British Journal of Pharmaceutical Research
10(3): 1-6, 2016, Article no.BJPR.23990
ISSN: 2231-2919, NLM ID: 101631759

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Toxic Effects of Aqueous Extract of *Plectranthus amboinicus* (Lour) Spreng on *Allium cepa*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CFCB, AACMC and JNP designed the study. Authors CFCB, MVOBA, IG and AACMC prepared the manuscript. Authors CFCB, ALGJ, MVOBA and MFCJP performed the sample extraction and biological assay. Authors IG, AACMC and JNP supervised the study. Author ABFF did the phytochemical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/23990
Editor(s):
(1) Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, Hunan, China.
Reviewers:
(1) Anthony Cemaluk C. Egbuonu, Michael Okpara University of Agriculture, Umudike, Nigeria.
(2) Israel Felzenszwalb, Universidade do Estado do Rio de Janeiro, Brazil.
(3) Yasin Eren, Suleyman Demirel University, Turkiye.
Complete Peer review History: http://sciencedomain.org/review-history/13239

Short Research Article

Received 30th December 2015
Accepted 23rd January 2016
Published 9th February 2016

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ABSTRACT

Aims: *Plectranthus amboinicus* (Lour) Spreng. belonging to the Lamiaceae family being extensively used in folk medicine for its anti-inflammatory effects, nonetheless, there is a lack of more substantial data on the toxicogenetic effects of this preparations. This study aimed to evaluate possible cytotoxic effects of aqueous extract of leaves of *Plectranthus amboinicus* on *Allium cepa* assay.
Methods: Distilled water was used as negative control and a solution of copper sulfate was used as positive control. Also a qualitative chemical screening for the identification of the major classes of active constituents were performed.
Results: The results demonstrate the presence of diterpenes and flavonoids, and a decreased in the mitotic index as the concentrations of extract increased.
Conclusions: These findings suggest that a toxic effect at high doses of *Plectranthus amboinicus* can be related to the presence of flavonoids and diterpenes.

Keywords: Allium cepa; diterpenes; flavonoids; in vitro; mitotic index; Plectranthus.

1. INTRODUCTION

There are growing concerns in medicinal plant research due to the discovery of natural compounds with pharmacological potential [1,2]. In this sense, the use of medicinal plants constitutes an ancient practice that shows encouraging effects [3]. However, toxicogenetics data from these preparations are still incipient [4].

Plectranthus amboinicus (Lour.) Spreng. is a perennial plant distributed throughout tropical Africa, Asia, Australia, and the Americas, including Brazil, where it is popularly known as "thick-mint leaf". Pharmacological studies have demonstrated antioxidant, cytotoxic, diuretic, anti-inflammatory, antibacterial and antitumor properties of this plant [5-7]. Phytochemical analyses reported the presence of phytochemical constituents like flavonoids, terpenoids, saponins, steroids, tannins and essential oils in *P. amboinicus* leaves [8].

As previously stated, *P. amboinicus* has been used in traditional medicine, with several benefits to the human health. However, it still lacks consistent data about mutagenic effects from preparations of this plant that can lead to deleterious effects on genetic material. Thus, studies addressing DNA damage, such as *Allium cepa* assay, are important to minimize the risk of inducing cell injury during medicinal use of the *P. amboinicus*. This study aimed to evaluate the possible cytotoxic effects of aqueous extract of leaves of *P. amboinicus* in *Allium cepa* assay.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

Leaves and flowers from *P. amboinicus* were collected in August (2011) at Fazenda Petrolina, city of Campo Maior – Piauí (Brazil) and the voucher specimen (N°27.312) was identified and deposited in the Graziela Barroso herbarium of the Universidade Federal do Piauí (UFPI). To obtain the extract, 100 g of fresh leaves of *P. amboinicus* that were never withered, old or yellowed, torn or damaged by insects, were collected and selected. The leaves were washed with distilled water at 100℃. The concentrated extract obtained in the rotary evaporator was filtered and diluted to 12.5, 25 and 50 mg/mL.

2.2 Phytochemical Screening

The plant material was subjected to qualitative chemical screening for the identification of the major classes of active constituents. The phytochemical profile of *P. amboinicus* leaves was determined according to the methodology described before [9,10]. The method consists of colorimetric reactions for qualitative detection of flavonoids, tannins, anthraquinones, alkaloids, saponins, coumarins and cardiac glycosides.

2.3 *Allium cepa* Assay

The Allium cepa assay was performed as described by Fiskejö [11], with adaptations. Meristematic cells of *A. cepa* were exposed to different concentrations (12.5, 25 and 50 mg/mL) of aqueous extract of the leaves of *P. amboinicus*. Distilled water was used as negative control and a solution of copper sulfate at 0.6 mg/mL was used as positive control.

The bulbs were separated according to size and washed in running water for 15 min. They were then placed to germinate at 18-22℃ at the different concentrations of *P. amboinicus* extract and controls. After 48 h of exposure, the roots

were measured in centimeters (cm) and the bulbs with growth above 2.0 cm were discarded. After their measurements, roots were placed in Carnoy fixative solution for 24 h, followed by 70% ethanol, and kept under refrigeration until the moment of preparation of the slides.

The assay was conducted in a controlled temperature of 20℃ on a bench without vibrations and without direct illumination. Two roots of each bulb were washed in distilled water to remove the fixative and went through hydrolysis with HCl (1N at 60℃ for 8 min). After hydrolysis, they were washed again in distilled water at room temperature and transferred into dark bottles, containing Schiff reagent, for approximately 2 h. The apical portion of the roots, with approximately 1 mm to 2 mm in length (root tips), were removed and stained with 2% Acetic Carmine for 10 min. The squash root tips were prepared in 45% acetic acid and two slides were prepared for each bulb. The slides were observed under an optical microscope (1000X) and 2000 cells (1000 per slide) were analyzed for each bulb. The parameters evaluated were: (a) root growth in centimeters, as an indication of toxicity; (b) the mitotic index (MI) for evaluation of cytotoxicity.

2.4 Statistical Analysis

The data obtained was analyzed through Analysis of Variance (ANOVA) with a significance level of $p < 0.05$ using the SPSS Program Version 20.0 (SPSS, Chicago, IL).

3. RESULTS

3.1 Phytochemical Analyses

Phytochemical analyses of *P. amboinicus* leaves indicated the presence of diterpenes and flavonoids.

3.2 Toxic and Cytotoxic Effects of *P. amboinicus* in *Allium cepa*

The aqueous extract obtained from the leaves of *P. amboinicus* at concentrations of 25 and 50 mg/mL had inhibitory effects (37.4% and 57.0% of grow inhibition, respectively) on root growth of *A. cepa* (Table 1). This indicates toxicity of the extract of *P. amboinicus* leaves at the aforementioned concentrations. However, no significant effect was observed at the concentration of 12.5 mg/mL, which showed only 3.8% inhibition of root growth, when compared to the negative control.

The mitotic index of *A. cepa* root tip cells treated with increasing concentrations of *P. amboinicus* extract was significantly decreased in comparison to the control group ($p<0.001$; Table 1). Indeed, at a lower concentration (12.5 mg/mL) this effect is comparable to the positive control. Moreover, the cytotoxicity observed in this study is dose dependent, and the more pronounced reduction of mitotic index was verified at a dose of 50 mg/mL (28 times less than negative control) (Table 1).

4. DISCUSSION

The *A. cepa* assay presents itself as an ideal biomonitor for a primary genotoxicity screening of herbal infusions for its low cost. The assay is well accepted to study the toxic effects of medicinal plants, with its operational viability allowing the evaluation of different sample concentrations concomitantly and in short time [12]. The *A. cepa* assay has shown high sensitivity and good correlation when compared with other test systems [13].

In the current study, the phytochemical analyses did not indicate the presence of tannins, anthraquinones, alkaloids, saponins, coumarins and cardiac glycosides. This data is in accordance with a study by El-hawary et al. [14]. Nevertheless, we detected diterpenes and flavonoids. In this line, it was demonstrated in others *Plectranthus* species, a variety of diterpenes [15]. On the other hand, there are few reports indicating the presence of flavonoids in *Plectranthus* species [14,16-18].

The toxicity of the extract of *P. amboinicus* leaves was observed in *A. cepa* for concentrations of 25 and 50 mg/mL. These results are compatible with toxicity testing in other eukaryotes assays. In an acute toxicity study, it was demonstrated that the aqueous extracts of *P. amboinicus* induced no mortality after 72 h at a dose of 10,000 mg/kg in mice [18]. The root growth after exposition to different concentrations of *P. amboinicus* extract was consistent with the mitotic index, except for the concentration of 12.5 mg/mL. Thus, these parameters suggest evidences of toxicity and cellular cytotoxicity at concentrations of 25 and 50 mg/mL, pointing to an interference with the DNA synthesis process [19].

Table 1. Root growth and mitotic index in root meristems cells of *Allium cepa* exposed to *Plectranthus amboinicus* aqueous leaves exctract

Group	Root growth (cm)[C]	Root growth (%)	Mitotic index[c]
Negative control[a]	1.1±0.3	100	42.2±0.7
Positive control[b]	0.3±0.07***	32.7***	4.7±2.5***
P. amboinicus 12.5 mg/mL	1.0±0.5	96.2	4.7±1.1***
P. amboinicus 25 mg/mL	0.7±0.2*	62.6***	3.4±1.3***
P. amboinicus 50 mg/mL	0.5±0.1*	43.0***	1.5±0.9***

*[a]Distilled water; [b]Solution of copper sulfate (0.0006 mg/mL); [c]Mean ± standard deviation. The symbol * indicates P = .05; *** P < .001; One-way ANOVA Tukey's Multiple Comparison Test*

The *P. amboinicus* aqueous extract, showed cytotoxicity in all tested concentrations, which can be identified by a reducing on the mitotic index. Our results showed that when the concentration doubled, mitotic index rate decreased 1.4 to 2.2 times. When the mitotic index of tested organisms dropped below 22% of the control, it caused a lethal effect [20]. In the current study, a lethal effect was observed with all concentrations tested.

Moreover, significant reduction in mitotic index, compared to the negative control, can indicate the cytotoxic action of chemical agents on the growth and development of the exposed organism [21]. Ali et al. [22] demonstrated that *Plectranthus* sp. extract is cytotoxic at high doses. It can be concluded that, together with the increase in *P. amboinicus* extract concentrations, the mitotic index of *A. cepa* root tip cells decreased due to the blocking effect on the G1 cell-cycle phase by some component(s) of the extract, or DNA synthesis inhibition at S-phase [23]. In this line there are a few studies demonstrating that certain flavonoids can interfere with DNA synthesis leading to cytotoxicity [24-26]. It was also demonstrated that particular diterpenes and flavonoids can induce a cell cycle arrest [27,28]. Indeed, abietane diterpenoids have been reported as the main constituents of some species of *Plectranthus* and is associated to the potential cytotoxic and antiproliferative activities observed against human pancreatic cancer cells [29]. This effect was also detected in others genus (*Salvia*, *Peltodon*, *Hyptis*, *Isodon*) from Lamiaceae reporting the presence of cytotoxic abietane diterpenes [30-33]. One example of these compounds is parvifloron D isolated from the aerial parts of *Plectranthus ecklonii* that act as a potent apoptotic inducer in human leukemia cells [34]. In the same way, isolated abietane diterpenoids from *Coleus xanthanthus* (*P. xanthanthus*), coleon U 11-acetate, xanthanthusin H, together with analogues, coleon U-quinone, 8α,9α-epoxycoleon U-quinone and xanthanthusin E were cytotoxic against K562 human leukemia cell line [35]. Thirty diterpenoids with an abietane or a halimane skeleton was analyzed against five human tumor cell lines and compounds with. abietane skeleton were the most cytotoxic compound in H460 human leukemia cell line [36]. Abietane diterpenes earn greater attention because several cancer chemotherapeutic agents possess this functional group [31]. Moreover, Burmistrova et al. [36] associated the strong cytotoxicity of this group against human leukemia cells with different death pathways.

5. CONCLUSIONS

In conclusion, this study points that the aqueous extract of this plant could be potentially toxic at high doses, and this effect can be related to the presence of flavonoids and diterpenes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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