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The effect of cisplatin administration on certain trace elements homeostasis in rats and the protective effect of silver birch (*Betula pendula*) sap

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ABSTRACT

Background: A clinically active structure with known antitumor activities is *cisplatin* (CDDP), but this it comes with toxicity characteristics which can be faded by the beneficial effects of Silver birch (*Betula pendula*) sap.
Objective: We aimed to assess the cisplatin activity on: Mn, Mg, Cu, Fe and Zn homeostasis in rats and to observe the effect of birch sap.
Methods: Healthy Wistar rats ($n = 10$/group) were divided in four groups: Control: receiving 1 mL saline I.P. way + water; E1: cisplatin 20 mg kgbw^{-1}, I.P.; E2: cisplatin 20 mg kgbw^{-1}, I.P. + birch sap and *Control sap group*: 1 mL saline I.P. + birch sap. Blood was collected: at the trial's start and after 48 h, and blood and organs (liver, kidney and spleen) for the cytoarchitecture investigation and readings were sampled after seven days. Samples were processed in nitric acid by microwave digestion and readings were completed by flame atomic absorption spectroscopy, the outcomes being statistically analyzed by ANOVA.
Results: Cisplatin produced a significant imbalance in the trace elements homeostasis, the birch sap administration recovering them usual homeostasis status. Comparatively with the Control, rats exposed to cisplatin presented a not significant ($p > 0.05$) decrease of Zn (−26.74%) and Mg (−10.25%), a significant ($p < 0.05$) decrease of Cu (−27.73%) at 48 h, a highly significant ($p < 0.01$) decrease of Cu (−56.08%) and Fe (−85.35%) at seven days after administration and a not significant ($p > 0.05$) increase of Mn (+28.16%). Birch sap administration after Cisplatin was followed by restoration or nevertheless significant increase ($p < 0.05$) of the investigated trace elements Zn (+56.88% to 48 h/+89.94% after seven days), Mg (+26.86%/+95.74%), Cu (+23.13%/+74.56%), Fe (+39.64%/+440.11%) and Mn (+4.30%/+15.87%), suggesting them defence against cisplatin. Histology revealed the order of main altered organs after the CDDP exposure: kidney, spleen and liver.
Conclusions: The study recommended the important protective role of *Betula pendula* sap against diverse cisplatin deleterious side-effects.

1. Introduction

Birch is a special tree known since ancient times, with plentiful healing valences. Externally, *birch bark* was used to intensify healing and alleviate pain, to treat skin inflammations and infections, such as eczema and psoriasis, maybe because the outer bark contains up to 20% betulin [1,2].

Similarly, birch bark was utilized in the traditional medicine as diuretic and is thought helpful in the treatment of many important health disorders like: hypertension, high-level cholesterol, obesity, gout, kidney stones, nephritis, cystitis, digestive disorders and respiratory syndrome. For these reasons, decoction of the bark or leaves it is frequently used [2,3].

The *buds* major components are the essential oils like: α-copaene (~10%), germacren D (~15%) and δ-cadinene (~13%) and also, they are containing additional triterpenoid substances which have been exposed anti-inflammatory, antiviral and anti-cancer activity [4,5].

Birch sap (or birch water) can be obtained from *Betula alba* (white birch), *Betula pendula*, *Betula lenta*, *Betula papyrifera* and *Betula fontinalis*, being considered a traditional drink in hemiboreale and boreal regions of the northern hemisphere and in northern China. When is fresh, birch sap is a clear and colourless often slightly, sweet, with a soft texture water. After two to three days, the fermentation starts and sap taste it becomes more acid [6,7].

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As chemical constituents, the birch sap includes about 1% heterozides (betulozides and monotopinozides) and can be used in a similar manner as maple syrup, consumed fresh, concentrated by evaporation or fermented, as "wine" [8–10]. Furthermore, it containing 17 amino acids, including glutamic acid, as well as minerals, enzymes, proteins, betulinic acid and betulin, antioxidants, sugars (xylitol, fructose and glucose) and vitamins (C and B group) [11–13]. Among the contained minerals, in sizeable quantities in the sap are detectable also: potassium (120 mg/100 mL), calcium (60 mg/100 mL), magnesium (11 mg/100 mL), phosphorus (6.4 mg/100 mL), manganese (1.1 mg/100 mL) and iron (0.1 mg/100 mL) [14].

Cisplatin or *cis-diamino*-dichlor-platin (CDDP) is one of the best clinically active structures known with antitumor activities, still in use. Its introduction in 1975 substantially changed the variety of malignancies that are known as specifically chemo sensitive like: lung and ovarian cancers, germinal tumours, etc. [15,16].

In therapy, CDDP is administered intravenously as sterile 0.9% NaCl solution and, once reached in the circulation, it remains intact due to the quite high concentration of Cl^- ions (~100 mM). Consequently, its neutral compound enters in the cell, both by passive diffusion as well as by cell distribution, and here, the neutral cisplatin molecule undertakes a hydrolysis process, in which, the Cl^- ligand is replaced by a water molecule, thus generating positively charged species. Hydrolysis emerges within the cell to a considerable reduced concentration of Cl^- ions (3–20 mM), and consequently, to higher water concentrations [17,18].

Once inside the cell, cisplatin has several known potential targets as: DNA [19], enzymes (such as sulphur-containing ones) [20], metallothionein and glutathione [15], essential trace elements [21–23], and them metabolic stages [24,25], nephron [26], colon [27], etc.

The consequences of cisplatin upshot in the mitochondria are not well understood yet, but it is possible that, the effects on the mitochondrial DNA to result in the cell's death. Also, the cisplatin interaction with the enzymes containing sulphur in them structure is, for the moment, not as much identified, while; it is well thought-out that these enzymes are involved in the cellular resistance to cisplatin [18].

Although it is a good chemotherapic mean, cisplatin has several severe side effects, the most common being the peripheral neurotoxicity, nephrotoxicity and ototoxicity [28].

In present study, we aimed to evaluate the activity of cisplatin after administration upon certain trace elements (Mn, Mg, Cu, Fe, Zn) homeostasis in the lab animals (Wistar albino rats) and also to observe the overall protective effect of silver birch (*Betula pendula*) sap, knowing that up mentioned bio elements are essential cofactors for miscellaneous enzymes acting in the body.

2. Materials and methods

2.1. Animals and protocol

Healthy Wistar albino rats (280–330 g) were obtained from the authorized Biobase of University of Medicine and Pharmacy "*Victor Babes*" Timisoara, Romania. The rats were housed in standard polycarbonate cages (l × w × h = 750 × 720 × 360 mm) and fed *ad libitum* with standard diet (Diet, Biovetimix, code 140-501, Romania). As bedding, wood shavings were used. The environmental temperature was maintained at 22 ± 2 °C and at a relative humidity of 55 ± 10%. During the experimentation period, the light cycle was: 12 h light and 12 h dark. Before the start of the experiment animals were kept in cages for one week to acclimatize and handled in accordance with Directive 2010/63/EU on the handling of animals used for scientific purposes [29] and guidelines of the National Research Council (NRC) [30]. The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine from Banat's University of Agricultural Science and Veterinary Medicine from Timișoara.

The rats were distributed in four experimental groups (n = 10/group) as follows:

- Group C – *Control;* saline 1 mL I.P. way + water *ad libitum.*
- Group E1 – *Experiment 1*; cisplatin administered I.P., 20 mg kgbw^{-1}.
- Group E2 – *Experiment 2*; cisplatin administered I.P., 20 mg kgbw^{-1} + birch sap *ad libitum.*
- Group CS – *Control Sap*; saline 1 mL I.P. + birch sap *ad libitum.*

All rats were euthanized in the same time period, from 08,00 to 09,00 h, by overdosing anaesthetic agents using 300 mg kg bw^{-1} of ketamine (Ketamine 10%, CP Pharma, Burgdorf, Germany) and 30 mg kg bw^{-1} of xylazine (Narcoxyl, Intervet International, Boxmeer, the Netherlands), in accordance with Directive 2010/63/EU [29], and SVH AEC SOP.26, Euthanasia of Mice and Rats [31] and samples for the histological assay were collected.

2.1.1. Histological technique

For the histological investigations, liver, kidney, and spleen were sampled. The tissue fragments were prepared after known technique: were fixed in 80° alcohol for seven days and then washed, dehydrated and included in paraffin. Paraffin blocks enclosing tissue fragments were sectioned using a microtome, resulting in 5-μm-thick sections. The sections were stained by the standard haematoxylin and eosin method (H&E). All histological images were captured using the Olympus CX 41 software program, at magnifications of 100× or 400×.

2.1.2. Birch sap

Birch sap was collected from Caransebes region, Caras-Severin County (Lat. 45.4136°; Long. N, 22.2219° E), by creating a hole in the tree trunk of about 4 mm in diameter and 3–5 cm in profundity. Then, a plastic straw attached to a flask was inserted into the hole and periodically it was changed when it's filling. Following gathering, the sap was kept in refrigerator as needed.

2.2. Samples analysis

For determination of copper, iron, manganese, magnesium and zinc, samples mineralization was performed by microwave digestion. The samples were deposited in the digestion bottles adding, 10 mL of concentrated nitric acid and 2 mL of hydrogen peroxide. The flasks were covered with a lid, and inserted into the protective sleeve and then submitted to microwave digestion system (Multiwave GO, Anton Paar, GmbH, Austria), the working schedule being settled to: 20 min, 120 °C and 800 W. After digestion, the samples were placed in flasks rated 25 mL, adding double-distilled water up to the mark. All reagents used for digestion were of high-purity grade (Suprapur, Merck).

Readings were made by flame atomic absorption spectroscopy, using a Varian model AA 240 FS spectrophotometer (Agilent Technologies Inc. USA). Selected elements Cu, Mn, Zn, Mg and Fe were quantifed in the sample by atomic absoption spectrometry using a Varian AA 240 FS with deuterium lamp background corrector, in the conditions presented in Table 1.

All reagents used in this study were suprapur grade and were purchased from E-Merk, Germany. Working standards were prepared by serial dilutions of a Merck CertiPur ICP 1000 mg/L stock standard solution.

Table 1
The conditions for atomic absoption spectrometry reading.

Parameter	Cu	Mn	Zn	Mg	Fe
Wavelenght (nm)	328.4	279.5	213.9	248.3	285.2
Slit width (nm)	0.5	0.2	1.0	0.5	0.2
Lamp current (mA)	4.0	5.0	5.0	4.0	5.0
ratio air: acetilene	13.5:2	13.5:2	13.5:2	13.5:2	13.5:2