Research paper

*Silybum marianum* (milk thistle) products in Wilson’s disease: a treatment or a threat?

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Background: *Silybum marianum* (milk thistle) or its active flavonolignan complex, silymarin is widely used in the treatment of liver diseases of toxic and viral origin, and also in Wilson’s disease to prevent chronic liver impairment.

Purpose: Based on the available data it is probable that milk thistle-based preparations include copper in significant concentration. The goal of this study was to assess the safety of different milk thistle preparations in the treatment of patients with Wilson’s disease.

Methods: The copper content of 15 milk thistle preparations was measured by an inductively coupled plasma mass spectrometer. The products contained purified silymarin, crude milk thistle extract or the ground plant material.

Results: The copper content of the daily doses of the analyzed preparations ranged between 0.01–114.18 μg. Silymarin-based products had a lower copper content than preparations containing ground milk thistle fruits or the crude extract.

Conclusion: The application of milk thistle products with a high copper content should be avoided for patients with Wilson’s disease.

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1. Introduction

Wilson’s disease is a rare genetic disease that causes serious copper poisoning. Accumulation of inorganic copper in different tissues leads to organ dysfunctions including hepatic failure. Though neurological problems are the key symptoms of the disease, patients tend to develop the hepatic disease significantly earlier than the neuropsychiatric manifestations would present themselves (Chanprasert and Scaglia, 2015). The most common manifestation of liver failure is chronic active or acute self-limiting hepatitis (the former may lead to cirrhosis), however rarely acute fulminant hepatic failure may also develop (Lang et al., 2007). Because of the low incidence of Wilson’s disease, the diagnosis may be challenging due to the varied characteristics and unpredictability of the symptoms of the disease in both types of presentation and severity when developed in patients. Anti-copper agents (e.g. α-penicillamine, trientine, dimercaptoposuccinic acid, zinc salts and tetrathiomolybdate) are commonly used to prevent copper accumulation (Li et al., 2016). For the prevention of hepatic failure, hepatoprotective agents like *Silybum marianum* (L) Gaertn are often used.

There is no special therapy to prevent hepatic impairment. For this purpose, aspecific hepatoprotective agents are used. One of the most widely applied medications is silymarin, a flavonolignan complex obtained from the fruits of *Silybum marianum* (milk thistle). The mechanism of action of silymarin is complex. It has a pronounced antioxidant activity, modulates inflammation and fibrogenesis (Loguerio and Festi, 2011). Although no human data is available to directly support the clinical efficacy of silymarin in Wilson’s disease, based on the acknowledged aspecific hepatoprotective effect of the flavonolignan complex, silymarin is usually applied as a long-term treatment.

Although the compound silymarin is commercially available as a licensed medicine in several European countries the number of products marketed as food supplements is increasing. In this product category, certain preparations contain ground plant material or milk thistle extracts rather than the flavonolignan complex silymarin derived from the small hard skinned fruit (achene) known as *Fructus silybi* that develops from the large...
purple flowerhead, known commercially as *Silybum marianum* seeds. Silymarin is usually produced by a multistep purification process from the *Silybum marianum* fruits, including extraction with ethyl acetate (Madaus et al., 1983). This ensures the enrichment of pharmacologically active flavonolignans and the removal of neutral (or undesirable) components. Since milk thistle belongs to the category of plants with a high copper concentration with a remarkable 17 µg/g copper content in the fruits (Lovkova and Buzuk, 2011), the way of processing can largely influence the copper content of the final product. In the case of patients suffering from Wilson's disease the benefit/risk ratio of silymarin or milk thistle treatment fundamentally relies on whether the processing enriches or decreases the copper content of the final product. The aim of this work was to analyze the copper content of milk thistle-based medicines and food supplements to assess their safety in the treatment of patients suffering from Wilson's disease.

### 1.1. Material and methods

The copper content of 15 commercial milk thistle products were examined in this study. Four samples were medicines (extracted from the fruits of *Silybum marianum*; S1–S4, capsules and tablets) and 11 samples were dietary supplements or healing products1 (S5–S8, S13 and S15 milk thistle extract; S9, S10, S12 and S14 powdered fruits and S11 oil) in the form of capsules, tablets and teas. A detailed description of all investigated products is provided in the Supplementary data.

Sample preparation was carried out in two different ways. Powdered tablets, capsules and also tea samples were extracted for 30 min in an ultrasonic bath with 10.0 ml 1 M HCl of trace analytical purity (Suprapur grade, Merck), followed by filtration through a membrane filter. Tea samples S12–S14 were extracted with 10.0 ml water in an ultrasonic bath for 30 min then filtered, to mimic tea preparation. Procedural blank samples were also prepared (the preparation was carried out with all the same reagents and labware, but without the sample). A Millipore Direct-Q UV3 labwater purifier (Millipore Corporation, Billerica, MA, USA) was used to produce trace quality deionized labwater for sample preparation.

Copper content determinations were carried out using an Agilent 7700x type inductively coupled plasma mass spectrometer (ICP-MS). The sample introduction system consisted of an I-AS type autosampler, a MicroMist pneumatic microconebulator and a Peltier-cooled (2 °C) Scott type double-pass spray chamber. The sample uptake rate was 200 µl/min. ICP plasma and interface parameters were set according to the standard hot plasma configuration. Fine tuning was performed before the analytical measurements using tuning solutions supplied by the manufacturer (G1820-60410, G5185-5959 Agilent Technologies). All measurements were carried out using the He mode of the ORS® collision cell, by monitoring the signal of 65Cu and 64Cu isotopes. 40Sc was used as internal standard. For calibration purposes, six standard samples were prepared from a certified ICP-MS standard stock solution (IV-ICP-MS 71A, Inorganic Ventures) via dilution with trace quality deionized labwater. QC samples were also used to ensure the accuracy of determinations. All labware (PE autosampler vials, PMP certified volumetric flasks, etc.) were thoroughly cleaned before use with dilute ultratrace quality nitric and hydrochloric acid (Ultrapure Normatom, VWR).

### 2. Results

To verify the sample preparation procedure milk thistle fruit was extracted with 1M HCl as previously described. The measured copper content (18.43 µg/g) was in agreement with the literature data as previously reported by Lovkova and Buzuk (2011) (17.00 µg/g).

The copper content of the daily doses of the preparations ranged between 0.01–114.18 µg (Table 1). Mean daily dietary copper intakes of adults in different European countries have been estimated to be 1.0–2.3 mg/day for males and 0.9–1.8 mg/day for females (Van Dokkum, 1995). For patients suffering from Wilson’s disease, milk thistle preparations containing a high concentration of copper significantly increase the copper load and therefore can be regarded as undesirable during the treatment. The copper concentrations of samples containing silymarin, the purified flavonolignan complex of milk thistle fruits (S1–S8 and S15), were rather low (0.01–3.51 µg in the daily dose). However, products based on (presumably crude) *Silybum* extract (S13) or ground plant material (S9, 10, 12, 14) contained copper at concentrations which were magnitudes higher than those of the silymarin-containing products (36.04 and 12.04–114.18 µg in the daily dose, respectively). In the case of milk thistle fruit oil, the copper concentration was low, nevertheless the silymarin content was also negligible.

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1 The category “healing products” is a special product category in Hungary, with a very similar legal background to that of traditional herbal medicinal products.
3. Conclusion

The pharmacotherapy of Wilson's disease focuses on the facilitation of urinary excretion of chelated copper (by administering e.g. penicillamine or trientine) or on the prevention of absorption from the intestine (by administering zinc salts). For the prevention of hepatic failure, the use of hepatoprotective agents like *Silybum marianum* is often suggested. Our results emphasize that milk thistle preparations may greatly vary in copper content. The method of extraction has major impact on the copper content therefore in the treatment of Wilson’s disease, products containing silymarin (where the copper/silymarin ratio is practically zero) should be preferred. Health professionals recommending milk thistle-based products for patients with Wilson’s disease should consider the formulation of the product which has been shown to influence the copper content. In the light of these results it may be worth including the copper content of herbal products used as hepatoprotective agents in the summary of product characteristics.

Conflict of interest

All authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhermed.2016.06.002.

References