Protective Effect of *Hypericum perforatum* L. Extract on Hexavalent Chromium Induced Toxicity in Rat Adrenal Gland

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This study was carried out to test the possibility of hexavalent chromium administration through drinking water to induce the structural damage in rat's adrenal glands and the possibility of *Hypericum perforatum* extract to faith against chromium aggression. Chromium induced cellular stress was determined by the expression level assessment of the Bcl2 genes family, known to modulate the apoptotic pathway. Obtained results showed that exposure to chromium altered adrenal glands morphology, by induction of apoptosis. When *Hypericum perforatum* extract was administered expression level of Bcl2 genes and histological lesions in adrenal glands were significantly reduced.

Keywords: chromium, adrenal gland, *Aronia melanocarpa*

Chromium (Cr) exists in several oxidation states, but the most biologically active are trivalent (III) and hexavalent (VI) forms. This metal is naturally found in soils, rocks, volcanic dust and gases, but has numerous uses in chemical industry, production of dyes and pigments, wood preservation, leather tanning, chrome plating, stainless steel industry and many others, which can cause environmental pollution. Cr VI is well known as oxidizing agent, and thus it acts as cell function disruptor [1]. In its hexavalent form, chromium crosses through cellular membranes and is rapidly reduced to Cr III and reactive oxygen species (ROS) [2]. Although evidences indicate that chromium can affect hypothalamus and anterior pituitary gland [3] there are no studies regarding Cr VI effects on adrenal glands.

Oxidative stress appears due to an imbalance between ROS generation and the antioxidant defense systems of the body. In that situation is necessary to assure exogenous intake of antioxidants. The richest sources of antioxidants are herbs, cereals, fruits and vegetables because of their content in polyphenols substances, flavonoids, carotenoids, C and E vitamin [4].

*Hypericum perforatum* L. known as St. John's wort is a perennial plant from *Hypericaceae* family native to Europe and Asia, and is commonly used in traditional medicine [5]. Some studies reported that *Hypericum perforatum* extracts are effective scavengers of superoxide radical [6]. A survey of the literature showed that *Hypericum perforatum* has antioxidant and neuroprotective effects and is efficient in management of inflammatory disorders, malignancies, bacterial and viral diseases [5].

Herein, we investigated the Cr VI possibility to induce structural changes in adrenal glands, considering its capacity to cross cell membranes and to easily produce ROS, and if so, the possibility of *Hypericum perforatum* (aqueous extract) to play a protective role, given to its demonstrated antioxidant activity.

**Experimental part**

**Animals and experiment design**

Adult Wistar male rats (220–240g) were purchased from Animal facility of University of Medicine and Pharmacy Victor Babes Timisoara, Romania. The Ethical Committee of Banat University of Agricultural Sciences and Veterinary Medicine King Michael I of Romania Timisoara approved experimental protocol for this study. The animals were housed in polycarbonate cages, at standard laboratory conditions (temperature 22±2°C, relative humidity 40-60%, 12 h light: 12 h dark schedule). Access to food and water was *ad libitum*.

Potassium dichromate (K2Cr2O7) was purchased from Sigma-Aldrich.

The plant material (dried flowers of *Hypericum perforatum*) was purchased from natural plant shop and used to prepare an aqueous extract. This extract was obtained by mixing dried plant with distilled water, at water/volume ratio of 0.25/10 (w/v). The mixture was heated at 90°C for 10 minutes and then filtered [7, 8].

Rats were equally divided in six groups. The control group received only distilled water during the experimental period. Second group (Cr) was treated with hexavalent chromium compound (K,Cr2O7) in distilled water for three months. Third group (CrH) received Cr VI in distilled water with *Hypericum perforatum* 2.5 % aqueous extract for three months. Fourth group (H) received *Hypericum perforatum* 2.5 % aqueous extract for three months. Fifth group (Cr2) was treated with Cr VI in distilled water for three months, followed by one month of distilled water administration. Sixth group (CrH2) was treated with Cr VI in distilled water for three months, followed by one-month administration of *Hypericum perforatum* 2.5 % aqueous extract only.

In our previous researches we tested Cr VI acute and chronic toxicity on male and female reproductive tracts. Exposure doses started from 25 ppm, which is considered to be LOAEL 9], and continued with 50 ppm (2xLOAEL) and 75 ppm (3xLOAEL) Cr VI. In all study designs chromium toxicity was reported, lesions being directly correlated with exposure level [10-13]. Therefore chromium level for this study was established to be 75 ppm Cr VI.

At the end of exposure period the animals were sacrificed and samples prepared for further analysis. The experiment and animal procedures were conducted in accordance with EU Directive 2010/63/EU regarding welfare and protection of animals used in experimental and other scientific purposes.

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perforatum extract against Cr VI effects. Obtained results were interpreted by 2-
GAPDH gene expression. The reactions were normalized with 150 ng of cDNA. Gene expression analysis was performed with GoTag qPCR Master Mix Kit (Promega), using the MX 3000P real-time PCR system (Agilent Technologies). The primer sequences from this experiment are listed in Table 1. The reactions were normalized with GAPDH gene expression. Obtained results were interpreted by 2-ΔΔCT method.

Results and discussions

Cellular stress induced by hexavalent chromium administration and the possible reduction of its toxic effects by administration of Hypericum perforatum extract was evaluated by the expression level assessment of the Bcl2 genes family, known to modulate the apoptotic pathway. Thus to determine the anti apoptotic activity produced by Hypericum perforatum extract, the Bcl2 gene expression was determined. Bcl2 proteins have the property to interfere in apoptosis process acting directly on Bax proapoptotic type proteins. In order to assess the proapoptotic action of chromium, the expression of Bax gene was determined. The evaluation of Bax/Bcl2 ratio emphasizes the organ’s ability to adapt to, or overcome, the action of Cr VI, and the effectiveness of Hypericum perforatum extract against Cr VI effects.

Proapoptotic Bax gene expression increased significantly in all studied experimental groups compared to the levels found in control group (C). The most noticeable increase of Bax gene expression was observed in Cr group. A significant reduction of expression of this gene is noticed in the case of Cr2 experimental group and the administration of toxic was followed by a month recovery time. Supplementation with aqueous extract of Hypericum perforatum in the one-month recovery period leads to a highly significant decrease in gene expression of Bax in the case of Cr experimental group compared to the C group. The in the case of CrH experimental group, who received concomitant both toxic and aqueous extract of Hypericum perforatum, it was noticed a significant decrease in the expression level of this gene compared with the group that received only toxic. In the case of this experimental group the level of Bax gene expression was closer to the value registered for control group, but still significantly increased, showing the stress produced by the aggressor agent on adrenal gland.

Antia apoptotic Bcl2 gene expression levels follows the same pattern as in the case of Bax gene, being significantly increased in all experimental groups compared with control one, thus counteracting the Bax gene overexpression and blocking the initiation of the apoptotic pathway.

The Bax/Bcl2 ratio favorable to Bax gene, observed in the case of hexavalent chromium administration for three months, is indicating the overcoming of adaptation processes and the installation of cell apoptosis process; the ratio value being Bax/Bcl2 - 2.3 (fig 1). This result confirms the structural alterations and cell damage emphasized by histopathology studies. Microscopic examination of adrenal slides from Cr group highlighted degenerative lesions like diffuse cortex cytoplasmic vacuolation, cell hypertrophy, cell apoptosis and capillary dilation (fig. 2). Initiation of cellular apoptotic processes can be explained by the action of hexavalent chromium on cellular DNA, causing its alteration, both, by direct pathway through reduction reactions, modification of nitrogenous bases, mono- or double-catena breaks, the formation of Cr-DNA, DNA-Cr-DNA or protein-Cr-DNA adducts, and the indirect pathway through the production of ROS [14, 15].

Also, in the case of Cr2 and CrH2 experimental groups, an overexpression of these two genes is noticed, with a Bax/Bcl2 ratio very similar between the two groups (Cr2 Bax/Bcl2 - 1.66, CrH2 Bax/Bcl2 - 1.59) indicating the action of stressor factor, Cr VI, on cells leading to the apoptotic mechanisms. The action of Cr VI is also emphasized by structural alterations discovered in the tissue. In the Cr2 group were observed localized cells of the adrenal cortex with vacuolar degeneration and cell apoptosis (fig. 3). However those alterations are more reduced than in the Cr group. But the adrenal glands morphology from CrH2 group (fig. 4) was similar to those of control group (fig. 5). The structure appeared normal, without degenerative lesions, but with capillaries dilation. This result is due to the recovery period but also, to the beneficial influence of the Hypericum perforatum extract administration, as agent for reducing oxidative stress, with beneficial effect in the post aggressed recovery.

CrH group has a gene expression level similar to that of the control group, a Bax/Bcl2 ratio around the numeric

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sense (5’-3’)</th>
<th>Antisense (5’-3’)</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>AAGGAAGAGCTGGCGCTACCT</td>
<td>AGGCCCTCCACGATGCACAAAGTTG</td>
</tr>
<tr>
<td>Bax</td>
<td>CCAAGACGGATTACCAACAAGAC</td>
<td>TGCCACACGGAAGAAGACCTTCTG</td>
</tr>
<tr>
<td>Bcl2</td>
<td>GGATGACTTCTTCTCTCTGCTTACCCT</td>
<td>A7CCCTGAAGAGTTCTTCACCAC</td>
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value of 1 (CRH Bax/Bcl2 -1.28), which leads us to conclude that continuous administration of aqueous extract of Hypericum perforatum, concomitant with the Cr VI, cause the blocking of apoptotic cascade initiations mediated by Bax and Bcl2 pathway. Histopathological lesions: intracellular vacuolation and hypertrophy of the cells from zona fasciculata and zona reticularis, sinusoid capillaries dilation (fig. 6), and slight fibrous septum formation, although present were significantly reduced than in Cr group, and considering the apoptotic cascade blockage are perhaps being produced through other mechanisms.

Resulting protective role of Hypericum perforatum extract is supported by in vitro studies that emphasize Hypericum perforatum antioxidant effects due to its protectiveness against mitochondrial dysfunction by maintaining transmembrane potential, thus reducing lipid peroxidation in mitochondria and its property to scavenge superoxide radicals and DPPH [6]. Also it was demonstrated that Cr VI induced oxidative stress can be reduced by administration of antioxidants like vitamin E, C, folic acid, curcumin and natural extracts [16-19].

Molecular and histological assessment of adrenal glands from H group, that received only Hypericum perforatum extract for three months, revealed the same results as those obtained from control group.

**Conclusions**

Cr VI administration in drinking water caused structural damage in adrenal glands, probably by production of ROS and induction of oxidative stress. But the results indicate that Hypericum perforatum aqueous extract provides protection against structural alterations of adrenal glands
caused by Cr VI administration, and thus it may be used as suppressant against chromium-adrenal toxicity.

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References

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