# Study on the Influence of *Sempervivum tectorum* and Melatonin on Glutathion Protective Effects in Rats Blood Exposed to Aluminum Sulphate

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**Abstract**
The present study was carried out to investigate the influence of *Sempervivum tectorum* aqueous extract and melatonin on reduced glutathione (GSH) protective effect in Wistar albino rat blood exposed to aluminium sulphate- $Al_2(SO_4)_3$. The rats were divided in one control group (C) and 7 experimental groups (E). The control group received tap water. The experimental rats were feed the following way: E1 group – aluminum sulphate, daily, for 3 months; : E2 group – *Sempervivum tectorum*, daily, for 3 months; : E3 group – melatonin, daily, for 3 months; : E4 group – aluminum sulphate with *Sempervivum tectorum*, daily, for 3 months; : E5 group – aluminum sulphate with melatonin, daily, for 3 months; E6 group – aluminum sulphate, daily, for 3 months, and thereafter with *Sempervivum tectorum* for 1 month; E7 group – aluminum sulphate, daily, for 3 month, and thereafter with melatonin for 1 month. This study showed that Aluminum toxicity induced lower GSH. The oxidative stress caused by aluminum, given individual, is more pronounced than in the case in which aluminum is administered simultaneously with *Sempervivum tectorum* or melatonin. Decreasing GSH value is very small if *Sempervivum tectorum* or melatonin is given for one month, three months after the administration of aluminum. Effect induced by melatonin is more favorable than that of *Sempervivum tectorum*.

**Keywords**: aluminum, GSH, melatonin, rat, *Sempervivum tectorum*.

## 1. Introduction

Aluminum is the most abundant metal and the third most abundant element in earth's crust, comprising about 8.8% by weight (88 g/kg). It is never found free in nature but is present in most rocks, particularly igneous rocks, as aluminosilicate minerals [1].

Aluminum is also present in air, water, and many foods. Aluminum enters environmental media naturally through the weathering of rocks and minerals. Anthropogenic releases are in the form of air emissions, waste water effluents, and solid waste primarily associated with industrial processes, such as aluminum production. Because of its prominence as a major constituent of the earth's crust, natural weathering processes far exceed the contribution of releases to air, water, and land associated with human activities [2].
Aluminum is not bioaccumulated to a significant extent. Certain plants can accumulate high concentrations of aluminum. For example, tea

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leaves may contain very high concentrations of aluminum, >5.000 mg/kg in old leaves [3].
Other plants that may contain high levels of aluminum include Lycopodium (Lycopodiaceae), a few ferns, Symplocos (Symplocaceae), and Orites (Proteaceae) [4].
Aluminum does not appear to accumulate to any significant degree in cow's milk or beef tissue [5].
Similarly, because of its toxicity to many aquatic organisms, including fish, aluminum is not in significant degree in aquatic organisms. [6].
While the intake of aluminum is mainly through the ingestion of food and drinking water, inhalation of ambient air represents a small contribution to an individual's exposure to aluminum [7].
Processed foods containing aluminum additives such as processed cheese and grain-based products constitute the foods with the largest quantities of aluminum and the largest components of the dietary intake of children [8].
Aluminum intake from aluminum-containing medication, such as antacids, buffered aspirins, and antidiarrheal agents are ordinary intakes [8].
Children may be exposed to aluminum from aluminum-containing medications, vaccinations, parenteral feeding, dialysis fluids, and treatment for hyperphosphatemia [9].
Aluminum is a neurotoxin [10].
Aluminum exposure promoted oxidative stress in different neural areas of the animals, including those in which aluminum concentrations were not significantly increased. [11].
This study was performed to investigate the influence of aqueous extract *Sempervivum tectorum* and melatonin, on the protective character of reduced glutathione (GSH) in the blood of Wistar albino rat, exposed to aluminum sulphate $Al_2(SO_4)_3$.

## 2. Materials and methods

The study was performed in compliance with good laboratory practice; in accordance to the European Convention principles for the protection of vertebrates animals used in experimental and other scientific purposes, adopted in 1986, in Strasbourg and the 2010/63/EU Directive of the European Parliament and of the European Council adopted in 22 September 2010, on the protection of animals used for scientific purposes, in accordance with Romanian law for animal experimentation and having the acceptance of the Scientific Ethic Committee of Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania" from Timisoara (no.3558/05.06.2012).
This study was carried out on 40 adult Wistar albino rats, male, having a weight of 250±10 g, purchased from the Animal House of University of Medicine and Pharmacy "Victor Babeş" Timisoara, Romania, divided in one control group (C) and 7 experimental groups ($E_1$, $E_2$, $E_3$, $E_4$, $E_5$, $E_6$, $E_7$).
The control group (C) received tap water, ad libitum.
The experimental rats were feed the following way: E1 group – aluminum ($Al^{3+}$), daily, for 3 months; E2 group – 6% *Sempervivum tectorum* infusion, daily, for 3 months; E3 group – melatonin (10mg/100ml water), daily, for 3 months; E4 group – $Al^{3+}$l with 10% *Sempervivum tectorum* infusion, daily, for 3 months; E5 group – $Al^{3+}$ with melatonin (10mg/100ml water), daily, for 3 months; E6 group – $Al^{3+}$, daily, for 3 months, and thereafter with 6% *Sempervivum tectorum* for 1 month; E7 group – $Al^{3+}$, daily, for 3 month, and thereafter with melatonin (10mg/100ml water) for 1 month.
$Al^{3+}$ administration was performed using aluminum sulfate, $Al_2(SO_4)_3$, in drinking water, 1000ppm. This harmful level has been found in drinking water around Alro SA Slatina [12].
After the experimentation period the rats were euthanatized with ketamin, 50 mg/kg b.w., the blood was collected in BD heparinized vacutainers (ref. no. 367885). Blood samples being used for reduced glutathione (GSH) determination. He was quantitatively measured at T60 uv-vis Spectrophotometer, through Beutler et al. method [13] at λ=412 nm of yellow color developed by adding 5,5'-dithiobis(2-nitrobenzoic acid), DTNB, to sulphahidryl compounds. The results are expressed as μmol GSH/g Hb by estimating hemoglobin concentration of original whole blood.
Results are expressed as average values ±SEM for each experimental group. The results were statistically analyzed by ANOVA method.

## 3. Results and discussion

Glutathione, the tripeptide γ-glutamyl-cisteinyl-glycine, has several important functions. It is a

reductant, conjugated to drugs to make them more water soluble. He is involved in transport of aminoacids across cell membranes, can be a cofactor in some enzymatic reactions and a helper in the rearranging of protein disulfide bonds. Usually inside the erythrocyte ratio of GSH - GSSG is 100:1 [14].

An important function of GSH in erythrocyte is to reduce $H_2O_2$ and organic peroxides, reactive oxygen metabolites, which may permanently damage the hemoglobin and cleave C-C bonds in the phospholipids that make up cell membranes [15].

The blood test results are presented in Table 1 and Figure 1.

**Table 1.** GSH mean values in the control group (C) and the experimental groups (E)

| Group | Control | E1 | E2 | E3 | E4 | E5 | E6 | E7 |
|---|---|---|---|---|---|---|---|---|
| $\bar{x}$ | 0.628 | 0.206 | 0.697 | 0.716 | 0.426 | 0.463 | 0.529 | 0.557 |
| ±S.E.M. | ±0.039 | ±0.002 | ±0.003 | ±0.003 | ±0.004 | ±0.007 | ±0.004 | ±0.007 |

Unit GSH: µmol GSH/g Hb

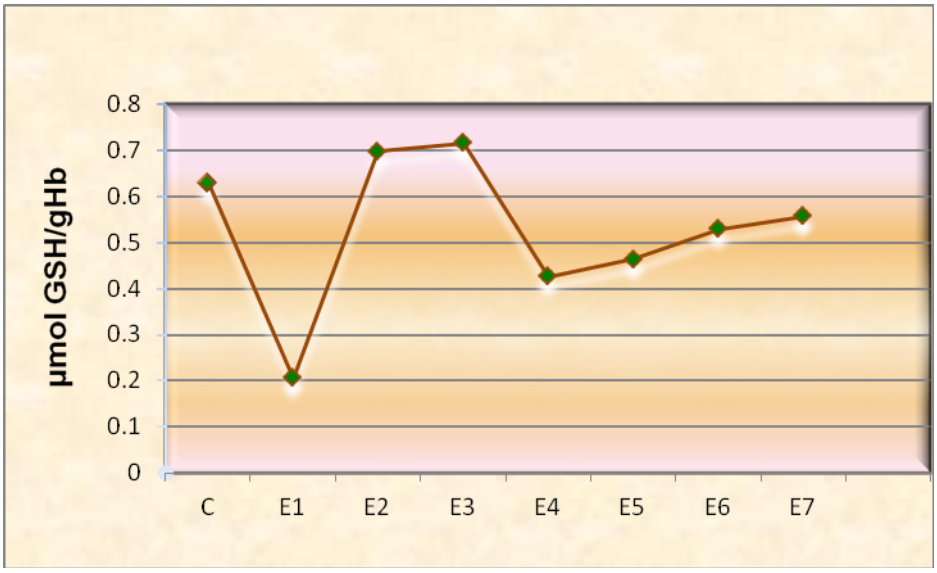


**Figure 1.** Concentration of reduced sugars after hydrolysis with enzymatic mixtures

Free radicals are produced mainly in biochemical redox reactions involving oxygen; there is a part of cellular metabolism through a series of monovalent reductions.

Glutathione is involved in many metabolic processes, effectively eliminating free radicals and other reactive oxygen species (hydroxyl radicals, lipid-peroxides, nitric peroxides and $H_2O_2$) directly and indirectly through enzymatic reactions.

GSH protects mitochondria and cell membranes from the damaging effects of reactive oxygen species (oxidative stress), protects the tertiary structure of proteins and activates the aminoacid transport through the cell membrane [16].

The most important function of GSH is the detoxification of xenobiotics and drug by GSH conjugation reaction [17].

GSH binds to heavy metals, like zinc, mercury, cadmium and copper and transport them to the liver, where they are further detoxified and excreted [18].

The oxidation state of Aluminum is 3+ [19] and GSH has electron donating property [20]. Aluminum metallic-element may interact with the GSH in the plasma and cytosolic fraction of blood. In the aqueous phase of blood GSH is present and Aluminum has strong affinity for GSH. This affinity mainly exists between Aluminum and sulfhydryl groups of GSH [21].

This affinity may decrease the reduced form of GSH. GSH depletion is leading to its synthesis from cysteine via γ-glutamyl cycle. Because GSH is not effectively synthesize GSH depletion continues due to the chronic exposure to metal [21].

*Sempervivum* species are well known plants in folk medicine for the treatment of ear inflammation [22].
Drinking tea prepared from leaves of *S. tectorum* is recommended for ulcer treatment [23].
Fresh juice squeezed from the leaves of some species of *Sempervivum* has been used as folk medicine in many countries almost exclusively for external purposes. It can be spread as a pack on wounds, burns, and abscesses and on painful areas attacked by gout as a refrigerant and astringent. *S. tectorum* extract reduced lipid levels in rats and has antimicrobial and in-vitro antioxidant properties [24-26].
Antioxidative and anti-inflammatory effects of *Sempervivum* have been previously described, though the mode of action is still unexplained and neither compound has been attributed to these effects. Phytochemical screening of Sempervivum extracts with different polarity proved the presence of notable quantity of polysaccharides, polyphenolic compounds, flavonoids and organic acids [27].
Melatonin, N-acetyl-5-methoxytryptamine, is the major product of the pineal gland in vertebrates. It is a well-known antioxidant and free radical scavenger. Moreover, its solubility in lipid and aqueous media, which allows it to cross morphophysiological barriers and enter subcellular compartments, permit melatonin to function as a highly effective inhibitor of oxidative damage [28]. It is a very potent and efficient endogenous free radical scavenger. It reacts with the highly toxic hydroxide radical and provide on–site protection against oxidative damage to different biomolecules [29]. It is also involved in the regulation of electron transfer, detoxifying reactive radical intermediates and control pre-oxidative processes [30].

## 4. Conclusions

GSH is the principal reducing agent in erythrocytes, the principal scavenger of oxygen species in mitochondria. The consequences of ad libitum $Al^{3+}$ administration, 1000ppm, due to his affinity for GSH – reduced form, induces the inhibition of the regeneration of GSH from the oxidized form (GSSG).
$Al^{3+}$ individual administration (E1) induced a pronounced decrease of GSH.
*Sempervivum tectorum* aqueous extract (E2) and melatonin (E3) administration induced a slightly increase GSH, compared with the control group (C).
The simultaneous administration of *Sempervivum tectorum* with $Al^{3+}$ (E4) and melatonin with $Al^{3+}$ (E5) induced a significantly increase of GSH in comparison with E1 group.
When *Sempervivum tectorum* and melatonin extract was administered one month (E6, E7), after the $Al^{3+}$ administration was stopped, GSH level was increased considerably relative E1 group, more than E4 and E5. In this case GSH level was also slightly lower compared to the control group (C).
Difference between the effects of *Sempervivum tectorum* and melatonin was insignificant.

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