

## BAICALIN INHIBITS FREE RADICALS PROCESSES INITIATED BY CHROMIUM IONS

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Chromium is essential in organism e.g., for the correct metabolism of glucose (an ingredient of the glucose tolerance factor – GTF), but it is also a toxic metal. Toxicity of chromium depends closely on its valence and is connected substantially with its oxidative properties. Cr(VI) is classified by IARC as carcinogenous for people (1, 2).

*Scutellaria baicalensis* is a Chinese plant rich in flavonoids, especially baicalin, baicalein and wogonosid (3, 4). The extract received from this plant – Antoxyd (Bioactive Products Factory) showed to be an active radicals scavenger, also inhibiting the lipid peroxidation caused by chromium compounds (5). It was interesting to know whether baicalin, abundantly present in Antoxyd, can be responsible for this effect.

In this study baicalin's influence on lipid peroxidation caused by chromium in human erythrocytes was evaluated. Also the scavenging ability of baicalin towards the hydroxyl radical generated by chromium ions in mitochondria of the human placenta was investigated. The aim of the study was to examine baicalin's interaction with chromium ions as well.

### EXPERIMENTAL

The materials used in the research were fresh blood and mitochondria. The blood taken on the sodium citrate was obtained from The Casualty Department of the Surgery patients of the Academic Hospital in Wrocław. It was centrifuged and the plasma was rejected. The erythrocytes were washed three times with physiological salt solution and then a 10% suspension of blood corpuscles in PBS buffer was prepared. The level of hemoglobin was measured by Drabkin method.

Mitochondria were received from the human placenta (Department of Reproduction and Obstetrics, University of Medicine in Wrocław) from physiological deliveries. Placenta after the initial purification were homogenized in the TRIS-HCl buffer pH 7.4 containing 0.23 M mannitol; 0.07 M sucrose; 1 mM EDTA and 0.2% bovine serum albumin (BSA) and were proceeded acc. to (6). The concentration of the protein was evaluated with Lowry method. The TBARS (thiobarbituric acid reactive species) level was measured using Stocks' method (7) with thiobarbituric acid (TBA) at the wavelength of 535 nm. The TBARS concentration was expressed in nmol/g Hb. The  $\cdot\text{OH}$  level (hydroxyl radical) was evaluated colorimetrically by the measurement of the deoxyribose degradation, at the wavelength of 532 nm (8). The concentration of the  $\cdot\text{OH}$  radical was expressed in nmol/mg proteins. Baicalin (Baicalin, 95%; Sigma-Aldrich) was used at the following concentrations: 1; 10; 20; 100 and 200  $\mu\text{mol/mL}$  in DMSO (dimethyl sulfoxide). Aqueous solution of salt chromium III ( $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  p.p.a.; Riedel de Haen) and chromium VI ( $\text{K}_2\text{Cr}_2\text{O}_4$  p.p.a.; POCh) at concentrations of 0.05; 0.5 and 1  $\mu\text{g/mL}$  were used.

The results were evaluated statistically using the one-factor analysis ANOVA and the *post hoc* RIR Tukey's test ( $p < 0.05$ ).

### RESULTS AND DISCUSSION

Baicalin at concentrations from 10  $\mu\text{mol/mL}$  to 200  $\mu\text{mol/mL}$  showed its antioxidative effect by the decrease of TBARS level in the erythrocytes ( $p < 0.001$ ) in comparison to the control K1 (Fig. 1). It was observed that there was a statistically significant linear negative correlation between the

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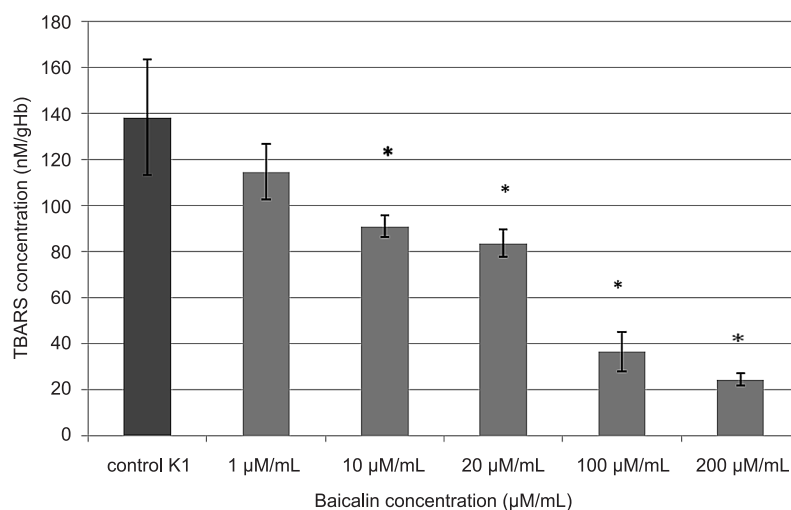


Figure 1 Antioxidative effect of baicalin on TBARS concentration in erythrocytes against control K1; \* statistically lower than control K1 ( $p < 0.05$ )

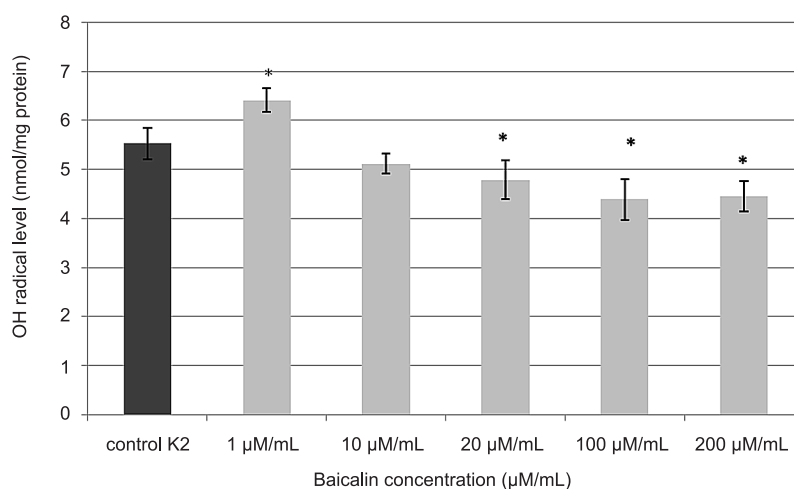


Figure 2. Effect of baicalin on OH radical level in mitochondria against control K2; \* statistically different from control K2 ( $p < 0.05$ )

baicalin dose and the TBARS concentration in the erythrocytes with the Pearson factor  $r = -0.9090$  ( $p = 0.000$ ). Baicalin also showed the ability to scavenge the hydroxyl radical and in concentrations from  $20 \mu\text{mol/mL}$  to  $200 \mu\text{mol/mL}$  it decreased the content of hydroxyl radicals in the mitochondrial suspension (in comparison to the control K2) (Fig. 2). Statistically significant linear negative dependence between the baicalin dose and the hydroxyl radical concentration in the mitochondrial suspension with the Pearson factor  $r = -0.6012$  ( $p = 0.000$ ) (Fig. 3) was noted. Chromium compounds (III) at the examined concentrations ( $0.05 \mu\text{g/mL}$ ;  $0.5$

$\mu\text{g/mL}$ ;  $1.0 \mu\text{g/mL}$ ;  $5.0 \mu\text{g/mL}$ ;  $10.0 \mu\text{g/mL}$ ) increased the lipid peroxidation, while chromium compounds (VI) exerted such an effect only in lower concentrations ( $0.05 \mu\text{g/mL}$ ;  $0.5 \mu\text{g/mL}$ ) ( $p < 0.001$ ) (5). Baicalin in the range of concentrations from  $10$  to  $200 \mu\text{mol/mL}$  has the ability to scavenge the hydroxyl radical generated in the mitochondrial suspension by chromium (III) and chromium (VI) ions at doses of  $0.05 \mu\text{g/mL}$ ;  $0.5 \mu\text{g/mL}$ ;  $1.0 \mu\text{g/mL}$  ( $p < 0.05$ ) (Tab. 1). The experiment demonstrated baicalin's effectiveness in inhibiting lipid peroxidation enhanced with Cr (III) and Cr (VI) ions. It seems probable that Antoxid's

Table 1. The mean value of ·OH radical level in mitochondria after combined exposure to Cr(III) or Cr(VI) in three doses (0.05; 0.5 and 1.0 µg/mL) with baicalin in four doses (10; 20; 100 and 200 µmol/mL)

Concentration of chromium ions [µg/mL]	Concentration of baicalin [µmol/mL]				
	0 (control)	10	20	100	200
Cr(III) 0.05	5.628 ± 0.189	5.114 ± 0.273*	4.692 ± 0.165*	3.951 ± 0.395*	3.453 ± 0.162*
Cr(III) 0.5	5.566 ± 0.182	5.297 ± 0.230	4.938 ± 0.276*	4.112 ± 0.307*	3.734 ± 0.144*
Cr(III) 1.0	5.400 ± 0.266	4.946 ± 0.271*	4.822 ± 0.112*	3.766 ± 0.104*	3.586 ± 0.108*
Cr(VI) 0.05	6.116 ± 0.166	4.896 ± 0.208*	5.044 ± 0.115*	3.988 ± 0.117*	3.866 ± 0.217*
Cr(VI) 0.5	5.858 ± 0.273	4.938 ± 0.197*	4.753 ± 0.132*	4.017 ± 0.157*	3.899 ± 0.180*
Cr(VI) 1.0	5.596 ± 0.263	5.031 ± 0.217*	4.994 ± 0.216*	3.903 ± 0.218*	3.734 ± 0.321*

\*Statistically significant in comparison to the control ( $p < 0.05$ )

Table 2. The mean value of TBARS concentration in erythrocytes after combined exposure to Cr(III) or Cr(VI) in three doses (0.05; 0.5 and 1.0 µg/mL) with baicalin in four doses (10; 20; 100 and 200 µmol/mL).

Concentration of chromium ions [µg/mL]	Concentration of baicalin [µmol/mL]				
	0 (control)	10	20	100	200
Cr(III) 0.05	246.053 ± 16.31	68.532 ± 16.381*	88.881 ± 53.708*	46.664 ± 20.763*	34.183 ± 11.355*
Cr(III) 0.5	250.591 ± 14.49	240.095 ± 30.050	226.578 ± 11.791	96.785 ± 33.011*	62.260 ± 20.101*
Cr(III) 1.0	258.552 ± 15.04	233.013 ± 7.282	202.456 ± 19.951*	104.324 ± 24.579*	55.676 ± 16.001*
Cr(VI) 0.05	263.133 ± 22.470	300.777 ± 22.174*	312.154 ± 18.668*	207.797 ± 26.694*	138.747 ± 17.776*
Cr(VI) 0.5	254.949 ± 13.150	115.124 ± 30.410*	227.620 ± 30.151	97.831 ± 14.294*	85.808 ± 14.303*
Cr(VI) 1.0	218.610 ± 16.870	219.782 ± 28.777	216.193 ± 19.266	106.218 ± 23.217*	61.459 ± 12.610*

\*Statistically significant in comparison to the control ( $p < 0.05$ )

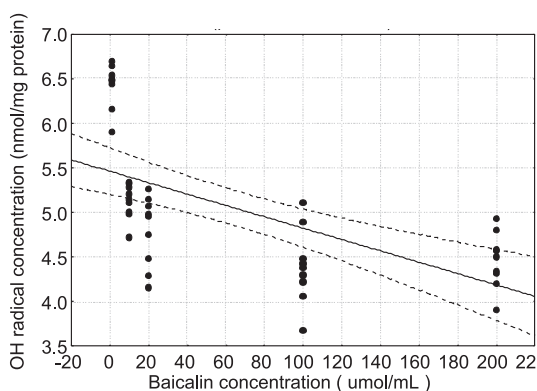


Figure 3. Linear correlation between the dose of baicalin and OH radical concentration in mitochondria ( $p = 0.000$ ;  $r = 0.6012$ )

beneficial effect shown earlier (5) is a consequence of the high content of baicalin. Baicalin's activity depended on its concentrations and the valences of chromium ions. Baicalin's activity was most effective in two doses: 100 and 200 µmol/mL during the

exposition to Cr (III) ions (Tab. 2). The research did not discover any dangerous interactions between baicalin and chromium ions.

*Scutellaria baicalensis* Georgi is a plant rich in flavonoids like baicalin, baicalein, wogonoside and wogonin. Baicalin seems to play a special role because of its large spectrum of biological activities (9–11). There are a lot of reports about the usefulness of antioxidants in the oxidative stress caused by some xenobiotics. The interactions of toxins with antioxidants should be examined in order to determine whether the supplementation with antioxidant in oxidative stress caused by chemicals is beneficial or harmful due to the possible occurrence of dangerous interactions.

Chromium can be present in several oxidation states. While Cr(III) is predominant in the environment, Cr(VI) originates rather from industrial use (12). The mechanism of Cr(VI)-induced toxicity and carcinogenesis is not well understood, although recent studies suggest the involvement of Cr(VI) in free radical formation. Chromium forms complexes with endogenous substances e.g., Cr-glutathione, Cr-DNA (13, 14).

In the present study, baicalin inhibits the lipid's peroxidation and hydroxyl radical generation in exposure to chromium (III) and chromium (VI) ions. The previous study showed that Antoxyd caused an effective decrease in lipid peroxidation induced by both Cr(III) and Cr(VI) ions (5). Antoxyd is very rich in baicalin (72%), that is why it could be supposed that baicalin plays an important role in its antioxidative activity.

The protective effect of baicalin in exposure to chemicals was examined by Song-Won Park et al. (15). This study showed for the first time that baicalin can prevent acute hepatic damage induced by CCl<sub>4</sub> (carbon tetrachloride) in mice. These results indicate that baicalin preserves the structural integrity of the hepatocellular membrane and protects against CCl<sub>4</sub>-induced hepatotoxicity in mice. The MDA level increased significantly in CCl<sub>4</sub>-injected mice and was then reduced by baicalin.

The effect of dietary baicalin supplementation on iron overload-induced oxidative injury of mouse liver was studied by Zhao et al. (16). They found that when mice were fed a baicalin containing diet for 50 days, hepatic lipid peroxidation decreased; while catalase activity and total antioxidant status increased. The protective effect of baicalin on the liver of iron overload mouse may be due to both the antioxidant and iron chelation activities of baicalin. These data provide preliminary experimental support for baicalin as medicine for iron overload diseases.

Flavonoids are known to scavenge free radicals, chelate metal ions and increase the expression of antioxidant proteins. Baicalin and baicalein being lipid-soluble, penetrate membranes easier than other flavonoids (17). Kimura et al. (18) reported that baicalin inhibited lipid peroxidation induced by ADP-NADP and Fe<sup>2+</sup> ascorbate in rat liver homogenates. Other authors reported that baicalin could scavenge reactive oxygen species (ROS), including superoxide, H<sub>2</sub>O<sub>2</sub> or hydroxyl radical generated from Fenton reaction and from the reaction system containing xanthine or xanthine oxidase (19).

Our investigation shows that baicalin effectively inhibited the toxicity caused by chromium ions, by reducing TBARS formation and hydroxyl radical concentration. The beneficial effect of baicalin towards chromium toxicity might be connected with its metal ions chelating activity but also a mechanism connected with hydroxyl radicals scavenging ability of baicalin has been observed in mitochondria. Regarding the effectiveness of Antoxyd in the previous study and high antioxidative properties of baicalin reported now, we can suppose that the

effect of Antoxyd could be connected with baicalin presence.

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