The aim of the study was to evaluate the protective status of an antioxidant combination (melatonin and Sempervivum tectorum extract) on aluminium accumulation in genital organs of male rats. The 12 weeks study was carried out on 35 Wistar male rats divided in five groups: C – control, receiving distilled water; E1 – aluminium sulphate 0.1 mg/L; E2 – melatonin 10 mg/kg b.w. in drinking water; E3 – 6% aqueous extract of Sempervivum tectorum; E4 – combination, melatonin and plant extract. The study pointed out the significant (p<0.001) accumulation of aluminium in testis and accessory glands comparative to control. The aluminium level was significantly (p<0.05) reduced in groups exposed to melatonin and S. tectorum extract compared to aluminium exposed group, remaining still significantly (p<0.05) higher than in control. The combination of used antioxidants (E4) was proven more efficient in protection against aluminium accumulation, especially in testis.

Key words: aluminium, sexual organs, antioxidants.

INTRODUCTION

Aluminium, considered the third most abundant element, was related to have negative effects that exceed the benefic effects being considered a trace element with toxic risk potential (Poston, 1991; Kiss and Szolnoki-Csikkel, 1993). Aluminium is released in the environment from various anthropogenic sources and by natural processes, being found in silicates as hydrous aluminium oxides and hydroxides and it is not found as free metal and has only oxidation state (Howe, 1998).

Aluminium compounds are widely used in medicine as antacids, phosphate binders, buffered aspirins, vaccines, antiperspirants and allergen injection (Kaehny et al., 1997; Exley, 1998).
Melatonin, also known as N-acetyl-5-methoxytryptamine, is a ubiquitous molecule and widely distributed in nature, with functional activity occurring in unicellular organisms, plants, fungi and animals (Pandi-Perumal et al., 2006). In most vertebrates, including humans, melatonin is synthesized primarily in the pineal gland and is regulated by the environmental light/dark cycle via the suprachiasmatic nucleus (Pandi-Perumal et al., 2006; Fyiad, 2007; Reiter, 1991). Melatonin has been shown to protect effectively against oxidative damage caused by a variety of toxins (Reiter et al., 2000).

*Sempervivum tectorum*, part of *Crasulaceae* family, is still used in folk medicine, either taken internally or used externally. It is extremely useful in skin diseases, inflamed eyes, and enlarged glands (Leyel, 2007). It is also used in ear inflammations, herpetic skin eruption, minor burns and wounds (Alberti et al., 2009).

In the present study we want to emphasize the protective effect of melatonin and *S. tectorum* on aluminium accumulation in sexual organs of male rats following subchronic exposure.

**MATERIAL AND METHODS**

The work was carried out on 35 white adult Wistar rats purchased from “Victor Babeș” University of Medicine and Pharmacy Timișoara animal facility. The rats were kept in plastic cages, fed with standard diet, 25°C controlled ambient temperature, with 12 h dark/light cycle.

The rats were divided in five groups: C – control, receiving distilled water; E₁ – aluminium sulphate 0.1 mg/L; E₂ – melatonin 10 mg/kg b.w. in drinking water; E₃ – 6% aqueous extract of *Sempervivum tectorum*; E₄ – combination, melatonin and plant extract. The time of exposure was three months.

At the end of the experiment the rats were euthanatized after ketamine 10%, 50 mg/kg b.w. anesthesia and the sexual organs (testis and epydidymis) and accessory glands (prostate, seminal vesicles and bulb-urethral glands) were collected. The organ samples were prepared by microwave digestion as follows: 1 g sample, 10 ml HNO₃ and 2 ml H₂O₂ in digestion plastic flask for 10 min, 120°C, 800 W using a CEM Mars X5 digestion accelerator. Aluminium was determined by atomic absorption spectroscopy at 309.3 nm wavelength using a Shimadzu AA6650 spectrometer (Shimadzu, Kyoto, Japan) with graphite furnace (pyrolytic graphite tube). Aluminium standard solution – Al(NO₃)₃ in HNO₃ 0.5 mol/l 1000 mg/l Al was purchased from Merck KGaA, Darmstadt, Germany.

For the evaluation of differences between studied groups, one-way ANOVA with Bonferroni’s correction was used, considering statistical difference when p<0.05 or lower. The values were expressed as mean ± SEM. The statistical software used was GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, USA).
RESULTS AND DISCUSSIONS

Previous experimental investigations on the status of aluminium storage in various organs revealed the protective role of melatonin in the accumulation of this trivalent metallic element in rats (Muselin et al., 2014).

Aluminium level in testis (Fig. 1) increased significantly (p<0.001) in rats exposed to aluminium compared to control (+316.15%). Administration of melatonin together with aluminium reduced significantly (p<0.01) the accumulation of aluminium in testis (-49.08%), the same situation was observed in case of S. tectorum (-49.55%) and in case of combination of the both antioxidants (-50.11%).

![Fig. 1. Aluminium levels in rat’s testis.](image1)

** *** p<0.001 compared to C
// p<0.01 compared to E1

In the rats epydidymis, aluminium concentration was significantly higher in exposed groups than in control (+212.65%, p<0.01). Administration of melatonin together with aluminium reduced significantly the accumulation of aluminium (-35.22%, p<0.05), the same situation being recorded in case of S. tectorum (-39.25%, p<0.05) or both antioxidants combined (-42.36%, p<0.05) – Fig. 2.

![Fig. 2. Aluminium levels in rat’s epydidymis.](image2)

** ** p<0.01 compared to C
/ p<0.05 compared to E1
Aluminium exposure was followed by a significant accumulation of aluminium in the prostate (Fig. 3) compared to control (+133.78%, p<0.001). Aluminium level decreased significantly when melatonin and *S. tectorum* were administered (E2/E1: -39.68%, p<0.01; E3/E1: -34.92%, p<0.01; E4/E1: -52.38%, p<0.001).

![Fig. 3. Aluminium levels in rat’s prostate.](image)

** p<0.05 compared to C
*** p<0.01 compared to E1
**** p<0.001 compared to E1

Exposure of rats to aluminium was followed by a significant accumulation of aluminium in the seminal vesicles (Fig. 4) compared to control (+129.09%, p<0.01). Aluminium level decreased significantly when melatonin and *S. tectorum* were administered (E2/E1: -44.44%, p<0.05; E3/E1: -40.12%, p<0.05; E4/E1: -47.13%, p<0.05).

![Fig. 4. Aluminium levels in rat’s seminal vesicles.](image)

** p<0.01 compared to C
/ p<0.05 compared to E1
In the bulbo-urethral glands, aluminium accumulated significantly compared to control (+97.18%, p<0.05) (Fig. 5). Aluminium level decreased when melatonin and *S. tectorum* were administered, but not significantly (E2/E1: -14.28%, p>0.05; E3/E1: -16.152%, p>0.05; E4/E1: -19.23%, p<0.05).

Fig. 5. Aluminium levels in rat’s bulbo-urethral glands. *p<0.05 compared to C*

In present study we observed that aluminium accumulated in studied organs and the accumulation was limited by the use of melatonin and *S. tectorum* extract. The mechanism by which melatonin decreases heavy metals concentration in the tissues is not known. The reduction of aluminium levels by *Sempervivum tectorum* infusion could be a consequence of tannin content of this plant, the tannins precipitating the aluminium in the digestive tract or even removing the aluminium from those tissues (Bunny, 1992).

Aluminium bioaccumulation can be produced, like in case of other chemical xenobiotics, by fixing on plasma proteins or on the protein receptor. Frequently competition for fixing on the protein can occur as a competition between two substances.

Accumulation of aluminium in internal organs was pointed out by many authors. Thus, Spencer et al. 1995 observed that aluminium presents higher levels in rat’s liver and kidney in one hour after 800 μg iv. administration compared to control. Szentmihályi et al. 2004 noted that aluminium accumulated in liver of hyperlipidemic rats compared to control and was reduced by *S. tectorum* administration. Guo et al., 2009 pointed out the aluminium accumulation in testis of mice’s that received 7 and 35 mg/kg aluminium chloride intra peritoneal for 14 days.

Some reports suggest a possible mechanism of melatonin influence upon metals accumulation. Limson et al. 1998 and Chwefatiuk et al. 2006, observed that in mice which received drinking water containing 50μg Cd/mL, or 50μg Cd/mL with additional 2,4 or 6 μg/mL melatonin for 8 weeks, melatonin co-treatment was followed by a dose dependent decrease in renal, hepatic and intestinal Cd
concentration. One possibility is that melatonin, which is capable to form stable complexes with metals, e.g. Cd, inhibits intestinal absorption of this metal, especially its uptake from the intestinal lumen into mucosa (Limson et al., 1998, Chwela’tiuk et al., 2006). Another possible explanation is that melatonin, which is highly lipid-soluble, can move freely across all cellular barriers, facilitating the removal of metal from soft tissues (Reiter et al., 2002).

CONCLUSIONS

Exposure of rats to aluminium sulphate has as consequences the accumulation of this trace element in testis, epididymis, prostate, seminal vesicles and bulbo-urethral glands in amounts significantly higher than in control group. Melatonin and S. tectorum extract had a protective effect, significantly reducing the accumulation of aluminium in studied organs, especially in testis.

Acknowledgements: This work was published during the project “POSTDOCTORAL SCHOOL OF AGRICULTURE AND VETERINARY MEDICINE”, POSDRU / 89 / 1.5 / S / 62371, co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007-2013.

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