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# Cu (II) (PICOLINYL-L-TRYPTOPHAN)$_2$ AS AN EFFECIENT MULTIFUNCTIONAL RADIOPROTECTOR

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Ionizing radiation (IR) exposure is triggering inflammation and increased rate of apoptosis leading to generation of tissue damaging agents and cell death. In the present study we investigated the effects of potential radioprotector, copper complex of L-tryptophan Schiff base derivative, Cu(II)(picolinyl-L-tryptophan)$_2$, on the blood levels of inflammatory and apoptotic markers, prostaglandin-$E_2$ and B-cell lymphoma 2-associated X protein, circulating immune complexes and key enzymes of the native antioxidant system superoxide dismutase and catalase in animal model of ionizing radiation. The results obtained demonstrated that Cu(II)(picolinyl-L-tryptophan)$_2$ possesses normalizing effects towards the IR-induced alterations in inflammatory response, antioxidant system activation and apoptotic cell death when administered to animals 1 hour before their exposure to IR. On the base of these results we concluded that Cu(II)(Picolinyl-L-tryptophan)$_2$ may be considered a multifunctional radioprotective agent.

*Antioxidant system enzymes – B-cell lymphoma 2-associated X protein – circulating immune complexes – Cu(II)(picolinyl-L-tryptophan)2 – ionizing radiation – prostaglandin-$E_2$*

Իոնիզանցնող ճառագայթումը (ԻՃ) առաջացնում է բորբոքային պրոցեսներ և բարձրացնում ապոպտոզի մակարդակը, ինչը հանգեցնում է հյուսվածք վնասող գործոնների ձևավորմանը և բջջի մահվան: Աշխատանքում մենք ուսումնասիրել ենք պոտենցիալ ռադիոպաշտպանիչ միացության՝ L-տրիպտոֆանի Շիֆֆի հիմքի ածանցյալ Cu(II)(պիկոլինիլ-L-տրիպտոֆանատ)$_2$-ի ազդեցությունը կենդանի մոդելների արյան մեջ բորբոքային և ապոպտոտիկ մարկերների՝ պրոստագլանդին $E_2$-ի, B բջջային լիմֆոմայի հետ ասոցիացված X սպիտակուցի, շրջանառու իմունային համալիրների և բնական հակաօքսիդանտային համակարգի առանցքային ֆերմենտների՝ սուպերօքսիդդիսմուտազի և կատալազի մակարդակների վրա իոնիզացնող ճառագայթման պայմաններում: Հետազոտությունների արդյունքները ցույց են տալիս, որ ճառագայթումից 1 ժ առաջ փորձարկվող կենդանիների օրգանիզմ ներարկված Cu(II)(պիկոլինիլ-L-տրիպտոֆանատ)$_2$-ը օժտված է ԻՃ հետևանքով առաջացած փոփոխությունները կարգավորող, հակաօքսիդանտային համակարգն ակտիվացնելու հատկություններով և առաջ է բերում ապոպտոտիկ բջիջների մահ: Հիմնվելով ստացված տվյալների վրա՝ եզրակացրել ենք, որ Cu(II)(պիկոլինիլ-L-տրիպտոֆանատ)$_2$-ը կարող է հանդիսանալ որպես բազմաֆունկցիոնալ ռադիոպաշտպանիչ ագենտ:

*հակաօքսիդանտային համակարգի ֆերմենտներ – B բջջային լիմֆոմայի հետ ասոցիացված X սպիտակուց – շրջանառու իմունային համալիրներ – Cu(II)(պիկոլինիլ-L-տրիպտոֆանատ)$_2$ – իոնիզացնող ճառագայթում – պրոստագլանդին-$E_2$*

Ионизирующая радиация (ИР) вызывает воспаление и повышает уровень апоптоза, что приводит к образованию разрушающих ткань факторов и гибели клетки. Нами исследовано влияние потенциального радиопротектора производного Шиффового основания L-триптофана, Cu(II)(пиколинил-L-триптофаната)$_2$ на уровни маркеров воспаления и апоптоза, простагландина-$E_2$, X белка ассоциирующего с лимфомой B- клеток, циркулирующих иммунных комплексов, и ключевых ферментов антиоксидантной системы супероксид-дисмутазы и каталазы в крови у животных, подвергшихся влиянию ИР. Результаты показали, что Cu(II)(пиколинил-L-триптофанат)$_2$, полученный за час до облучения, нормализует изменения, вызванные воспалительным ответом, активирует антиоксидантную систему

и регулирует апоптотическую гибель клетки. На основании полученных результатов мы заключили, что Cu(II)(пиколинил-L-триптофанат)$_2$ может быть использован как мультифункциональный радиозащитный агент.

*Ферменты антиоксидантной системы – X белок ассоциирующий с лимфомой B-клеток – циркулирующие иммунные комплексы – Cu(II)(пиколинил-L-триптофанат)$_2$ – ионизирующая радиация – простагландин-$E_2$*

Exposure to ionizing radiation (IR) causes serious injury to human health, damaging important functional systems of organism and increases the risk of acquiring cancer and other diseases.

Humans are continually exposed to ionizing radiation from many sources. This is called natural background radiation. For most people more than half of their natural background radiation dose comes from radon, a radioactive gas found in natural sources including the ground, underground water, and the atmosphere. Other natural background radiation sources include cosmic radiation from space, naturally occurring radioactive minerals in the ground and in the air, and medical diagnostics and treatments. In general, the health risks associated with this kind of exposure are minimal. If, however, a catastrophic event or accident occurs that results in the exposure of all or mostly all of the body to a high dose of penetrating radiation over a very short period of time, the result is a severe illness known as acute radiation syndrome (ARS). In these circumstances, the key protective systems of the body, such as the immune and antioxidant, mobilize all the reserves of the organism, but finally deplete.

The effects of IR on the immune system depend on the dose received. Massive cell death, inflammation, and infection are the acute effects of high-dose radiation exposure.

Irradiation was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals ($O^{-2}$), hydroxyl radicals ($OH^-$), and hydrogen peroxides ($H_2O_2$) [19], which react rapidly with almost all structural and functional organic molecules, including proteins, lipids, and nucleic acids causing disturbance of cellular metabolism [14]. One of the protective mechanisms here is the enzymatic system, which operates through the sequential and simultaneous actions of a number of enzymes including catalase (CAT) and superoxide dismutase (SOD) [10]. SOD, which is localized in various cell compartments, dismutes $O^{2-}$ to $H_2O_2$ and oxygen [13]. CAT is synthesized in a tissue specific and age dependent manner, scavenges $H_2O_2$ generated during photorespiration and ß-oxidation of fatty acids [11].

Induction of the arachidonic acid cascade with subsequent production of eicosanoids through the cyclooxigenase and lipooxigenase pathways is one of the indirect effects of (IR). Eicosanoids affect different systems of the organism regulating cell growth, differentiation, and apoptosis. Although, prostaglandins, a family of eicosanoids, produced in response to IR, are known as immune system suppressors and radio-sensitivity-enhancing agents, recently data indicates that prostaglandins, particularly prostaglandin-$E_2$ ($PGE_2$), are radio-protective for normal cells, but only when administered before irradiation [4, 9, 18]. Endogenous prostaglandins act as inflammatory mediators [3]. In addition, promising findings suggest that prostaglandins, especially $PGE_2$, are involved in a new early mechanism of control in the activation of B-cell lymphoma 2-associated X protein (BAX) during apoptosis [9, 18, 8]. IR exposure is triggering inflammation and increase rate of apoptosis-leading to generation of tissue damaging agents and cell death [7]. IR also destroys native antioxidant protective system [1]. One of the primary goals of radioprotective therapy is to develop efficient radioprotective agents diminishing the effects of IR at multiple levels. Earlier, it has been shown that copper complexes with organic compounds exhibit anti-inflammatory and radioprotective activities and are as efficient as radio-protectors [1, 2, 6]. Cu(II) (picolinyl-L-tryptophan)$_2$, a newly synthesized L-tryptophan Schiff

base derivative, was shown to decrease the mortality rate of experimental animals in the conditions of IR [12].

Here, we studied the effects of Cu(II)(picolinyl-L-tryptophan)$_2$ on the blood levels of the inflammatory, apoptotic, and antioxidant mediators including different sub-populations of circulating immune complexes (CIC), the specific activities of the key enzymes of antioxidant system CAT and SOD, $PGE_2$ and BAX in the conditions of IR.

***Materials and methods.*** Animal model of IR. Experiments were performed using white male rats with 160-180g of body weight. The animals were divided into the following groups: 1) intact animals (IA), 2) irradiated animals, 3) irradiated animals treated by the Cu(II)(picolinyl-L-tryptophan)$_2$ (per oral administration of 10mg per kg of body weight 1 hour before IR). The animals were subjected to a single-dose IR using "RUM-17" X-ray unit (conditions: dose 5 Gy, dose rate 1.78 Gy/min, voltage 200 kV, and current strength 20 mA, without filters and with focal distance 50 cm).

Isolation of serum and erythrocyte from the blood samples. To obtain serum, the blood was collected after decapitation of the animals on the 3$^{rd}$, 7$^{th}$, 14$^{th}$ and 28$^{th}$ days after irradiation, left until clotting at room temperature, and then centrifuged at 3000 g for 10 min. The serum was collected and used in further experiments. To isolate erythrocytes, the blood was collected in the tubes with anticoagulant, then centrifuged at 3000 g x 4$^0$C, 10 min. Precipitated erythrocytes were washed with saline and use in further experiments.

Determination of CIC levels. Total concentration of CIC and a level of large-sized CIC in the serum was determined earlier developed assays [16, 17] and expressed as absorbance units at 280 nm ($A_{280}$). Serum concentration of CIC subpopulations containing C1q products of the complement system (C1q-CIC) was determined by the the enzyme-linked immunosorbent assay (ELISA), using commercial kit "IMTEC-C1q-CIC" (Human GmBH, Germany) according to manufacturer's instruction. The levels were expressed in □g per ml of serum (mg/ml).

Determination of SOD and CAT activities. SOD activity in hemolyzed erythrocytes was determined by the earlier developed spectrophotometric assay estimating the extent of inhibition of autooxidation of adrenaline in the alkaline medium by SOD [15]. Specific activity was expressed in the International units of SOD activity per ml of hemolyzed erythrocytes (U/ml).

The acticity of CAT in hemolyzed erythrocytes was determined by using earlier described spectrophotometric assay estimating the decrease in the content of hydrogen peroxide in incubation medium in the presence of catalase [5]. Specific activity was expressed in the International units of CAT activity per ml of hemolyzed erythrocytes (U/ml).

Determination of $PGE_2$ and Bax levels. $PGE_2$ and Bax levels in the blood serum were measured by ELISA using commercially available kits (Cayman Chemical Company, USA and Uscn Life Science Inc., China, respectively) according to manufacturers' protocols. The levels were expressed in pg per ml of serum (pg/ml).

Statistical analysis was performed by Student's unpaired t-test and by calculation of Spearman's rank correlation coefficient (Rs). P values ≤0.05 were considered as statistically significant.

***Results and Discussion.*** The influence of Cu(II)(picolinyl-L-tryptophane)$_2$ on the levels of different sub-populations of CIC. Beginning from day 14 after exposure to IR a significant increase of the total CIC and large-sized CIC serum levels were observed. Pretreatment of animals with Cu(II) (picolinyl-L-tryptophane)$_2$ before IR decreased the levels of CICs beginning from day 14 after irradiation, as compared to untreated irradiated animals. The obtained results are shown on tabl. 1.

Influence of Cu(II)(picolinyl-L-tryptophane)2 on specific activity of SOD and CAT. According to the data obtained, a statistically significant decrease in SOD activity of hemolyzed erythrocytes was detected in irradiated animals non-treated with Cu(II) (picolinyl-L-tryptophane)$_2$ prior to irradiation, as compared to intact animals. In case of CAT, a significant decrease in this enzyme activity was detected in irradiated animals, as compared with non-irradiated. Pre-treatment with Cu(II) (picolinyl-L-tryptophane)$_2$ before

exposure to IR resulted in increase of SOD activity up to control level. The same effect was observed in case of CAT, thereby increasing the stability of enzymes to ionizing radiation. The obtained results are shown on tabl. 3.

**Tabl. 1.** The levels of total CIC (mean±SD) in the blood serum of the experimental animals treated and non-treated with Cu(II)(picolinyl-L-tryptophane)$_2$ before irradiation and in intact non-irradiated animals

| Group | [Total CIC], $A_{280}$, □g/ml |
|---|---|
| Irradiated animals non-treated with Cu(II)(picolinyl-L-tryptophane)$_2$ before exposure to IR | 0.29□0.05 (a) |
| Irradiated animals treated with Cu(II)(picolinyl-L-tryptophane)$_2$ before exposure to IR | 0.23□0.02 (b) |
| Intact animals | 0.13□0.01 (c) |

*The results represent data obtained on day 28 after exposure to IR. a vs. b ($p < 0.05$); a vs. c ($p<0.005$); b vs. c ($p<0.003$).*

**Tabl. 2.** The levels of C1q-CIC (mean±SD) in the blood serum of the experimental animals treated and non-treated with Cu(II)(picolinyl-L-tryptophane)$_2$ before irradiation on different time-points after irradiation and in intact non-irradiated animals

| Group | [C1q-CIC], □g/ml |
|---|---|
| Irradiated animals non-treated with Cu(II)(picolinyl-L-tryptophane)2 before exposure to IR | 2.14□0.16 |
| Irradiated animals treated with Cu(II)(picolinyl-L-tryptophane)$_2$ before exposure to IR | 0.77□0.18 |
| Intact animals | 1.07□0.76 |

*The results represent data obtained on day 28 after exposure to IR. a vs. b ($p < 0.05$).*

**Tabl. 3.** Comparative analysis of superoxide dismutase (SOD) and catalase activity (mean±SD) in the lysate of erythrocytes irradiated animals at a dose of 500 R on day 7$^{th}$ on the background of prior (1 hour before irradiation) subcutaneous injection Cu(II)(picolinyl-L-tryptophane)$_2$

| Group | SOD specific activity, U/ml | CAT specific activity, U/ml |
|---|---|---|
| Irradiated animals non-treated with Cu(II)(picolinyl-L-tryptophane)2 before exposure to IR | 296.76□87.76 | 22572□542 |
| Irradiated animals treated with Cu(II)(picolinyl-L-tryptophane)2 before exposure to IR | 431.24□69.3 | 16884□1536.1 |
| Intact animals | 559.36□62.2 | 11232□1347.7 |

Influence of Cu(II) (picolinyl-L-tryptophane)$_2$ on $PGE_2$-dependant mechanism of Bax-activation. The obtained results indicated that IR-exposure significantly decreases the serum levels of both $PGE_2$ and Bax, and that pre-treatment with Cu(II)(picolinyl-L-tryptophane)$_2$ diminished these effects (fig.1, 2). In addition, we found a significant positive correlation ($R_s = 0.961$; $p < 0.05$) between the levels of $PGE_2$ and Bax in irradiated animals treated and non-treated with Cu(II)(picolinyl-L-tryptophane)$_2$before exposure to IR.

The results obtained in the present study indicated that Cu(II) (picolinyl-L-tryptophane)$_2$ can normalize the harmful effects of IR on the immune and antioxidant systems as well as on the apoptotic rate. This particularly applies to the levels of different subpopula tions of CIC, important mediators of the immune response and inflammation, SOD and CAT, the key enzymes of the native antioxidant protective system, and $PGE_2$-dependant mechanism of Bax-activation. Cu(II) (picolinyl-L-tryptophane)$_2$ might be considered as a prospective multifunctional radio-protective agent.

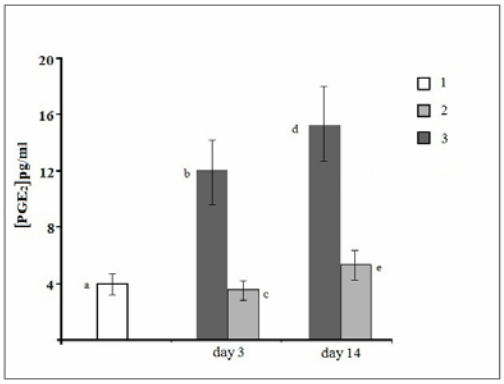


**Fig. 1.** Serum $PGE_2$ levels (M±SD) in intact animals (1) as well as in irradiated animals treated (2) and non-treated with Cu(II)(picolinyl-L-tryptophane)$_2$ (3).
a vs. b ($p<0.05$); a vs. d ($p<0.05$); a vs. c ($p>0.05$); a vs. e ($p>0.05$)

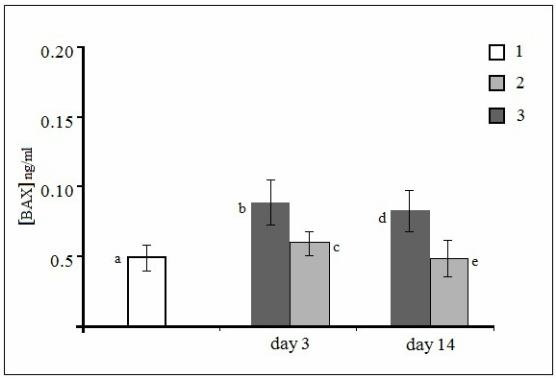


**Fig. 2.** Postradiation changes in the serum Bax levels (M±SD) in intact animals (1) as well as in irradiated animals treated (2) and non-treated with Cu(II)(picolinyl-L-tryptophane)$_2$ (3).
a vs. b ( $p<0.05$); a vs. d ( $p<0.05$); a vs. c ( $p>0.05$); a vs. e ( $p>0.05$)

## REFERENCES

1. *Arakelova E., Ayvazyan V., Zhamharyan L., Hovsepyan T., Boyajyan A., Malakyan M., Bajinyan S.* Influence of ionizing radiation on the immune and antioxidant systems of the organism. Georgian Chem. J., *10*, 4, p. 59-62, 2010.
2. *Arakelova E., Ayvazyan V.,Zhamharyan L., Hovsepyan T., Boyajyan A., Malakyan M., Bajinyan S.* Comparative analysis of the influence of the aminoacid Schiff bases and their Copper complexes on the immune and antioxidant status of the organism under ionizing radiation. Proceedings of the International Conference “Physical research methods in medicine”, Tbilisi, p. 13-17, 2011.
3. *Boone D.L., Currie W.D., Leung P.C.* Arachidonic acid and cell signaling in the ovary and placenta. Prostaglandins Leukot Essent Fatty acid, *48*, p. 79-87, 1993.
4. *Choy H., Milas L.,* Enhancing radiotherapy with cyclooxigenase-2 enzyme inhibitors: a rational advace? J Natle Cancer Inst., *95*, 19, p. 1440-1452, 2003.
5. *Gota L.* A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta, *196*, p.143-152, 1991.
6. *Iakovidis I, Delimaris I, Piperakis SM.* Cooper and its complexes in medicine: a biochemical approach. Mol. Biol. Int., p.1-13, 2011.

7. *Kim K., McBride W.H.* Modifying radiation damage. Curr Drug Targets, *11*,11, p.1352-1365, 2010.
8. *Lalier L., Cartron P-F., Olivier C., Loge C., Bougras G., Robert M., Oliver L., Vallette FM.* Prostaglandins antagonisticallt control Bax activation during apoptosis. Cell Death and Differentiation, *18*, p. 528-537, 2011.
9. *Lalier L., Pedelaborde F., Braud C., Menanteau J., Vallette F.M., Olivier C.,* Increase in intracellular $PGE_2$ induces apoptosis in Bax-expressing colon cancer cell. BMC Cancer, *11*, p. 153, 2011.
10. *Larson, R.A.* The antioxidants of higher plants. Phyto Chem., *27*, p. 969-978, 1988.
11. *Lin, C.C. and C.H. Kao.* Effect of NaCl stress on $H_2O_2$ metabolism in rice leaves. Plant Growth Regul., *30*, p. 151-155, 2000.
12. *Malakyan M.* Investigation of antiradiation activity of Cu(II) complexes with schiff bases derived from L-tryptophan and isomeric pyridinecarboxaldehydes. First International Conference on Radiation and Dosimetry in Various Fields of Research (RAD 2012), Niš, Serbia, p.166, 2012.
13. *Salin, M.L.* Toxic oxygen species and protective systems of the chloroplast. Physiol. Plant. *72*, p. 681-689, 1987.
14. *Salter, L. and Hewitt C.N.* Ozonehydrocarbon interactions in plants. Phytochem., *31*, p. 4045-4050, 1992.
15. *Sirota TV*. A new approach to studying the autoxidation of adrenaline: possibility of the determination of superoxide dismutase activity and the antioxidant properties of various preparations by polarography. Biomed Khim, *58*, 1, p. 77-87, 2012.
16. *Struchkov P.V., Konstantinova N.A., Lavrentov V.V., Chuchalin A.G.* Screening test for evaluating the pathogenetic properties of immune complexes. Laboratornoe Delo (Moscow), 7, p.410-412, 1985.
17. *Tarnachka B., Gromadzka G., Czlonkowska A.* Increased circulating immune complexes in acute stroke: the triggering role of Chlamidia pneumonia and cytomegalovirus. Stroke, *33*, 4, p. 936-940, 2002.
18. *Tessner TG., Muhale F., Riehl TE., Anant Sh., Stenson WF.* Prostaglandin $E_2$ reduces radiation-induced epithelial apoptosis through a mechanism involving AKT activation and bax translocation. J Clin Invest, *114*, p. 1676-1685, 2004.
19. *Xienia, U., G.C. Foote, S. Van, P.N. Devreotes, S. Alexander and H. Alexander. Differential developmental expression and cell type specificity of dictystelium catalases and their response to oxidative stress and UV light. Biochem. Biophys. Acta. 149, p. 295-310, 2000.*

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