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**The status of some bioelements in rats with oxidative stress induced by cisplatinum and aluminum**

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**Abstract**. The paper presents data regarding the status of some important bioelements that act as co-factors for different enzymes in rats with induced oxidative stress by the administration of aluminum and cisplatinum. The study was made on thirty-six Wistar albino young rats (three months old) divided randomly in four groups as follows: C – Control, 1 ml of physiologically saline (P.S); E1 – administration of 100 mg/kg bw AlCl3; E2 – administration of 10 mg/kg bw cisplatinum; E3 – administration of 100 mg/kg bw AlCl3 and 10 mg/kg bw cisplatinum. All administrations were via the intraperitoneal route (i.p.), once a week, for a four weeks period. Were measured the oxidative stress enzymes CAT (catalase), SOD (superoxide dismutase), glutathione peroxidase (GPx), Glutathione reductase (GSH-r) and the blood levels of iron, manganese, magnesium, copper, zinc, and selenium. In all experimental groups were observed the significant decrease of CAT, the significant increase of GPx and GSH-r, not significant increase of SOD, not significant fluctuations of manganese and magnesium, a significant decrease of zinc and copper and not significant decrease of selenium. We can conclude that aluminum and cisplatinum can impair the normal status of the main bioelements through induced oxidative stress.
**Key words**: rats, bioelements, trace elements, oxidative stress, cisplatinum, aluminium.

**Introduction.**

Numerous platinum compounds are widely used as chemotherapeutic agents, cisplatinum being one of them, with use in the largest covering pallet of tumors including testis, ovary, lung and bladder cancers (Dilruba S and Kalayda GV, 2016; Fuertes MA et al., 2003). There are many studies that pointed out the capacity of cisplatinum to induce oxidative stress by different mechanisms (Smigic J et al., 2018; Muselin F et al., 2018).

Aluminum, an abundant metal in the Earth crust, is known as a very potent neurotoxic and reproductive toxic trace element (Liaquat L et al., 2019; Sood PK et al., 2011), also with a more and more studied ability to induce oxidative stress (Yousef MI, 2004; Muselin F et al., 2014; Benyettou I et al., 2017), not directly but, in some cases, through some bioelements such as iron and copper (Halliwell B and Gutteridge JMS, 1992).

The essential bioelements were defined as the elements whose dietary deficiency can change adversely and consistently the biological function from optimal and this change could be prevented or reestablished by physiological amounts of the element (Nielsen FH, 2003).

There is known that aluminum and cisplatinum have the ability to induce oxidative stress disturbing the activity of numerous antioxidant enzymes (Muselin F et al., 2018; Yousef MI, 2004; Muselin F et al., 2014), and by this affecting the normal status of some bio-element in the body.

The aim of the presented study was to emphasize the status of some bioelements in rats blood in the condition of induced oxidative stress as a consequence of aluminum and cisplatinum administration.

**Materials and methods.**

For this experiment were used thirty-six Wistar albino rats aging three months and 200±10 g, obtained from the authorized animal facility of University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania, housed in standard polycarbonate cages and fed *ad libitum* with the standard diet. They were maintained in 12 h light/dark cycle at 22 ± 2 °C and 55 ± 10% relative humidity. Prior to the start of the experiment, animals were kept one week for acclimatization being handled in accordance with

Directive 2010/63/EU on the handling of animals used for scientific purposes (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX: 32010L0063&rid=2). The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine from BUASMV Timisoara (No.120/2018).

The rats were randomly distributed in four experimental groups (n=9) as follows: C – Control; 1 ml of physiologically saline (P.S) via i.p administration; E1 – administration i.p of $AlCl_3$ (100 mg/kg bw); E2 – administration i.p of cisplatinum (10 mg/kg bw); E3 – administration i.p of $AlCl_3$ (100 mg/kg bw) and cisplatinum (10 mg/kg bw). The i.p administration was once a week for a four weeks period. At the end of the experiment all rats were euthanized by overdosing anesthetic agents (Ketamine 10%, CP Pharma, Germany, and Narcoxyl, Intervet International, Netherland), and blood was collected into heparinized BD Vacutainer and centrifuged for 10 min at 3000×g to separate erythrocytes and plasma and processed according to the manufacturer of the kit, being washed three times with saline solution and the separates were stored at $-80^0$C until the analyses were performed.

The assessed parameters were the activity of catalase (CAT), glutathione reductase (GSH-r) and superoxide dismutase (SOD) in erythrocytes, the activity of glutathione peroxidase (GPx) in whole blood, using a Randox RX-Daytona automated analyzer (Randox, Crumlin, UK) and commercially available kits (Randox, Crumlin, UK). The activities of the antioxidant enzyme were measured at 37 $^0$C and were expressed in U/g Hb. The CAT activity was assessed by a method described by Hadwan (Hadwan MH, 2018).

. The levels of the main bioelements such as manganese (Mn), selenium (Se), magnesium (Mg), zinc (Zn), copper (Cu), and iron (Fe) in blood were assessed by atomic absorption spectroscopy (AAS) using a Perkin Elmer AAanalyst800 spectrophotometer (Perkin Elmer Inc. USA).

Samples preparation for AAS analyses were performed by microwave digestion (Multiwave GO, Anton Paar, GmbH, Austria), adding 10 mL of concentrated nitric acid and 2 mL of hydrogen peroxide over 1 g of sample with the parameter set as 120 °C, 800 W for 20 min. All used reagents were a high-purity grade (Suprapur Merk, Germany) and the calibration standards were prepared from a Merck CertiPur ICP 1000 mg/L stock standard solution.

The obtained results were expressed as mean ± SEM by one-way ANOVA with the Bonferroni correction considering the differences are statistically provided when $P\leq0.05$ or lower. The software used was GraphPad Prism 6.0 for Windows (GraphPad Software, San Diego, USA).

**Results and discussions.**

Some of the bioelements act as co-factors for multiple anti-oxidant enzymes as are superoxide dismutase (Cu, Zn, Mn), catalase (Cu, Fe), and glutathione peroxidase (Se), and by this could have the ability to reduce oxidative damage in living organisms (Méplan C, 2011).

As is presented in Figure 1, in our study we observed the increase of GPx, SOD and GSH-r in groups exposed to aluminum (+32.77%, $P\leq0.01$; +18.35%, $P\geq0.05$; +55,81%, $P\leq0.001$) and cisplatinum (+34.45%, $P\leq0.01$; +15.29%, $P\geq0.05$; +63.55%, $P\leq0.001$) and the significant decrease of CAT (-21.57%, $P\leq0.0001$; -29.32%, $P\leq0.0001$) comparative to control group.

Administration of aluminum chloride and cisplatinum increased significantly the GPx (+56.69%, $P\leq0.001$), SOD (+26.74%, $P\leq0.05$), GSH-r (+90.21%, $P\leq0.0001$) and decreased significantly the CAT activity (-37.21%, $P\leq0.0001$) comparative to control group. Even so, the values of enzymes activity in E3 group were not significantly ($P\geq0.05$) different comparative to E1 and E2 groups, except the CAT activity which was significantly ($P\leq0.01$) decreased in E3 comparative to E1 (-19.93%).

Aluminum and cisplatinum have the capacity to release the free iron ions from biological complexes and by this, via Fenton's reaction, catalyzing the hydrogen peroxide decomposition to hydroxyl radicals initiating cellular damage **(**De Zwart LL et al., 1999; Ward RJ et al., 2001).

In our study (Figure 2), the Fe level in the blood of rats exposed to aluminum and cisplatinum was increased, sigmificantly only in groups that received cisplatinumum or both substances comparative to control group (E1/C: +18.16%, $P\geq0.05$; E2/C: 26.49%, $P\leq0.05$; E3/C: +31.22%, $P\leq0.05$), observing not

significant (p>0.05) differences between experimental groups (E2/E1: +7.05%, E3/E1: +11.06%; E3/E2: +3.74%).

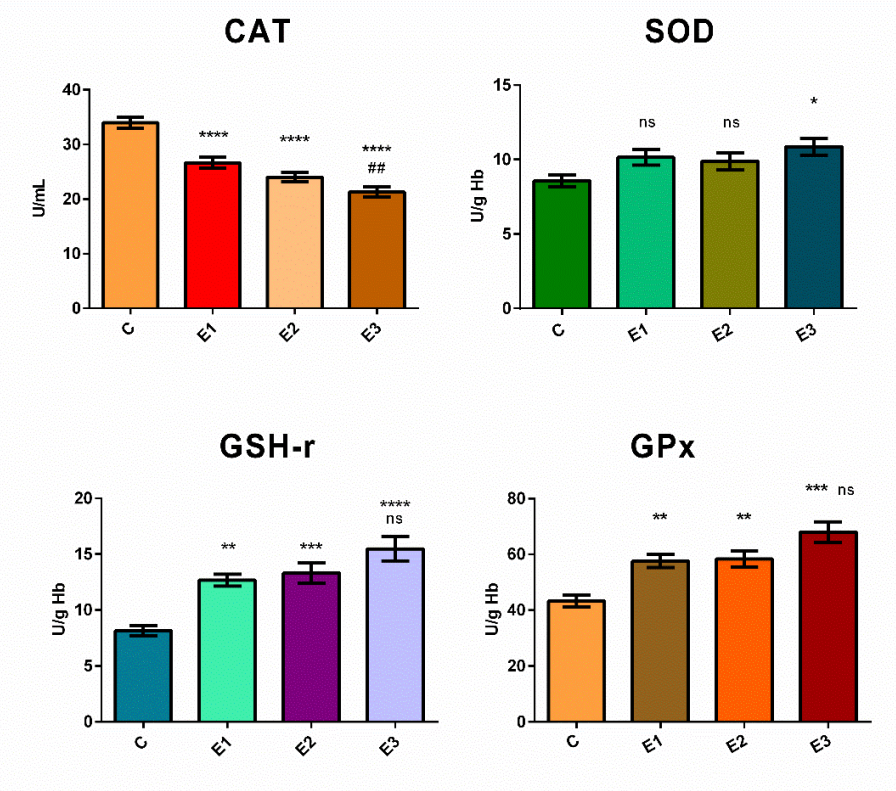


**Figure 1.** Oxidative stress biomarkers enzymes in rats exposed to aluminum and cisplatinum.
Comparative to C group: ** P≤0.01, *** P≤0.001, **** P≤0.0001
Comparative between experimental groups: ns – not significant
Comparative between E3 and E1: ## - P≤0.01

Iron plays a key role in a proper CAT activity (Skrajnowska D et al., 2013), and we observed an indirect relation between the activity of this enzyme and iron level in rats exposed to aluminum and cisplatinum.

Both aluminum and cisplatinum can interfere also with Se (Francescato HDC et al., 2001; Guo CH et al., 2009), affecting either direct or indirect, the homeostasis of this bioelement in the living organisms. We observed a decrease of blood Se levels in the groups that received aluminum and cisplatinum, significantly only in the group that received both substances (E1/C: -3.98%, P≥0.05; E2/C: -11.44%, P≥0.05; E3/C: -24.17%, P≤0.05).

GSH-r acts together with GPx, and both are Se dependent (Mohammadirad A and Abdollahi M, 2011; Shazia Q et al., 2012; Staneviciene I et al., 2017) for proper protection against free radicals. In the present study was observed an indirect correlation between Se levels and both enzyme activities.

The imbalance of Mg homeostasis or chronic Mg deficiency may have as a consequence the excessive formation of oxygen-derived free radicals (Barbagallo M and Dominguez LJ, 2014). We observed a not significant (P≥0.05) decrease of Mg blood level in groups that received only aluminum (E1/C: -7.49%) or both substance (E3/C: -15.04%), and a significant increase (P≤0.05) of its level in group that received only cisplatinum (E2/C: +18.74%).

SOD is a metalloenzyme that contains Cu, Zn or Mn as a cofactor, representing the front line of defense against reactive oxygen species (ROS) (Miller AF, 2001; Younus H, 2018). In our study we ob-

served a decrease of Cu, significantly only in group that received cisplatinum or combination of substances (E1/C:-17.24%, P≥0.05; E2/C: -25.90%, P≤0.05; E3/C: -26.28%, P≤0.05), the decrease of Zn, significantly only in groups that received aluminum or combination of aluminum and cisplatinum (E1/C: -33.30%, P≤0.01; E2/C: -13.41%, P≥0.05; E3/C:-37.39%, P≤0.001). Regarding the Mn levels, we observed a not significant increase (P≥0.05) in groups that received aluminum (E1/C: +19.21%) and cisplatinum (E2/C: +22.35%) but in group that was administrated both substances we have noted a not significant (P≥0.05) decrease of Mn level comparative to control group (E3/C: -9.41%).

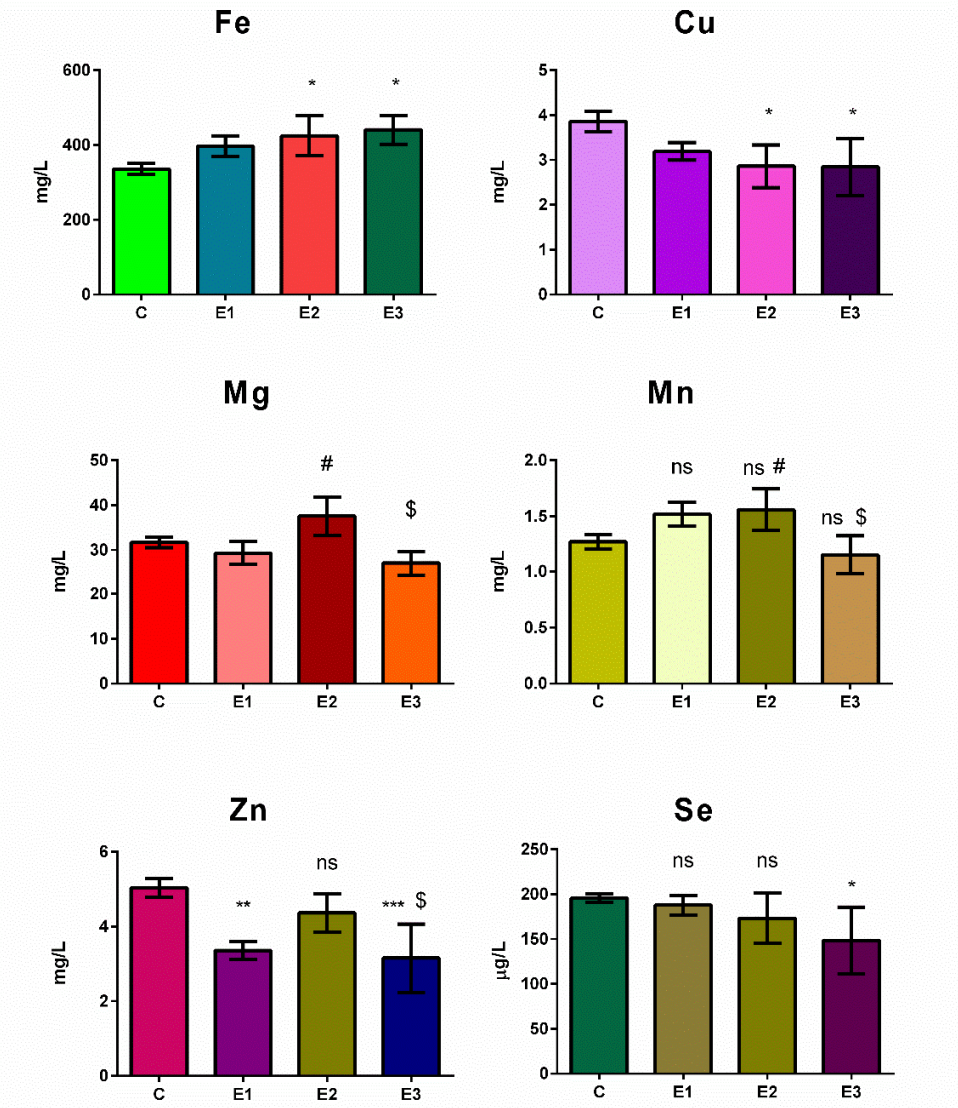


**Figure 2.** The levels of studied trace elements in blood of rats exposed to aluminum and cisplatinum.
Comparative to C group: * P≤0.05, ** P≤0.01, *** P≤0.001, ns – not significant
Comparative between E2 and C: # - P≤0.05
Comparative between E3 and E2: $ - P≤0.05

Pezonaga I et al , noted that administration of cisplatinum, in human patients with cancers, was followed by the decrease of Zn, Cu, and Mg plasma levels, in turn, Nakamura T, et al (2016) did not observe significant changes of Mn, Fe, and Cu, but noted a slight decrease of Zn in cisplatinum treated patients comparative to non-treated ones.

Regarding aluminum administration, in a study of Khanna P and Nehru B (2007), was pointed out the interaction between Al and Zn and the interference with the activity of SOD enzyme. They noted that higher Al concentrations can affect Zn and Se homeostasis, which in turn, can lead to a decrease of Zn and Se concentrations and an increase of oxidative stress.

**Conclusion.**

Administration of aluminum and cisplatinum to rats was followed by the increase of SOD, GSH-r, GPx and the decrease of CAT activity suggesting the induction of oxidative stress.

In this condition, in rats from the group that received aluminum was observed the increase of Fe, Mn and the decrease of Cu, Mg, Se, and Zn; in the group that received cisplatinum has noted the increase of Fe, Mg, Mn and the decrease of Cu, Zn, and Se.

We can conclude that aluminum and cisplatinum can impair the normal status of the main bioelements through induced oxidative stress.

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