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Towards improved valorisation of (*Betula pendula)*: preliminary study of the genotoxic, antigenotoxic and cytotoxic potential of a commercial aqueous silver birch leaf extract

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Birch is a tree, of the genus *Betula* belonging to the *Betulaceae* family and it is known for its various pharmaceutical properties. At the same time it is a suitable tree species for renewable forests. In the present preliminary study an aqueous commercially available leaf extract of silver birch (*Betula pendula)* was studied to evaluate its potential genotoxic and cytotoxic activity as well as its antigenotoxic properties against the mutagenic agent *mitomycin-C* by employing the *in vitro* Cytokinesis Block Micro Nucleus (CBMN) assay. Human lymphocytes were treated with *Betula pendula* with and without *mitomycin-C*. *Betula pendula* did not increase the frequency of micronuclei, and showed cytotoxic potential. All mixtures of *Betula pendula* and *mitomycin-C* demonstrated a decrease in the micronuclei frequencies, with the lowest and highest concentrations inducing a significant antigenotoxic activity. Therefore, the birch product studied showed antigenotoxic and cytotoxic potential which could render it useful in various medicinal applications.

Key words: natural medicines, anticancer properties, silver birch leaf-extracts, CBMN assay

INTRODUCTION

Silver birch (*Betula pendula* Roth.) is one of the most important forest-forming and timber production trees. In Poland in particular, approximately 2.5 million cubic metres of birch wood timber is sourced annually by the State Forests. Birch wood is being used for paper and furniture production, as firewood and for certain pharmaceutical, cosmetics and food products. However, all parts of the silver birch can form valuable biomass for biofuel production. Towards that direction its composition has been characterised by Lachowicz *et al.* [1]. Uri *et al*. [2] have studies the carbon sequestration ability of the same species, which is important to know when trees are to be used for biofuel production. However, biomass is most valuable when it is first used as pharmaceuticals or natural additives, with food and animal feed to follow, chemicals production to form the third option and biofuels production as the ultimate between the aforementioned one.

Birch is a tree source of natural pharmaceutical products. As such, the valorisation of birch biomass should start from the extraction of valuable products. Since ancient times natural products have found multiple applications in various fields and demonstrated remarkable pharmacological and medicinal properties. A great deal of the knowledge of those properties is lost; additionally such knowledge was frequently based on empirical data and the science underpinning their beneficial properties has to be established. Their thorough study could lead to the discovery of more beneficial properties and their establishment as promising agents against several diseases. This work focuses on such properties of the birch, to demonstrate the added value products which can be obtained from such biomass before its exploitation for energy generation.

Birch is a tree, of the genus *Betula* belonging to the *Betulaceae* family and is most common in the northern hemisphere. It is known for its pharmaceutical properties while many studies have been conducted indicating that the plants themselves as well as components from various birch species possess antimicrobial, antioxidant and anticancer activity (Rastogi *et al*. [3]).However, most research papers have focused on the chemical composition and various properties of the birch bark and its constituents (Zhanataev *et al.* [4], Sami *et al*, [5], Calliste *et al*, [6]). There is very limited scientific work on the properties of the birch leaves.

In this research, the possible genotoxic and cytotoxic activity of an aqueous leaf extract of birch (*Betula pendula)*, as well as its antigenotoxic potential was studied in cultured human lymphocytes applying the cytokinesis block micronucleus (CBMN) assay for the detection of micronuclei in the cytoplasm of interphase human lymphocytes. Micronuclei may originate from acentric chromosome fragments or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. Thus, this assay detects the potential clastogenic and

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aneugenicactivity of chemicals in cells that have undergone cell division after exposure to the test chemical (OECD, [7], Kirsch-Volders *et al*. [8]).The antigenotoxic effect of the birch product was studied against the genotoxic damage induced by mitomycin C (MMC), an antitumor compound that has a range of genotoxic effects including the inhibition of DNA synthesis, mutagenesis and clastogenesis.

MATERIALS AND METHODS

Chemicals

The aqueous leaf extract of *Betula pendula* was purchased by Abnoba GmbH (http://www.abnoba.de/, Pforzheim, Germany; Betula folium D3 Abnoba, batch-no. 706 A41 was used for the investigations). Mitomycin C and Cytochalasin-B (Cyt-B) were purchased from Sigma (St. Louis, MO, USA). Ham's F-10 medium, foetal bovine serum and phytohaemaglutinin were commercially supplied (Gibco, UK). All other chemicals and solvents were of the highest grade commercially available. Stocks of the compounds and solutions were stored at 4°C until use.

Ethics Statement

The study was approved by the Ethical Committee of the University of Patras. After informed consent healthy, non-smoking male individuals (less than 30 years), were used as blood donors to establish whole blood lymphocyte cultures. According to the donors' declaration, they were not exposed to radiation, drug treatment or any viral infection in the recent past.

CBMN assay in human lymphocytes in vitro

The CBMN assay was performed according to standard procedures (OECD 487 [7]) with minor modifications. Human blood samples were obtained from two non-smoking, healthy individuals not undergoing any drug treatment, having viral infection, or having X-ray exposure in the recent past. Whole blood (0.5 mL) was added to 6.5 mL of Ham's F-10 medium containing 1.5 mL of fetal bovine serum and 0.3 mL of phytohaemagglutinin to stimulate cell division. The birch product was studied at four different doses i.e. 0.5, 1, 2 and 5% (v/v) of the total culture volume and *Mitomycin C*(0.05 μg/ml) was given along with each of the reported doses of the birch-leaf product, 24 h after culture initiation.

After 44 h of incubation, cytochalasin-B (final concentration of 6 μg/ml) was added to the cultures to block cytokinesis of dividing cells. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 72 h; 72 h after the initiation of culture, cells were harvested and collected by centrifugation. A mild hypotonic treatment with 3:1 solution of Ham's medium and milli-q H_2O was left for 3 min at room temperature which was followed by 10 min fixation (for at least 3 times) with a fresh 5:1 solution of methanol/acetic acid. Cells were stained for 10 min with 7% Giemsa (Vlastos *et al.* [10]). Binucleated cells with preserved cytoplasm were scored per experimental point to calculate the micronuclei frequency according to standard criteria (Fenech [11] and Fenech *et al.* [12]).

To determine possible cytotoxic effects, the cytokinesis block proliferation index (CBPI) was evaluated (Surrallés *et al.* [13]). CBPI is given by the equation:

$$CBPI = \frac{M1 + 2M2 + 3(M3 + M4)}{N} \quad (1)$$

where M1, M2, M3 and M4 correspond to the number of cells with one, two, three and four nuclei, respectively and N is the total number of counted cells.

RESULTS

As mentioned above, the birch-leaf product was studied at four different doses i.e. 0.5, 1, 2 and 5% (v/v) of the total culture volume and the same doses were tested combined with *Mitomycin C* at a concentration of 0.05 μg/ml for *Betula pendula* in order to identify its antigenotoxic effect against the genotoxic damage induced by *Mitomycin C*. Treatment with the *Betula pendula* did not induce micronuclei as compared to control. Treatment with 0.05 μg/ml of *Mitomycin C* induced an increase in micronuclei frequencies, as expected, compared to control. A decrease in micronuclei frequency was found when the lowest and highest concentration of *Betula pendula* was given along with the concentration of *Mitomycin C*.

The cytotoxic effect of the *Betula pendula* and its mixture with *Mitomycin C* was evaluated by the determination of CBPI. Regarding the cytotoxic index, significant differences on CBPI were detected between control cultures and the two highest doses of the *Betula pendula*. Moreover, significant cytotoxic effect appeared between *Mitomycin C* and one of the *Betula pendula* concentrations with *Mitomycin C*.

DISCUSSION

Natural products have been used since antiquity and are known for possessing remarkable medicinal and pharmaceutical properties. In the present study

the genotoxic and cytotoxic effects of a birch (*Betula pendula*) aqueous extract (*Betula pendula*) was screened to evaluate its antigenotoxic potential. For this purpose the *in vitro* Cytokinesis Block MicroNucleus (CBMN) assay was employed.

The lack of genotoxicity showed by the *Betula pendula* could be explained by its major phenolic components which were found to be quercetin-monoglycosides by the phytochemical analysis of the specific product conducted in a previous study (Gründemann *et al*, [14]). Quercetin's genotoxic potential has been evaluated in various studies and it has been concluded that this naturally occurring flavonol is not considered genotoxic (Da Silva *et al*. [15]; Utesch *et al*. [16]).

The potential antigenotoxic activity of the *Betula pendula* was examined by co-treatment of human lymphocytes with the *Betula pendula* and the mutagenic inducer *Mitomycin C*. *Mitomycin C* is an antibiotic that transforms into an alkylating agent and affects DNA synthesis, causes inter-strand cross-links in DNA and formation of DNA adducts (Iyer and Szybalski [17], Waring [18], Dall'Acqua *et al* [19], Bargonetti *et al* [20]). It has been found to be genotoxic in all *in vitro* and *in vivo* test systems in mammalian cells and animals, thusbeing considered as carcinogenic agent (Lorge *et al*, [21]; Mazumdar *et al*, [22]). Accordingly, *Mitomycin C* was found to be genotoxic, inducing statistically significant increase in micronuclei and BNMN.It was observed that all concentrations of the *Betula pendula* led to a decrease in micronuclei frequency which was statistically significant in the lowest and highest concentrations tested. The induction of antigenotoxic potential could be attributed to the antigenotoxic activity of *Betula pendula*'s most abundant flavonol, quercetin. Specifically, quercetin prevented DNA damage and had antiproliferative properties in human hepatoma cell line (HepG2) against *tert*-butyl hydroperoxide (*t*-BHP), in addition to increasing the rate of DNA repair (Ramos *et al* [23]). Moreover, quercetin exhibited antigenotoxic effect *in vivo* against chromium trioxide induced micronuclei in polychromatic erythrocytes of mouse peripheral blood (García-Rodriguez *et al*. [24]).

As far as the cytotoxicity of the *Betula pendula* is concerned, a significant decrease of CBPI values was observed at the two highest concentrations of the *Betula pendula*, as well as at one concentration of the *Betula pendula* with *Mitomycin C*. The induction of cytotoxicity by the *Betula pendula* can be corroborated by past studies concerning various Betula species. In a previous study by Goun *et al* [25], the methylene chloride and the methanol extracts of *Betula pendula* were evaluated for their activity against leukemia. A cytotoxicity assay, determining the inhibitory effect of test samples on the growth of mouse leukemia cells (L1210), was applied, with the extracts exhibiting a high level of cytotoxicity against leukemia. *Betula pendula* fractions were tested and showed antiproliferative activity against B16 melanoma cells (Calliste *et al* [6]). Another Betula species, *Betula platyphylla* var. *japonica* was tested and its extract induced apoptotic cell death in human promyelocytic leukemia (HL-60) cells, a cancer cell line (Ju *et al* [26]).

Since most natural substances constitute a mixture of various components, their potential beneficial effects could be attributed to the synergism among the different components (Liu RH, [27], Koutsoudaki *et al*, [28] Vlastos *et al* [29]). In addition to that, the mixture of different substances or natural products could also lead to enhanced effects (Huh *et al* [30]).

CONCLUSION

In conclusion, our preliminary study provides evidence on the antigenotoxic potential, induction of cytotoxicity and the lack of genotoxic activity of a birch product under the present experimental conditions. Further research could be suggested to identify potential beneficial properties of birch. As such, the production of added value product from birch, before it is used for energy generation is possible and should be examined. This study was employing a commercially available leaf extract. However, the conditions of production may have an effect on the qualities of the final product and as such, its properties will be examined under a variety of extraction methods.

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REFERENCES

1. H. Lachowicz, H. Wro´blewska, M. Sajdak, *Cellulose*, **26**, 3047 (2019).
2. V. Uri, Varik M., J. Aosaar, A. Kanal, M. Kukumägi, K. Lõhmus, *For. Ecol. Manage*, **267**, 117 (2012).
3. S. Rastogi, M. M. Pandey, A. Kumar, S. Rawat, *J Ethnopharmacol*, **159**, 62 (2015).
4. A K. Zhanataev, G A. Presnova, A. N. Chistyakov, A. D. Durnev, *Bull ExpBiol Med.* **138**, 475 (2004).
5. A. Sami, M. Taru, K. Salme, Y. K. Jari, *Eur. J. Pharm. Sci.,* 29, 1 (2006).
6. C A. Calliste, P. Trouillas, D. P. Allais, A. Simon, J. L. Duroux, *J Agric Food Chem*., **49**, 3321 (2001).

7. OECD.. *Test No. 487*: *In Vitro Mammalian Cell Micronucleus Test*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing. 10.1787/9789264224438-en. (2014).
8. M. Kirsch-Volders, I. Decordier, A. Elhajouji, G. Plas, M. J. Aardema, M. Fenech *Mutagenesis,* **26**, 177 (2011),
9. Abnoba GmbH http://www.abnoba.de/
10. D. Vlastos, C G. Skoutelis, I T. Theodoridis, D R. Stapleton, M I Papadaki. *J Hazard Mater.* **177**, 892 (2010).
11. M. Fenech, *Mutat Res*. **392**, 11 (1997).
12. M. Fenech, W P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi, E. Zeiger, *MutatRes*., **534**, 65 (2003).
13. J. Surrallés, N. Xamena, A. Creus, J. Catalan, H. Norppa, R. Marcos, *Mutat Res*. **34**, 1169 (1995)
14. C. Gründemann, C.W. Gruber, A. Hertrampf, M. Zehl, B. Kopp, R. Huber, *J Ethnopharmacol.* **136**, 444 (2011).
15. J. Da Silva, S. M. Herrmann, V. Heuser, W. Peres, N. Possa Marroni, J. González-Gallego, B. Erdtmann *Food ChemToxicol.*, **40**, 941 (2002).
16. D. Utesch, K. Feige, J. Dasenbrock, T. H. Broschard, M. Harwood, B. Danielewska-Nikiel, T.C. Lines, *MutatRes.*, **654**, 38 (2008).
17. V. N. Iyer, W. Szybalski *Science*, **145**(3627), 55 (1964).
18. M. J. Waring *Nature*. **219** (5161), 1320 (1968).
19. F. Dall'Acqua, S. Caffieri, G. Rodighiero, *Photochem.Photobiol*., **27**, 77 (1978).
20. J. Bargonetti, E. Champeil, M. Tomasz, *J Nucleic Acids*. art. no. 698960, doi: 10.4061/2010/698960 (2010).
21. E. Lorge, V. Thybaud, M. J. Aardema, J. Oliver, A. Wakata, G. Lorenzon, D. Marzin, *Mutat Res.*, **607**, 13 (2006).
22. M. Mazumdar, S. Giri, A. Giri, *Mutat Res.*, **721**, 147 (2011).
23. A. A. Ramos, C. F. Lima, M. L. Pereira, M. Fernandes-Ferreira, C. Pereira-Wilson. *Toxicol Lett.*. **177**, 66 (2008).
24. MdC. García-Rodriguez, T. Nicolás-Méndez, A. R. Montaño-Rodriguez, M. A. Altamirano-Lozano *J Toxicol Environ Health, Part A*. 77: 324 (2014).
25. E. A. Goun, V. M. Petrichenko, S. U. Solodnikov, T. V. Suhinina, M. A. Kline, G. Cunningham, C. Nguyen, H. Miles, *J Ethnopharmacol.*, **81**, 337 (2002).
26. E. M. Ju, S. E. Lee, H. J. Hwang, J. H. Kim, *Life Sciences*. **74**, 1013 (2004).
27. R. H. Liu, *J Nutr,* **134**, 3479S (2004).
28. C. Koutsoudaki, M. Krsek, A. Rodger, *J Agric Food Chem.*, **53**, 7681 (2005).
29. D. Vlastos, D. Mademtzoglou, E. Drosopoulou, I. Efthimiou, T. Chartomatsidou, C. Pandelidou, M. Astyrakaki, E. Chalatsi and P. Mavragani-Tsipidou *PLoSOne*. **8**, e69494 (2013).
30. J. E. Huh, Y. H. Baek, S. R. Ryu, J. D. Lee, D. Y. Choi, D. S. Park *Int Immunopharmacol.*, **9**, 230 (2009).