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Research Article

In Vitro Cytotoxic Analysis of Ethanolic Extract from Leaves of *Kalanchoe blossfeldiana* Poelln (Crassulaceae)

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Abstract

The idea of harmlessness "Natura doesn't hurt" is quite common, due to the lack of knowledge of the floral biology of some species, especially ornamental ones, as these studies are recent in Brazil. The present study aimed to perform an in vitro cytotoxicity analysis of the leaves of the ornamental plant *Kalanchoe blossfeldiana* Poelln on human red blood cells. After approval of the research project by the Brazilian Ethics Committee, identification of the plant by an Herbarium and selection of volunteers for the research by signing the TCLE and TCPE, the plant was purchased from a local florist and taken to the laboratory, since it is a plant grown in florists. The leaves were washed and 200 g were standardized, followed by grinding, maceration of the leaves in 95% ethanol and evaporation of the solvent. The cytotoxicity test was performed on red blood cells, in which the extract was diluted in six different concentrations and incubated with red blood cell washes for 1 hour. Immediately after, the samples were centrifuged and the absorbance of the supernatant was measured at 540 nm by spectrophotometry. From the results, the hemolysis percentages and statistical analysis were calculated using GraphPad Prism 7.0® software, with results at $p<0.01$ considered statistically significant. Hemolysis was observed in all concentrations compared to the control and that this hemolysis percentage increased proportionally to the extract concentration. It is concluded that the leaf extract shows signs of toxicity, revealing that care must be taken when cultivating the species, so that its leaves are not ingested.

Keywords: *Plant Poisoning; Statistical Analysis; Kalanchoe.*

Introduction

The study of floral biology encompasses several important aspects, such as morphology, reproductive systems, phenology, pollination, and flower fertilization. Interactions between plants and pollinators are essential for maintaining the structural and functional balance of natural ecosystems, protecting endangered species, and enabling the use of plant species for medicinal purposes and as ornamentals in urban environments [1].

This line of research in Brazil is relatively recent, with its first record dating back to the 1950s, conducted by the German professor Stephan Vogel, among the foreign researchers who came to Brazil, he was the most prominent. Subsequently, several studies were carried out by other explorers in the field; however, research in this area only became consolidated in the mid-1970s through the work of Professor Marlies Sazima at the State University of Campinas [2].

Existing research provides a scientific basis for the floral biology of wild plants, although many species, particularly ornamental plants, remain understudied. A better understanding of the ecology of cultivated and decorative plants may lead to increased productivity and more efficient use of various species [3].

The common belief that "nature is harmless" remains prevalent among the general population and is concerning, as some species exhibit toxic levels that can pose risks to individuals unfamiliar with them, despite their harmless appearance with colorful and vibrant leaves and flowers. In this context, further research on plant toxicity is necessary to contribute to effective measures for their rational use [4].

It is worth noting that secondary metabolites are classified into three main molecular groups: nitrogen-containing compounds, terpenes, and phenolic compounds. The nitrogenous group includes alkaloids, glycosides, cyanogenic compounds, and non-protein amino acids. Among these, alkaloids are one of the most important nitrogenous compounds, found in approximately 20% of vascular plant species [5].

Terpenes, in turn, play functional roles such as reducing plant–insect interactions, as they are bioactive compounds containing volatile allelochemicals. Phenolic compounds display great chemical diversity, allowing them to perform a wide range of functions in plants, such as attracting pollinators, providing mechanical strengthening of cell walls, and offering defense against herbivores, among others [6].

It is important to highlight that plant secondary metabolites may vary according to seasonal and daily changes, as well as intra-plant, inter-, and intraspecific variation, despite their genetic control. The expressions mentioned in the previous paragraphs may undergo such modifications as a result of biochemical, physiological, ecological, and evolutionary interactions. Therefore, their synthesis is often influenced by environmental conditions [7].

Kalanchoe blossfeldiana Poelln is a succulent plant belonging to the Crassulaceae family, characterized by abundant flowering and a wide range of colors, including red, orange, white, pink, and yellow, among others. It is native to Africa and Madagascar. In Brazil, it is popularly known as "Calandiva" or "flor-da-fortuna." Due to its attractive flowers and water-storing leaves, this species has become increasingly popular in flower shops and specialized stores [8].

K. blossfeldiana Poelln is the most commonly commercialized species of *Kalanchoe* in Brazil and, for this reason, was selected as the object of study in the present research. Therefore, further scientific investigation is needed to determine whether this species presents any toxicological risks, since its frequent use as an ornamental item increases the likelihood of accidental ingestion, particularly by domestic animals and small children.

Methodology

Collection of *Kalanchoe blossfeldiana* Poelln

Kalanchoe blossfeldiana Poelln was collected from a flower shop in the city of Juazeiro do Norte, Ceará, Brazil. It is popularly known as "flor-da-fortuna" (flower of fortune) and is a widely commercialized ornamental plant in the region. Although several units were available for sale, only one fully grown specimen was purchased for the study.

Preparation of the Ethanolic Extract

The ethanolic extract was prepared following the steps described below:

1. The leaves of *Kalanchoe blossfeldiana* Poelln were removed and washed under running water;
2. The leaves were then placed on paper towels at room temperature and allowed to dry completely;
3. After drying, 200 g of leaves were weighed on an analytical balance and cut into small pieces with scissors. The material was manually ground and macerated in 95% ethanol, using a ratio of 4 L of ethanol for every 200 g of leaves;
4. The mixture was left to stand for 96 hours;
5. After this period, the material was filtered through qualitative filter paper (80 g/m^2) and transferred to a transparent glass flask;
6. Finally, the solvent was completely evaporated using a water bath at 40°C, yielding the crude ethanolic extract of *K. blossfeldiana* Poelln leaves.

Blood Collection

The evaluation of the osmotic fragility of human red blood cells was carried out after approval of the research project by the Human Research Ethics Committee. Following approval, volunteer participants were selected, informed about the study, and provided written informed consent (ICF) and free and informed consent for participation in research (TCLE/ TCPE).

Inclusion criteria consisted of participants aged over 18 years, regardless of sex. Individuals with a history of hematological disorders were excluded. After volunteer selection, venipuncture was performed for vacuum blood collection using citrate anticoagulant tubes.

Evaluation of Cytotoxicity of the Ethanolic Extract

Cytotoxicity assessment was performed according to the methodology described by [9], following the steps below:

1. Red blood cell suspensions were previously washed with saline solution and incubated with the extract diluted in 1% dimethyl sulfoxide (DMSO) at six concentrations: 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL;
2. The samples were then incubated in a water bath at 37°C for 1 hour;
3. Subsequently, they were centrifuged at 1,500 rpm for 15 minutes, and the supernatants were isolated and read in a spectrophotometer at 540 nm.

Note: Distilled water was used as the positive control, and 0.9% NaCl as the negative control.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software, version 7.0® for Windows. Data were expressed as Mean ± Standard Error of the Mean (SEM). Hemolysis percentages and statistical analyses were calculated, and results with $p < 0.01$ were considered statistically significant.

Results

After conducting cytotoxicity tests on the crude ethanolic extract of K. blossfeldiana Poelln leaves, statistical analyses were performed for a better visualization of the results. Therefore, hemolysis was observed at all concentrations described in the following figure:

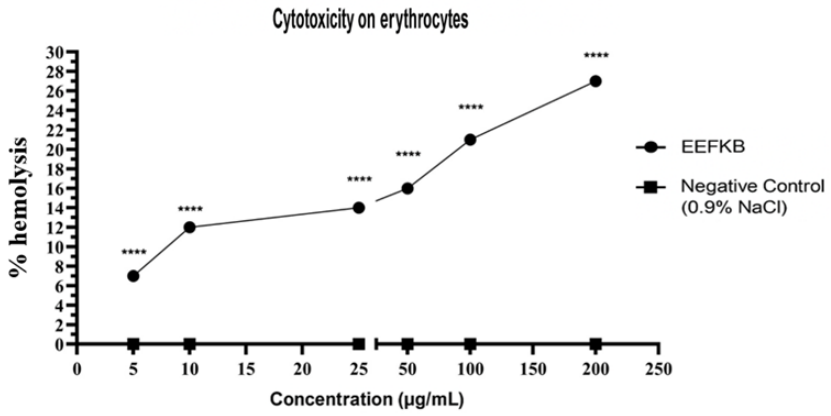

Figure 1: Hemolysis (%) resulting from cytotoxicity tests of the ethanolic extract of *Kalanchoe blossfeldiana* Poelln leaves.

Discussions

The results obtained revealed an increase in hemolysis at all concentrations when compared with the negative control. Moreover, the percentage of hemolysis increased progressively with each higher concentration of the extract. It is noteworthy that the presence of glycosidic compounds related to toxicity has already been described in the literature for *Kalanchoe blossfeldiana* Poelln and other species of the same genus.

The presence of the glycoside bufadienolides promotes sodium retention, leading to an increase in intracellular water content, which may explain the hemolytic effect observed for the plant extract. This compound has the ability to enhance the contractile force of cardiac muscle by inhibiting the enzyme Na^+/K^+-ATPase, resulting in sodium and calcium retention in myocytes and potassium expulsion, which can cause cardiac lesions [10].

There are approximately 150 native species of this genus, most of which are ornamental plants used domestically or found in the wild. However, not all species of *Kalanchoe* exhibit toxic effects. Nonetheless, a case report in the literature describes cattle poisoning in Pernambuco (Brazil) associated with the ingestion of toxic leaves of *K. blossfeldiana* Poelln during periods of forage scarcity [11].

In contrast, a study conducted with the administration of *K. blossfeldiana* Poelln extract in dogs expected that, due to the presence of cardiotoxic substances such as bufadienolides—common in most species—it would induce toxic effects, leading to hepatic and cardiac lesions. However, no alterations were observed in biochemical parameters or electrocardiograms [12].

Indeed, plants possess natural metabolites that serve as protective compounds and provide important nutritional and pharmacological benefits for both humans and animals. Nevertheless, the discovery and study of the roles of these compounds in plants and their interactions with animals remain relatively recent but are essential for ensuring safe use [5].

In this context, *Symphytum officinale* (comfrey) is a plant whose leaf tea was once popularly used as a "miracle remedy" to purify the blood and treat respiratory and other ailments. However, after its popularization in the United States, the plant was found to contain several pyrrolizidine alkaloids, such as lasiocarpine and symphytine, and their N-oxides, which are toxic. Consequently, it was banned in several countries and is currently restricted in Brazil to topical use only, according to the guidelines of ANVISA (Agência Nacional de Vigilância Sanitária) [13].

Furthermore, Brazilian *boldo* species have not yet been extensively studied regarding their toxicity. However, the main species are not recommended for use during pregnancy, as research indicates a possible association with gastric irritation. In addition, *Peumus boldus* (Chilean boldo) has been reported to be teratogenic and abortifacient in animal studies when extracts of its dried leaves were used [14].

With regard to *Averrhoa carambola* (star fruit), cases of loss of consciousness, mental confusion, and even fatal outcomes have been reported in uremic patients undergoing hemodialysis after ingestion of the fruit. This toxicological condition was later attributed to the presence of a neurotoxin—a toxic substance that inhibits and causes the destruction of nervous tissue, particularly neurons, which are responsible for the conduction of impulses in the central nervous system [15, 16].

In addition, in northern and northeastern Brazil, a species popularly known as *bucha paulista* is cultivated and commercialized for the treatment of rhinitis and sinusitis. However, its use may lead to increased irritation and nasal hemorrhage (in cases of acute or subacute exposure), contributing to cases of intoxication that are often unrecognized or not associated with its use, as many users are unaware of the potential harm caused by *bucha paulista* [17].

Conclusion

It can be concluded that the crude ethanolic extract of *Kalanchoe blossfeldiana* Poelln leaves exhibits a high cytotoxic effect on human erythrocytes due to the observed increase in hemolysis. This finding highlights the importance of carefully selecting the location where this plant is cultivated, ensuring it is not easily accessible to small children, as ingestion of its leaves may pose potential health risks.

Conflict of Interest

The authors declare no conflict of interest.

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