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Short communication

Toxicity screening of weight loss tea products by Brine Shrimp Lethality Assay

Ana Marija Bajatovska[1], Zoran Zhivikj[1*], Kristina Shutevska[1], Marija Karapandzova[2], Tanja Petreska Ivanovska[1], Lidija Petrushevska-Tozi[1], Tatjana Kadifkova Panovska[1]

[1]*University Ss Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Applied Biochemistry, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*
[2]*University Ss Cyril and Methodius in Skopje, Faculty of Pharmacy, Institute of Pharmacognosy, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*

Introduction

Tea is one of the most widely consumed beverages in the world. Significant share of tea sales is intended for weight loss and preventing weight gain and obesity. However, evidence-based data for the efficacy of weight loss teas is missing due to the conflicting results obtained in different studies (Rothenberg et al., 2018). Furthermore, these products are subjected to much less rigorous regulation compared to prescription medications (Farrington et al., 2019), and can lead to inconsistent quality and questionable safety. Commercially available weight loss teas are usually herbal mixtures that can result in adverse or toxic effects due to possible interactions of multiple active ingredients. The aim of our study was to determine the potential toxicity of weight loss tea products from different manufacturers using the Brine Shrimp Lethality Assay (BSLA).

Materials and methods

In this study, five commercially available weight loss teas (Product 1-5) were examined. Their composition and the contribution of each herbal component are shown in Table 1. To conduct the BSLA, tea infusions were prepared to which the *Artemia salina* larvae were exposed to the following concentrations: 10, 5, 3, 1, 0.5, 0.1, and 0.01 mg/mL obtained by mixing alkaline salt water (brine shrimp medium) and examined tea infusions. The dead nauplii were counted at various time intervals: 1, 2, 3, 4, 5, 6 and 24 h. The median lethal concentration (LC_{50}) was calculated by using the probits derived from the percentage of mortality against the logarithm of the sample concentration.

Based on the LC_{50} values, the tea samples were classified using Meyer's and Clarkson's toxicity scales (Meyer et al., 1982; Clarkson et al., 2004). According to both scales, toxicity presents at a value for LC_{50} less than 1000 µg/mL, and Clarkson's scale describes the toxicity further ranging from high (0 - 100 µg/mL), moderate (100 - 500 µg/mL) to low (500 - 1000 µg/mL) toxicity.

Results and discussion

The obtained results from the samples, according to the criterion of both scales have shown toxic potential for only Product 1. Prolonged exposure of brine shrimps to this sample resulted in greater toxicity. Considering Clarkson scale Product 1 was classified with moderate toxicity (LC_{50} value of 143 µg/mL). Under the same conditions, other examined samples (Products 2, 3, 4 and 5) have been found to be non-toxic (LC_{50} > 1000 µg/mL). The observed toxicity of Product 1 could not be attributed to particular herbal component (Table 1) since no respective data for BSLA-examined toxicity was previously reported. However, this basic *in vivo* model was selected because brine shrimps (*Artemia salina*) are used as the most adequate test organism for preliminary evaluation of toxicity. In terms of general toxicity, aqueous chamomile extracts exerted toxic effect on bioluminescent photobacterium *Vibrio fischeri* which is often used for monitoring toxicity of herbal extracts (Sotiropoulou et al.,

*zzivic@ff.ukim.edu.mk

2020). Product 4 has not displayed toxicity on brine shrimps even though it contains *Chamomillae flos* with equal contribution to the mixture as Product 1. Licorice, another Product 1 component, has been reported to be safe, but some studies indicated the possibility of adverse events depending on the dosage and duration of use (Wahab et al., 2021). The rest of the components present in Product 1 can be considered safe since no reports evidenced their toxic effects; however, there is a possibility, combination of few non-toxic herbal components to be responsible for the observed toxicity, due to numerous potential interactions. In this context, it is quite confusing the fact that Product 1 contains six herbal components, while Products 2, 3 and 5 have more diverse composition and exhibit non-toxic behavior to brine shrimps.

Table 1. Composition and the contribution of each herbal component in studied weight loss teas.

Tea product	Herbal component (%)
Product 1	*Frangulae cortex* (20%), *Taraxaci officinalis radix* (20%), *Ononidis radix* (20%), *Liquiritiae radix* (15%), *Chamomillae flos* (15%), *Foeniculi dulcis fructus* (10%)
Product 2	*Mentha pip. folium* (N/A), *Silybi mariani fructus* (20%), *Taraxaci officinalis radix* (15%), *Foeniculi dulcis fructus* (N/A), *Petroselini folium* (N/A), *Sambuci flos* (N/A), *Juglandis folium* (N/A), *Uvae-ursi folium* (5%), *Liquiritiae radix* (N/A)
Product 3	*Melissae folium*, *Betulae folium*, *Urticae folium*, *Petroselini folium*, *Equiseti herba*, *Thymi herba*, *Hybisci sabdariffae flos*, *Mentha pip. folium*, *Taraxaci officinalis radix*, *Rosae pseudo-fructus*, *Saturejae herba* (all N/A)
Product 4	*Sennae folium* (45%), *Frangulae cortex* (25%), *Mentha pip. folium* (15%), *Chamomillae flos* (15%)
Product 5	*Camelliae sinensis non fermentatum folium* (45%), *Coicis semen* (18%), *Guaranae semen* (12%), *Zingiberis rhizoma* (10%), *Taraxaci officinalis radix* (8%), *Citrus fructus* (7%)

The most interesting phenomenon was noticed when the nauplii were exposed to non-toxic samples (Products 2-5) consisting of salt water as a medium and tea infusion in a ratio 1:1 (concentration of 5 mg/mL). In these conditions no dead nauplii were observed within the first 6 h, while after 24 h the mortality was very low ranging between 5 and 20%. The ratio of salt water and tea infusion (1:1) probably shifts the balance towards favorable living conditions for brine shrimps by increasing the availability of antioxidants and other nutrients such as carbohydrates and minerals derived from the tea infusion.

Conclusion

BSLA is a simple low-cost bioassay as a first-pass tool to pinpoint cytotoxic characteristics of herbal compounds/extracts and as an indicator of preliminary toxicity. Herein, the study revealed that one of the five examined teas showed toxic potential. This supports the applicability of BSLA for screening toxicity of weight loss tea products since they are over consumed and often under regulated.

References

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., Folb, P. I., 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J. Ethnopharmacol. 92(2-3), 177–191. doi: https://doi.org/10.1016/j.jep.2004.02.011

Farrington, R., Musgrave, I.F., Byard, R.W., 2019. Evidence for the efficacy and safety of herbal weight loss preparations. J. Integr. Med. 17(2), 87-92. doi: https://doi.org/10.1016/j.joim.2019.01.009

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(5), 31–34. doi: https://doi.org/10.1055/s-2007-971236

Rothenberg, D.O., Zhou, C., Zhang, L., 2018. A review on the weight-loss effects of oxidized tea polyphenols. Molecules 23(5), 1176. doi: https://doi.org/10.3390%2Fmolecules23051176

Sotiropoulou, N.S., Megremi, S.F., Tarantilis, P., 2020. Evaluation of antioxidant activity, toxicity, and phenolic profile of aqueous extracts of chamomile (Matricaria chamomilla L.) and sage (Salvia officinalis L.) prepared at different temperatures. Appl. Sci. 10(7), 2270. doi: https://doi.org/10.3390/app10072270

Wahab, S., Annadurai, S., Abullais, S.S., Das, G., Ahmad, W., Ahmad, M.F., Kandasamy, G., Vasudevan, R., Ali, M.S., Amir, M., 2021. Glycyrrhiza glabra (Licorice): A comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology. Plants (Basel) 10(12), 2751. doi: https://doi.org/10.3390/plants10122751