Plant Foods for Human Nutrition
https://doi.org/10.1007/s11130-019-00725-x

SHORT COMMUNICATION

Duckweed for Human Nutrition: No Cytotoxic and No Anti-Proliferative Effects on Human Cell Lines

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Abstract
Duckweeds (Lemnaceae) possess good qualitative and quantitative profiles of nutritional components for its use as human food. However, no studies have been conducted on the probable presence or absence of any adverse effects. The extracts from seven duckweed species (*Spirodela polyrhiza, Landoltia punctata, Lemna gibba, Lemna minor, Wolffiella hyalina, Wolffia globosa*, and *Wolffia microscopica*) covering all five genera of the plant family were herewith tested for cytotoxic effects on the human cell lines HUVEC, K-562, and HeLa and for anti-proliferative activity on HUVEC and K-562 cell lines. From these assays, it is evident that duckweeds do not possess any detectable anti-proliferative or cytotoxic effects, thus, the high nutritional value is not diminished by such detrimental factors. The present result is a first step to exclude any harmful effects of highly nutritious duckweed for human.

Keywords Cytotoxicity · Duckweed · Human cell lines · Lemnaceae · Nutrition · *Wolffia*

Introduction

Duckweeds represent the fastest growing angiosperms producing large amount of biomass. In some countries, like Thailand, Laos, and Cambodia, duckweeds have been used for ages as human food. Comprehensive data concerning nutritional values of several duckweed species were recently highlighted [1–3]. However, until now, studies on the probable presence or absence of toxic components in duckweeds are completely missing.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11130-019-00725-x) contains supplementary material, which is available to authorized users.

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Materials and Methods

All plants were taken from the stock collection of the Matthias Schleiden Institute of the University of Jena, Germany. Seven species of duckweed (Table 1) were cultivated under standard conditions [4] (see also supplementary material). For each sample, 500 mg fresh weight of whole plant was freeze-dried and homogenized in methanol. The crude extracts were purified using solid-phase-extraction (Chromabond, Macherey-Nagel, Germany), dried and dissolved in DMSO. In order to analyse the DMSO solutions, we measured the cytotoxicity (cell death) and the anti-proliferative activity (retardation of cell proliferation) as described in standard protocols [5] (for further experimental details see Supplementary material 2).

Results and Discussion

Whole plant extracts of seven duckweed species were tested for the probable presence/absence of cytotoxic and anti-proliferative effects on human cell lines. Cell lines HUVEC, K-562 and HeLa were used as targets for cytotoxic assays and thereafter HUVEC and K-562 for anti-proliferative assays as *per* standardised procedures [6]. All extracts were applied in a

Published online: 18 March 2019

Table 1 Test for effects of extracts from duckweed samples on human cell lines and their cultivation media: HUVEC (ATCC CRL-1730) in DMEM (CAMBREX 12-614F), and K-562 (DSM ACC 10) in RPMI 1640 (CAMBREX 12-167F). CC_{50}/ GI_{50} = concentration for cytotoxicity (cell death)/ growth inhibition by 50%

Duckweed (Species, clone ID, origin)	Anti-proliferative effect	
	HUVEC CC_{50}/ GI_{50} [μg/ml]	K-562 CC_{50}/ GI_{50} [μg/ml]
Spirodela polyrhiza, 7498 (USA)	> 50	> 50
Landoltia punctata, 9589 (India)	> 50	> 50
Lemna gibba, 7742 (Italy)	> 50	> 50
Lemna minor, 9441 (Germany)	> 50	> 50
Wolffiella hyalina, 9525 (India)	> 50	> 50
Wolffia globosa, 5537 (Thailand)	> 50	> 50

series of dilutions up to the maximal possible dose of 50 μg ml^{-1}. In no case, any cytotoxic, anti-proliferative, or proliferation-stimulating effects were observed (Table 1). Therefore, the half-maximal dose of cytotoxic (CC_{50}) and anti-proliferative (GI_{50}) effects must be higher than this dose (*i.e.*, > 50 μg ml^{-1}) and it can be concluded that duckweed whole plant extracts do not have any detectable adverse effects on human cell lines. Presently, duckweed belongs to the category of "novel food" and the required investigations according to the novel food regulation have to be observed to enter the commercial market. Our previous papers [1, 2] emphasized on the nutritional richness of a large number of duckweed species and suggested that duckweeds are a beneficial source of human nutrition in coherence with the traditional cuisine of some of the Asian countries. The proof that there are no toxic effects, as presented from this study, is a necessary step to ensure global use of duckweed as a component of human nutrition. Investigations of further potential anti-nutritive components like nitrates, oxalates, tannins, phytates and cyanogenic glycosides [7] in duckweed are in progress.

Compliance with Ethical Standards

Human and Animal Rights This article does not contain any studies with human or animal subjects.

Conflict of Interest The authors declare that they have no conflict of interest.

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