Current Trends in Natural Sciences Vol. 8, Issue 15, pp. 191-195, 2019

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

PROTECTIVE ROLE OF TANNIC ACID AGAINST COPPER-INDUCED LIPID PEROXIDATION IN RATS

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Abstract
The present study was designed to evaluate the protective role of tannic acid against copper-induced alteration in lipid peroxidation and antioxidant function in rats. The results show the toxic effect of copper indicated by increasing of lipid peroxidation markers and decrease in the levels of plasma enzymatic and non-enzymatic antioxidants. Administration of tannic acid at different doses (20, 40 and 80 mg/kg body weight) show a significant modification of lipid peroxidation markers and the levels of plasma enzymatic and non-enzymatic antioxidants. This study demonstrated the protective role of tannic acid in reducing the toxic effects of copper in experimental rats.

Keywords: Copper, Lipid peroxidation, Tannic acid.

1. INTRODUCTION

Copper is an essential element to all animals. At elevated concentrations, it is toxic and can participate in the formation of reactive oxygen species, leading to cellular damage (Nugroho and Frank, 2012). Copper ions are highly redox active and might contribute to tissue damage by the generation of reactive oxygen species (ROS) such as hydroxyl radicals via Fenton (equation 1) and Haber-Weiss reactions. Furthermore, in the presence of copper or other transition metal ions and hydrogen peroxide (H_2O_2) can give rise to the highly reactive ($^{\bullet}OH$) (hydroxyl radical) species (Monnier, 2001).

$$Cu^{+1} + H_2O_2 \rightarrow Cu^{+2} + {}^{\bullet}OH + OH^- \quad \text{..... (1)}$$

Trace elements such as copper, is capable of redox cycling in which a single electron may be accepted or donated by the metal. This action catalyzes reactions that produce reactive radicals and can produce reactive oxygen species. The presence of such metals in biological systems in an uncomplexed form can significantly increase the level of oxidative stress Reactive oxygen species (ROS) are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress." Regulation of reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function. Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed (Birben et al., 2012).

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Phenolic acids are secondary metabolites widely distributed in the plant kingdom and are second only to flavonoids in terms of their dominance. Tannic acid, a naturally occurring plant polyphenol, is composed of a central glucose molecule derivatized at its hydroxyl groups with one or more galloyl residues, is a plant polyphenol which has been shown to possess antioxidant ,antimutagenic and anticarcinogenic properties. The antioxidant mechanism of tannic acid is still far from being fully understood, in the presence of copper ions, tannic acid acts as an antioxidant, suppressing hydroxyl radical formation (Gülçin et al., 2010). This study aimed to investigated the protective role of tannic acid in reducing the toxic effects of copper in experimental rats.

2. MATERIALS AND METHODS

Experimental animals: Thirty two healthy male rats (Wistar albino) weighing (235 ± 25 g) of 8-9 weeks old, at the beginning of the experiment, were used in the present study. Animals were housed in individual cages under standard laboratory conditions (temperature 22°C ± 2°C; relative humidity 45-55% and 12 h light-dark cycle). The concentration of $CuCl_2$ was determined according to the oral median lethal dose for rats (LD50).

Experimental design: After one week, Experimental animals were randomely divided into eight groups 4 rats per each group) upon the following designed: Group 1 (control) received saline (0.3 ml/kg BW); Group 2 was treated with copper (as $CuCl_2 \cdot 2H_2O$ at a concentration of 560 mg/L, p.o.) via drinking water for 5 weeks; Group 3 (Tannic acid) was treated with intraperitoneal injections (i.p.) of tannic acid (20 mg/ kg BW, in 0.3 ml double-distilled water) ; Group 4 (Tannic acid) was treated with intraperitoneal injections (i.p.) of tannic acid (40 mg/ kg BW, in 0.3 ml double-distilled water); Group 5 (Tannic acid) was treated with intraperitoneal injections (i.p.) of tannic acid (80 mg/ kg BW, in 0.3 ml double-distilled water),every third day for the last 3 weeks of the experiment. Group 6 (Cu + tannic acid) was treated with copper (as $CuCl_2 \cdot 2H_2O$ at a concentration of 560 mg/L, p.o.) via drinking water for 5 weeks, and with tannic acid (20 mg /kg BW, in 0.3 ml double-distilled water) i.p.; Group 7 (Cu + tannic acid) was treated with copper (as $CuCl_2 \cdot 2H_2O$ at a concentration of 560 mg/L, p.o.) via drinking water for 5 weeks, and with tannic acid (40 mg /kg BW, in 0.3 ml double-distilled water) i.p.; Group 8 (Cu + tannic acid) was treated with copper (as $CuCl_2 \cdot 2H_2O$ at a concentration of 560 mg/L, p.o.) via drinking water for 5 weeks, and with tannic acid (80 mg /kg BW, in 0.3 ml double-distilled water) i.p. every third day for the last 3 weeks of the experiment (Mladenović et al., 2015).

Analytical procedures: Blood sample were drawn from each animals (5 mL) from heart puncture method after 12 hours fast, then the blood transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at 2-8ºC until the time of assay which were triplicated for each sample. Lipid Peroxidation Assay Kit (Colorimetric/Fluorometric) is a robust and sensitive kit for detection of malondialdehyde (MDA (produced as an end product of lipid peroxidation. In this assay, free MDA present in the sample reacts with Thiobarbituric Acid (TBA) and generate a MDA-TBA adduct, which can easily be quantified colorimetrically (OD 532 nm) or fluorometrically (Ex/Em = 532/553 nm). Superoxide dismutase (SOD) activity was determined by Superoxide Dismutase (SOD) Assay Kit (MyBioSource ELISA Kit) by WST-1 method.Water-soluble tetrazolium, the sodium salt of 4-[3-(4iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1), was used as a detector of superoxide radical generated by xanthine oxidase and hypoxanthine. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined by a colorimetric method. Catalase activity (CAT) activity was determined

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by Catalase (CAT) Colorimetric Assay Kit .The reaction that catalase (CAT) decomposes H_2O_2 can be quickly stopped by ammonium molybdate. The residual H_2O_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity can be calculated by production of the yellowish complex at 405 nm. The DetectX® Glutathione kit is designed to quantitatively measure glutathione (GSH), and oxidized glutathione (GSSG) present in a variety of samples. Thoroughly mix sample with an equal volume of cold 5% SSA. Incubate for 10 minutes at 4°C. Centrifuge at 14,000 rpm for 10 minutes at 4°C. Collect the supernatant. The supernatant must be diluted 1:2.5 with Assay Buffer by mixing one part with 1.5 parts of Assay Buffer to bring the SSA concentration to 1%. The sample will have been diluted 1:5 at this point. All final dilutions are made in Sample Diluent. The concentration of GSH can be determined either as an endpoint read of the color developed at 405 nm or by measuring the rate of color development at 405 nm.

Statistical analysis was carried out by one way ANOVA-test which used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant. The results were expressed as mean ± standard deviations (mean ± SD).

3. RESULTS AND DISCUSSIONS

The potential toxicity of copper results indicated in Table 1 which show a significant increasing of lipid peroxidation markers and decrease in the levels of plasma enzymatic and non-enzymatic antioxidants. Administration of tannic acid at different doses (20, 40 and 80 mg/kg body weight) show a significant modification of lipid peroxidation markers and the levels of plasma enzymatic and non-enzymatic antioxidant. The prolonged exposure of copper caused an adverse e□ects, although it is a main element in many biological processes (Fuentealba and Aburto, 2003).

Table 1. Changes by Mean and Standard Deviation values for all biochemical parameters of control and treated groups of rats after 5 weeks of treatment

Experimental groups	***Parameters***			
	MDA (nmol/L)	***SOD (U/mL)***	***CAT (U/mL)***	***GSH (µM/mL)***
Group 1	0.77 ± 0.05	84.35 ± 3.88	55.30 ± 0.04	158.27 ± 9.05
Group 2	3.04 ± 0.09*	67.14 ± 1.36*	34.55 ± 0.42*	139.02 ± 2.44*
Group 3	0.94 ± 0.15	74.04 ± 0.38	41.73 ± 0.51	148.89 ± 0.33
Group 4	0.82 ± 0.39	76.18 ± 0.48	45.26 ± 0.18	152.51 ± 0.52
Group 5	0.78 ± 0.10**	81.63 ± 0.72**	52.40 ± 0.41**	156.48 ± 0.22**
Group 6	1.36 ± 0.47	69.37 ± 0.25	37.57 ± 0.26	142.43 ± 0.36
Group 7	1.04 ± 0.44	72.89 ± 0.17	43.33 ± 0.81	146.28 ± 0.99
Group 8	0.91 ± 0.33**	78.12 ± 0.37**	49.48 ± 0.17**	150.95 ± 0.43**

Values are given as mean ± SD. P ≤ 0.05, signifcantly di□erent from control. Valuses were triplicated for each sample. *p<0.05, signifcantly di□erent from control (group 1); **p<0.05, signifcantly di□erent from copper group (group 2).

Our results indicated that the treatment with copper induce lipid peroxidation reflexed by the signifucant increasing in MDA (Table 1). while this treatment reduced the antioxidants by the significant decreasing in SOD, catalase and GSH in group 2. The redox active metals includig copper (Cu) undergo redox cycling reactions and possess the ability to produce reactive radicals in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and

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subsequently induces modification caused numerous diseases. The underlying mechanism of action for Cu involves formation of the superoxide radical, hydroxyl radical (mainly via Fenton reaction) and other ROS, finally producing malondialdehyde (MDA).The mechanism of metal-induced formation of free radicals is tightly influenced by the action of cellular antioxidants. Many low-molecular weight antioxidants such as glutathione (GSH), carotenoids, flavonoids, and other antioxidants) are capable of chelating metal ions reducing thus their catalytic activity to form ROS. A novel therapeutic approach to suppress oxidative stress is based on the development of dual function antioxidants comprising not only chelating, but also scavenging components. Parodoxically, two major antioxidant enzymes, superoxide dismutase (SOD) and catalase contain as an integral part of their active sites metal ions to battle against toxic effects of metal-induced free radicals (Jomova and Valko, 2011).

Prospective studies suggest that tnnic acid may protect against oxidative stress that induced tissue damage (Omar et al., 2003). Our results demonestred that the treatment with tannic acid improved the antioxidant system by the signifigant increasing in the levels of SOD, catalase and GSH in dose dependent manner. Antioxidants are substances that when present at very low concentration inhibits the oxidation of a molecule. It has the capacity to nullify the ill effects of oxidation caused by free radicals in the living organisms. The unpaired electrons of these free radicals are highly reactive and neutralize the harmful reactions of human metabolism. Protection of the body against free radicals is provided by some enzymes which come under a distinctive group, concerned solely with the detoxification of these radicals. Superoxide dismutase (SOD) and catalase are the key enzymatic antioxidants of this defense system by which the free radicals that are produced during metabolic reactions are removed (Jeeva et al., 2015). Also tannic acid show an antioxidant and scavenging free radicals property by significant decreasing in MDA levels in dose dependet manner. However, improvement was noticed in the rats treated with tannic acid in addition to copper chloride. Various prospective studies have indicated the antioxidant potency of tannic acid in several models. However, the antioxidant properties of tannic acid remains potent regardless of the lipid sources and pro-oxidants employed for the oxidative assault (Jimoh et al., 2016). This study proved that tannic acid has a role as an antioxidant in protection of rats from the copper toxicity.

4. CONCLUSIONS

Tannic acid have the ability to sustaine antioxidant effect which result in decreased oxidative stress and cellular damage initiated through free radical and ROS production and depltion in endo-antioxidant by copper chloride.

5. ACKNOWLEDGEMENTS

The author is highly thankful to the participants for the completion of this work.

6. REFERENCES

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organ J, 5(1*), 9–19.

Fuentealba, C., Aburto, E. M. (2003). Animal models of copper-associated liver disease. *Comparative Hepatology 2, 5*.

Gülçin, İ., Huyut, Z., Elmastaş, M., Aboul-Enein, H. Y. (2010). Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry, 3(1)*, 43–53.

Jeeva, J. S., Sunitha, J., Ananthalakshmi, R., Rajkumari, S., Ramesh, M., Krishnan, R. (2015). Enzymatic antioxidants and its role in oral diseases. *J Pharm Bioall Sci, 7*, suppl s2:331–3.

Jimoh, T. O., Ogunmoyole, T., Aladejana, E. A., Kade, I. J. (2016). Antioxidant potentials of tannic acid on lipid peroxidation induced by several pro-oxidants in cerebral and hepatic lipids. *International Journal of Ethnopharmacology, 2(1)*, 014–020.

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Jomova, K., Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology, 283(2-3)*, 65–87.

Mladenović, J. M., Paunović, M. G., Matić, M. M., Knežević, V. S., Ognjanović, B. I., Štajn, A. Š., Saičić, Z. S. (2015). Copper-induced changes of lipid peroxidation and hemato-biochemical parameters in rat blood: Protective role of flavonoids. *Archives of Biological Sciences, 66(3)*, 1271–1279.

Monnier, V. M. (2001). Transition metals redox: reviving an old plot for diabetic vascular disease. *J Clin Invest, 107*, 799–801.

Nugroho, A. P., Frank, H. (2012). Effects of copper on lipid peroxidation, glutathione, metallothionein, and antioxidative enzymes in the freshwater mussel Anodonta anatina, *Toxicological & Environmental Chemistry, 94(5)*, 918–929.

Omar, H. M., Khadiga, A. H., Abd-Elghaffar, S. Kh., Ahmed, E. A. (2003). Aluminium toxicity in rats : the role of tannic acid as antioxidant. *Ass. Univ. Bull. Environ. Res, 6(2)*, 1-14.

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