European Journal of Medicinal Plants

24(3): 1-4, 2018; Article no.EJMP.42780
ISSN: 2231-0894, NLM ID: 101583475

Castanea sativa Mill. Extract Cytotoxicity

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/EJMP/2018/42780
Editor(s):
(1) Dr. Shanfa Lu, Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Bing Hu, Longhua Hospital Shanghai University of Traditional Chinese Medicine, China.
(2) Mustafa Sevindik, Gaziantep University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/25686

Short Research Article

Received 4th May 2018
Accepted 12th July 2018
Published 26th July 2018

ABSTRACT

Castanea sativa Mill. leaves infusion/decoction has been traditionally used in the northern region of Portugal to treat cough in children, diarrhoea, infertility and hypertension. However, no information exists about its possible toxic effects.

This study aims to investigate the toxicity of *C. sativa* Mill. extract by the determination of population growth impairment, generation time*, LC50,* morphometric changes in *Tetrahymena* pyriformis and MTT test.

Generation time, growth and MTT assay are affected by chestnut tree leaves extract and these effects are dosage dependent. The morphometry of the *Tetrahymena pyriformis* cells was also affected. According to the obtained LC50 value, the extract may be considered as mildly toxic.

The usage of *C. sativa* leaves extract must be exercised with caution especially when it comes to children.

Keywords: Castanea sativa Mill.; herbal medicines; toxicity; Tetrahymena pyriformis.

1. INTRODUCTION

The use of plants for medicinal care and disease prevention is one of the most ancient forms of medicine practice in humanity. According to the World Health Organization [1], between 70 to 95% of the population in developing countries depend on the use of medicinal plants as the only form of therapy for most diseases, especially due to costs of modern allopathy.

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Medicinal plants contain a variety of chemical compounds that exhibit a variety of biological activities such as anti-microbial and antioxidant [2], [3].

Traditionally speaking, it is accepted that the use of natural products used by ancients is not harmful. Traditional medicine does not normally have strict criteria, whether in terms of preparation and usage of the remedies or in the dosages and adverse effects. Thus, the increasing use of medicinal plants is currently a source of concern in relation to the prepared remedies. Attention should be drawn to the indiscriminate usage of medicinal plant extracts, especially to the imperative need of controlling for possible unwanted effects of chronic use on the organism. In fact, medicinal plant extracts can be toxic depending on their use, time of treatment and form of preparation; hence, the toxicity of medicinal plants is, nowadays, a serious problem to public health.

Castanea sativa Mill. (Fagaceae) is a long-lived deciduous tree commonly called "chestnut", "sweet chestnut" or “Portuguese chestnut". Infusions/decoctions of chestnut leaves are traditionally widely used in the northern region of Portugal to treat cough in children, diarrhoea, infertility and hypertension [4]. Despite this, very little is known about their toxicity and thus, it is very important to verify the toxicological properties of these infusions/decoctions especially because they are used in children who can have an immature immune system.

Protozoa like *Tetrahymena pyriformis* have shown to be an interesting model in the toxicological study of carcinogens [5], inorganic and organic chemicals [6], bioflavonoids [7], phenol derivatives [8], pharmaceutical drugs, phototoxicity and environmental radiation evaluation [9].

This study aims to assess the lethal concentration 50 (LC50) and cytotoxic effects of the aqueous extract of *C. sativa* Mill. on the physiology, morphology and biochemistry of *T. pyriformis* considering the significant use and the popular belief that is harmless.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *C. sativa* Mill. (plant name checked with www.plantlist.org in 27/03/2017) were collected from an uncultivated farmland in northern Portugal, in the councils of Montalegre (east) and Chaves (west) respectively in a study area included within the square of UTM coordinates 29TPH52. Identification was performed by the Department of Botanical Sciences of the Universidade de Trás-os Montes e Alto Douro (UTAD) where a voucher specimen (N° 3758) has been deposited.

2.2 Preparation of Extracts

Dried and powered chestnut leaves were macerated in the dark for 48h at room temperature. The macerate was filtered first through Whatman filter paper, then through 450 µm carbonates filters and afterwards it was lyophilized and stored at 4°C.

2.3 Toxicological Tests

Axenic *T. pyriformis* (strain GL, ref. CCAP/1630/1F from Strains of Culture Collection of Algae and Protozoa, UK) was cultured as described elsewhere [10].

To check the toxicity effect of chestnut extract, exponentially growing cells (10^4 cells/ml) were inoculated in the medium with the extract, at the desired concentration, and incubated in 96-well. Chestnut extract concentrations used were 45,2 mg/ml, 4,52 mg/ml; 0,45 mg/ml; 0,23 mg/ml and 0,045 mg/ml.

Controls were made with non-exposed cells to the extract.

Population growth impairment, generation time determination and morphometric changes (cell area width/length) were in accordance with the procedures described by [10].

The MTT test followed the procedure described by [11]

2.4 LC50 Determination

LC50 values were calculated using the Probit method [12].

3. RESULTS AND DISCUSSION

Aqueous extracts of *C. sativa* leaves were screened for their cytotoxicity against cells of *T. pyriformis* at different concentrations. Addition of chestnut leaves extract to *T. pyriformis* culture showed visible effects in generation time and population growth 24 hours after (Table 1).

Table 1. Effect of extract toxicity in *Tetrahymena pyriformis* with a 24-h exposure

Concentration (mg/ml)	Generation time (h)	Growth (% of control)	MTT reduction (%of control)
45,2	0.0±0.0	0.0±0.0	5.3±1.7
4,52	22.7±1.9	26.1±4.5	31.7±2.8
0,452	14.9±1.5	68,2±4.8	73.1±4.3
0,23	10.7±2.0	73.8± 4.0	79.4±3.1
0,045	7.6±1.2	97.2±2.9	85.6±2,5
Control	6.9±1.4	100.0±0.0	100.0±0.0

Each value is the mean of two independent assays ± standard deviation

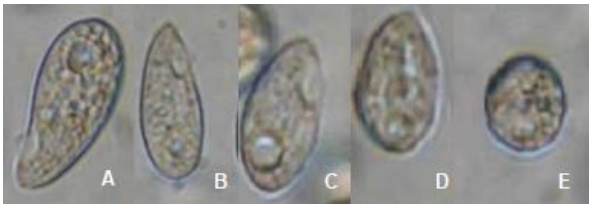

Fig. 1. Morphologic changes in *T. pyriformis* after 24 h-experiment with chestnut extracts

At the lowest concentration (0,045 mg/mL) *T. pyriformis* population is not significantly affected. However, as the chestnut leaves extract concentration increased, the generation time and the population impairment increased. This impairment is dosage dependent. At the highest concentration (45,2 mg/mL) there is no cell division in *T. pyriformis*.

Concerning the MTT test, results show that MTT reduction decreases with increasing concentration of the plant extract in a dose dependent response. At lower concentrations, there is a production of intensely coloured formazan as a result of the MTT reduction. For higher concentrations, 4,52 mg/mL and 45,2 mg/mL, the MTT reduction is significantly lower but it is never suppressed. This result suggests that, although most cells are severely affected, there is still some activity of the mitochondrial dehydrogenase.

The results obtained are similar to those obtained by [13] who found some mitochondrial activity in two-day damaged cells.

Fig. 1 (A to E) shows the results of the action of chestnut leaves extract in the morphology of *T. pyriformis*. As seen, the morphology of the cell of *T. pyriformis* is not affected at the lowest concentration of chestnut leaves extract (A); however when the extract concentration increases, the ratio of shortest and longest axis (W/L) changes (B,C,D) and the cells become rounder. At the highest concentration (45, 2 mg/ml), the cells are round suggesting their death. In fact, according to [14] the rounding off of the cells of *T. pyriformis* is due to changes occurring in the microfilament architecture leading to the cell death.

The lethal concentration 50 (LC50) of the tested chestnut crude extract is 0.825 mg/ml. According to [15], crude extracts with LC50 values between 0.500 mg mL^{-1} and 1.000 mg mL^{-1} are mildly toxic. Therefore, the results seem to support that the use of these extracts, especially in children, must be done with some precaution regarding the dosages and long ingestion cycles.

4. CONCLUSION

The use of medicinal plants is widespread all over the world due to easy access, low cost and because there is the false idea that what is natural does not cause harm. However, this idea may be very dangerous because plants can be toxic due to the mixtures of active compounds that they contain. This makes the study of their toxicity essential. The results of this study have shown that caution must be exercised in the oral administration of infusions and decoctions of *C. sativa*, especially when it comes to children.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25686