Al (293.64 %, $p < 0.0001$), serum superoxide dismutase (SOD, 14.84 %, $p > 0.05$), glutathione peroxidase (G-Px, 31.25 %, $p < 0.001$) and thiobarbituric reactive substances (TBARS, 21.37 %, $p < 0.001$) but significantly decreased catalase (CAT, 49.59 %, $p < 0.001$), glutathione (GSH, 13.49 %, $p < 0.001$) and glutathione reductase (GSH-r, 23.08 %, $p < 0.0001$) levels. These results indicate that Al-induced oxidative stress in the rats. Following administration of *Sempervivum tectorum* extract, the levels of the oxidative stress biomarkers were restored to normal, indicating good antioxidant properties of the extract. Al blood level was also significantly reduced by 33.45 % ($p < 0.001$) in rats exposed to the extract. Rat body weight was not significantly ($p > 0.05$) affected by the extract.

Conclusion: *S. tectorum* extract possesses antioxidant activity against free radicals produced in blood as a result of aluminium exposure.

Keywords: Aluminium exposure, *Sempervivum tectorum*, Biomarkers, Free radicals, Oxidative stress.

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INTRODUCTION

Sempervivum tectorum (*Crassulaceae* family), known as houseleek, is an evergreen plant with perennial root, crowned with imbricated fleshy leaves, which are smooth on both sides and ciliated at the margin, with the stem rising from the center of the tuft of leaves and terminated

with a cymose corymb flowers [1]. It has been used for many years in the treatment of ear inflammation [1, 2], insect bites, burns and ulcer [2] having also an antinociceptive activity [3]. The extract from this plant contains approximately 20 different flavones and flavonol mono- and diglycosides [2-4]. The main flavonoid glycoside is kaempferol [4-6].

The aim of the present work was to determine if the aqueous extract of *Sempervivum tectorum* can offer protection against the oxidative stress induced by aluminium exposure in rats.

EXPERIMENTAL

Animals

Thirty five adult Wistar albino rats, (six months old), were purchased from the Animal House of University of Medicine and Pharmacy "Victor Babeş" Timisoara, Romania. The rats were housed in plastic cages, at constant room temperature of 23 ± 2 °C, 12 h light/dark cycle and fed *ad libitum* with standard diet. They were kept in the cages for one week to acclimatize before the start of the experiment, and handled in accordance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7]. The experiment was approved by the Ethical Committee of Banat's University of Agricultural Science and Veterinary Medicine, Timisoara (no. 3558/05.06.2012).

Plant material

Sempervivum tectorum (St), identified by Dr. Muselin Florin (Head, Department of Vegetal Biology and Medicinal Plants, Faculty of Veterinary Medicine), was collected during Spring/Summer seasons of 2012 from the field of Timis County, Romania and was compared for identification with a herbarium specimen deposited in the Department of Vegetal Biology and Medicinal Plants, Faculty of Veterinary Medicine Timisoara, Romania (voucher no. 112). The fresh leaves were removed from the rosette and roots, washed and dried in oven at 50 °C, to a constant weight. The extract was performed from *S. tectorum* dry leaves with 0.4 mm particle size in tap water (the ratio w/v 0.8/10), in Erlenmeyer flasks. The mixture was heated at 90 °C for 10 min in water bath and after, twice filtered [8]. The level of aluminium in the plant material was determined by atomic absorption spectroscopy.

Animal grouping

The rats were randomly divided into five groups treated as shown: Group I (C, control i.e., tap water containing 50 µg/L Al); Group II (Al) – 1 mg/L Al as aluminium sulphate (AS); Group III (1mg/L AS + 8 % *S. tectorum* extract; Group IV (8 % *S. tectorum*); Group V (1mg/L AS for three months and thereafter, 8 % *S. tectorum* for one month. At the end of the experiment, three months for groups I to IV and four months for group V, the rats were weighed and euthanized

by exsanguinations after administering ketamine 50 mg/kg b.w. anesthesia (10 % Ketaminol, Intervet International BV, Boxmeer, Holland) and blood was collected in BD vacutainers - Plasma Heparinized (LH 102 IU) BD Vacutainer (ref no. 367885) for plasma samples and Serum Plain BD Vacutainer (ref. no. 367837), without anticoagulant for serum samples.

Aluminium analysis and estimation of oxidative stress biomarkers

Aluminium was determined by atomic absorption spectroscopy at 309.3 nm wavelength, after nitric acid digestion (1 mL sample and 10 mL nitric acid in digestion flasks for 20 min at 120 °C, 600 W in CEM MARS X5 microwave digestion oven) using a Shimadzu AA6650 spectrometer (Shimadzu, Kyoto, Japan) with graphite furnace (pyrolytic graphite tube) by the method of Gitelman and Alderman [9]. Aluminium standard solution - Al(NO₃)₃ in 0.5 mol/L nitric acid (1000 mg/L Al, Merck KGaA, Darmstadt, Germany). Detection limit for Al was 0.2 µg/L. *The blood samples, collected in BD vacutainers with lithium heparin (ref no. 367885), were centrifuged for 10 min at 4000 rpm, plasma separated and the packed erythrocytes washed 3 times with normal saline to obtain the hemolysate.* Reduced GSH was assessed in the hemolysate according to the method of Beutler *et al* [10] based on the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound with maximum absorbance at 412 nm. The antioxidant enzyme G-Px in hemolysate was evaluated according to the method of Paglia and Valentine [11]. G-Px catalyzes the oxidation of GSH by NADP⁺. In the presence of GSH-r and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form (GSH) with concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance was measured at 340 nm. GSH-r activity in hemolysate was assayed using the method of Beutler [12]. GSH-r catalyzes the reduction of GSSG in the presence of NADPH, which is oxidized to NADP⁺. The decrease in absorbance was measured at 340 nm.

Catalase activity in blood samples was assayed according to the method of Sinha [13] based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide, the green colored produced compound has the maximum absorbance at 570 nm. SOD activity in serum was assayed by a method of Beuchamp and Fridovich [14]. The principles of SOD assay was based on the inhibition of NTB (nitroblue tetrazolium) reduction by superoxide radicals to blue colored formazan

with maximum absorbance at 560 nm. TBARS was measured according to method of Ohkava modified by Turgut *et al* [15]. Hemoglobin (Hb) was determined by the cyanomethemoglobin method, using the Drabkin reagent (Sigma-Aldrich, Saint Louis, Missouri, USA). All measurements were made with a Shimadzu UVmini 1240 spectrophotometer (Shimadzu, Kyoto, Japan).

Statistical analysis

The results are expressed as mean \pm SEM. For the evaluation of differences between groups, one-way ANOVA with Bonferroni's correction was used, with statistical difference set at $p < 0.05$. The statistical software used was GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, USA).

RESULTS

The mean aluminium content of the plant was 4.736 $\mu\text{g/g}$ while it was 157 $\mu\text{g/L}$ in the aqueous extract.

Body weight, blood aluminium levels and hemoglobin

The body weight was slightly increased in all experimental groups compared to control, but the differences were not significant ($p > 0.05$).

Blood Al level was significantly ($p < 0.001$) increased in rats exposed to AS compared to control (293.64 %). When the rats were exposed to AS in combination with *S. tectorum* extract, Al level decreased significantly ($p < 0.001$) comparative to the group given AS alone (33.45 %) but was still increased comparative to control (161.95 %, $p < 0.001$). In rats exposed to extract alone, Al level was lower than in control group but the differences were not significant (10.18 %, $p > 0.05$). The administration of the extract during one month after AS exposure was stopped did not significantly reduce blood Al level comparative to Al group (7.8 %, $p > 0.05$), remaining still significantly ($p < 0.001$) higher than control (262.74 %). Compared to Al+St

group, the blood Al level was significantly ($p < 0.05$) higher (38.47 %). The differences regarding Hb levels were not significant ($p > 0.05$).

Oxidative Stress Biomarkers

GSH level (Fig 1) decreased significantly ($p < 0.01$) in the Al group compared to control (13.49 %) and increased when the rats were exposed only to extract (55.63 %, $p < 0.01$). Administration of the extract together with AS restored the GSH level but it still remained lower than in control group (3.94 %, $p > 0.05$). When the extract was administered one month after the AS administration was stopped the GSH level was significantly increased ($p < 0.05$) relative to the Al group (14.88 %) but was not significantly ($p > 0.05$) different from the Al+St (3.47 %) and control (0.61 %) groups.

A significant decrease ($p < 0.0001$) in the GSH-r level in rats exposed to AS, compared to control (23.08 %) was seen (Fig 1). When AS was administered together with extract GSH-r increased but still remained significantly ($p < 0.01$) lower than in control (12.06 %). The same profile was seen when the extract was administered after AS exposure was stopped (7.90 %, $p > 0.05$). Compared to Al group, GSH-r levels were higher in Al+St (14.32 %, $p < 0.01$), St (40.28 %, $p < 0.001$) and Al+1mSt (19.73 %, $p < 0.001$) groups. No significant ($p > 0.05$) differences in GSR-r were seen between control and St group (7.90 %) and also between Al+St and Al+1mSt (4.37 %).

G-Px increased significantly ($p < 0.001$) in rats exposed to AS (31.25 %) but the decrease when AS was administered together with extract, was *not significant*. Not significant ($p > 0.05$) differences in G-Px level were observed between the group exposed to extract and control group (11.64 %). Administration of the extract for a month after the AS intake was stopped, resulted in G-Px recovery to a level not significantly ($p > 0.05$) different from control (2.44 %). G-Px levels were lower in Al+St (22.30 %, $p < 0.001$), St (32.68 %, $p < 0.001$) and Al+1mSt (21.95 %, $p <$

Table 1: Body weight, Al blood levels and hemoglobin (mean \pm SEM) in rats exposed to aluminium and *Sempervivum tectorum* extract

Parameter	C	Al	Al + extract	St	Al + extract for 1 month
Body weight (g)	380.0 \pm 14.61	406.7 \pm 11.16 ^a	383.3 \pm 10.85 ^a	403.3 \pm 15.2 ^a	403.4 \pm 8.81 ^a
Al blood level ($\mu\text{g/L}$)	9.158 \pm 0.56	36.05 \pm 2.77 ^b	23.99 \pm 1.46 ^{b,c}	8.225 \pm 0.56 ^a	33.22 \pm 2.19 ^{b,d}
Hb (g/L)	2.26 \pm 0.07	1.88 \pm 0.04	2.27 \pm 0.22	2.72 \pm 0.30	2.11 \pm 0.02

Comparison with control group: ^a = not significant; ^b $p < 0.001$; comparison with Al group: ^c $p < 0.01$; comparison between Al+St and Al+extract for 1 month: ^d $p < 0.05$

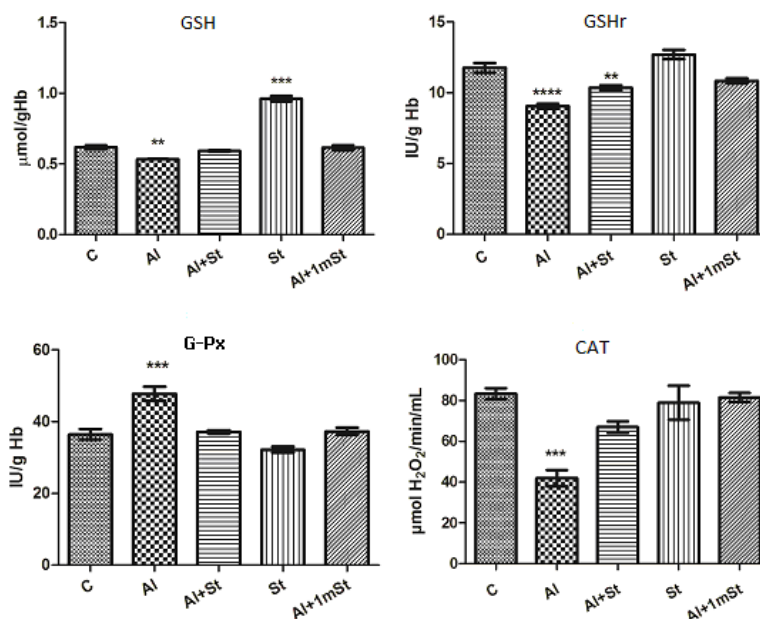


Fig 1: GSH, GSH-r, G-px and CAT levels in rats exposed to aluminium and *Sempervivum tectorum* extract; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with control

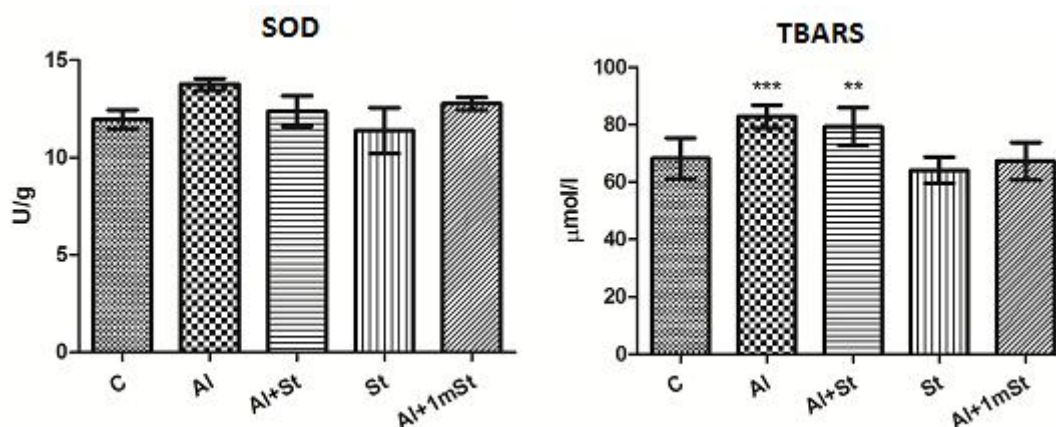


Fig 2: SOD and TBARS levels in rats exposed to aluminium and *Sempervivum tectorum* extract; ** $p < 0.01$, *** $p < 0.001$ compared with control

0.001) groups compared to Al group. G-Px activity was not significantly different in Al+1mSt group compared to Al+St group (0.45 %, $p > 0.05$).

CAT activity was reduced, significantly ($p < 0.001$) in group exposed to AS (49.59 %) but not significantly ($p > 0.05$) when AS was administered together with extract (19.60 %). The activity was recovered in the presence of the extract when it was administered one month after AS administration was stopped even though it still remained lower than control (2.18 %, $p > 0.05$). This activity was significantly higher in Al+St (59.50 %, $p < 0.001$), St (87.97 %, $p < 0.001$) and Al+1mSt (94.06 %, $p < 0.001$) groups compared to Al group.

In all experimental groups, the difference in SOD levels (Fig 2) was not significant ($p > 0.05$) when compared to control.

TBARS values (Fig 2) significantly ($p < 0.001$) increased in Al group compared to control (21.37 %). The extract reduced TBARS level when was administered together with AS, but the values were still significantly ($p < 0.01$) increased compared to control (16.28 %). Administration of the extract after AS exposure was stopped, significantly ($p < 0.001$) decreased the TBARS level, being even lower than control.

DISCUSSION

The release of free iron ions from biological complexes by aluminium can catalyze hydrogen peroxide decomposition to hydroxyl radical via

Fenton's reaction, initiating cellular damage [16-18]. Aluminium is able to inhibit NADPH-generating enzymes such as glucose-6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase, and because the reduced NADP is a co-enzyme for GSH regeneration, the decreased GSH level could be due to insufficient supply of NADPH [19]. We observed a significant decrease in the levels of erythrocyte GSH in groups exposed to AS, which were restored in all groups by extract administration. Moreover, the activities of erythrocyte antioxidant enzymes SOD and G-Px were increased in rats exposed to AS compared but this was reversed when AS was administered together with *S. tectorum*. SOD is an important antioxidant enzyme neutralizes superoxide anion. This increase of SOD might be an adaptative response.

G-Px plays a significant role in the peroxy scavenging mechanism and in the maintenance of the integrity of erythrocyte cell membrane [20]. The increase of GSH-Px activity could be due to its induction to counter the effect of increased oxidative stress induced by aluminium exposure. We observed that SOD and GPx levels were reduced when the extract was administered alone or together with AS. CAT protects the cells from the accumulation of hydrogen peroxide by converting it to form water and oxygen or by using it as an oxidant which works as a peroxidase [21]. Other authors have suggested that exposure to aluminium was followed by a decrease in the activities of main antioxidant SOD and G-Px in blood (not supported by our findings) and also a decrease in blood CAT activity (which is in accordance with our findings) [22,23].

As a response of oxygen species, a great diversity of aldehydes are formed in biological system that oxidize polyunsaturated fatty acids, and lead to lipid peroxidation. These metabolites lower the body defense system especially by the disturbance of SOD [24]. Regarding the lipid peroxidation, we observed a rise of TBARS levels as consequence of AS exposure, these levels were recovered after extract administration. Elevation of TBARS concentration is due to increased peroxidation of lipids in plasma membranes and is an indicator of oxidative stress [25]. Raise of TBARS could be due to the excessive oxidative damage induced by aluminium exposure. The increase of TBARS in plasma was also noted in a study of Sallam et al. [26] in rabbits exposed to 34 mg/kg b.w. aluminium chloride, and when it was used an antioxidant (ascorbic acid), the levels of TBARS were restored. The same dynamics of TBARS and GSH was recorded in a study of Turgut et al.

[15] in serum and liver of mice exposed to 300 mg/kg b.w. AS. They observed that TBARS and GSH levels were recovered by administration of a well known antioxidant – vitamin E.

Flavonoids have been shown to alleviate the oxidative stress by increasing the endogenous antioxidant status, protecting cells against free radicals damage by increasing resistance to oxidative stress [27]. *S. tectorum* contains flavonoids such as kaempferol [5, 6]. Blazovics et al. [28] emphasized that the active compound of *Sempervivum tectorum* extract were able to influence the changed cellular redox status in mucosa of all parts of the intestinal tract in experimental bowel disease and pointed out the protective effect of natural polyphenols and flavonoids of *S. tectorum* in this disease. Kéry et al. [29] noted that *S. tectorum* extract exhibits a strong inhibition of chemiluminescence, indicating a non-specific free radical scavenging activity. The extract had true superoxide scavenger activity in all free systems and this suggest that it may also act as a direct scavenger of superoxide anions [29].

Regarding the blood Al level, in the present study we observed that it was significantly increased by AS exposure and decreased in presence of the extract. The significant reduction of Al levels by *S. tectorum* a.e. only in Al+St group can be due to the tannin content of this plant [30], the tannins reducing the Al absorption by precipitation in digestive tract. Administration of *S. tectorum* one month after AS administration was stopped had not the same effect on Al level, which was not significantly lower than in Al group and was significantly higher than in Al+St group. Our results were in accordance with Szentmihályi et al. [31] which observed a significant reduction of Al level in liver of hyperlipidemic rats following *S. tectorum* extract administration due to elevation of Al excretion.

CONCLUSION

The results of our study indicate that aluminium exposure leads to oxidative stress due to the production of free radicals, increased lipid peroxidation and decreased catalase activity. The aqueous extract of *S. tectorum*, due to its good antioxidant properties, protects against the free radicals produced by aluminium exposure.

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