International Journal of Science Academic Research
Vol. 02, Issue 05, pp.1564-1566, May, 2021
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

EVALUATION OF IN VITRO CYTOTOXICITY AND INVESTIGATION OF IMMUNOSTIMULANT ACTIVITY OF *BAUHINIA RUFESCENS* AND *EUPHORBIA HIRTA*

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Received 17th March 2021; **Accepted** 25th April 2021; **Published online** 30th May 2021

Abstract

Background: Herbal medicine is a natural medicine. However, phytotherapy does not have only beneficial effects. Like any active product, it can have undesirable, toxic effects. Plants with very interesting potentials on the therapeutic level can present a toxicity which limits their use. The objective of this study is to evaluate the cellular toxicity of hydroethanolic extracts of *Bauhinia rufescens* and *Euphorbia hirta* and to investigate their immunostimulant activities. **Methodology:** Cytotoxicity evaluation was performed on a cell culture for 24h under 5% CO2.Cell death was evaluated by the propidium iodide exclusion method by flow cytometry. The immunostimulating activity was measured on the cell culture supernatant for 24 hours under 5% CO2.the TNFα cytokine was measured on the cell culture supernatant collected after 24 hours by Sandwich ELISA technique. **Results:** The hydroethanol extract of *Bauhinia rufesccens* showed a dose-dependent cytotoxicity. The highest concentrations of the extract (500 µg/mL to 25 µg/mL) were found to be highly toxic compared to 100% DMSO. The cytotoxicity of the hydroethanol extract of *Euphoria hirta* does not show a dose-dependent relationship. This relationship is established only from the concentration 12.5 µg/mL. Only the concentrations of 50 µg/mL, 25 µg/mL, 3.125 µg/mL, 1.5625 µg/mL and 0.78125 µg/mL are significantly less toxic than 100% DMSO. The hydroethanolic extract of *Bauhinia rufescens* cultured with peripheral blood mononuclear cells showed a dose-dependent immunostimulatory capacity. The concentration of 500ug/mL induced a high production of TNF compared to the positive control (PHA). This significant immunostimulatory activity was observed also for the concentrations of 250 µg/mL, 125 µg/mL and 0.78125 µg/mL for *Euphorbia hirta*, that the concentration of 0.78125µg /ml significantly stimulated the production of proinflammatory cytokine, TNFα compared to phytohemagglutin (PHA). **Conclusion:** the studied plants showed toxicity at high concentration. Compared to the immunostimulatory activity, *Euphorbia hirta*, the lowest concentration caused high production of TNFα. These results may pave the way on further studies for possible use of *Bauhinia rufescens* and *Euphorbia hirta* as natural immunostimulants.

Keywords: Cytotoxicity and immunostimulation, *Bauhinia rufescens* and *Euphorbia hirta.*

INTRODUCTION

Africa has a significant diversity of medicinal plants. Indeed, out of the 300,000 plant species recorded on the planet, more than 200,000 species are found in tropical African countries and have medicinal properties (Salhi *et al.,* 2010). Medicinal plants are a valuable heritage for humanity and especially for the majority of poor communities in developing countries who depend on them for their primary health care and livelihoods (Jiofack *et al.,* 2010). Thus, there has been a growing interest in both developed and developing countries in herbal medicines over the past two decades (Laplante, 2003). Herbal medicine is often presented as a natural medicine. However, herbal medicine does not have only beneficial effects. Like any active product, it can have undesirable, toxic and allergic effects. Plants with very interesting therapeutic potential may present a toxicity that limits their use, while other plants may have an immunostimulant potential. In Chad, the vastness of the country (1,284,000 square kilometers) makes access to medicines difficult. This difficulty is exacerbated by the uneven distribution of private pharmacies throughout the country, and the great distances between drug supply centers and localities. The shortage of medicines in terms of quality and quantity is constant in the various health structures. Some peripheral health structures are inaccessible during the rainy season due to the poor state of the roads, so the population resorts to phytotherapy for treatment.

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Plants such as *Bauhinia rufescens* and *Euphorbia hirta* are among the most commonly used plants in the Chadian pharmacopoeia for the treatment of gastroenteritis in both adults and children. However, there is little information on their biological activities such as cytotoxicity and ability to stimulate the immune system. The objective of this study is to evaluate the cellular toxicity of hydroethanolic extracts of *Bauhinia rufescens* and *Euphorbia hirta* and to investigate their immunostimulating activities.

METHODS

Type of study
This was an experimental study

Biological material: The biological material consisted of venous blood collected on EDTA from 18 volunteer donors.

Plant material: The leaves of *Bauhinia rufescens* and the whole plant of *Euphorbia hirta* were collected in September 2020 at 25 km from the city of Ndjamena (Chad) in a village called Marra. The collected plant material was authenticated at the herbarium of Institut de Recherche pour le Développement (IRD) of Farcha at the University of Ndjamena (Chad).

Methods

Preparation of hydroethanol extracts: The leaves of *Bauhinia rufescens* and the whole plant of *Euphorbia hirta* were dried at room temperature away from sunlight and dust,

then crushed in a clean mortar before being reduced to a fine powder using an electric mill. 500 g of *Bauhinia rufescens* powder and 300 g of *Euphorbia hirta* powder were macerated in an ethanol/water mixture (70/30). The resulting mixture was incubated for 48 hours at laboratory temperature and frequently shaken. The macerate was then successively filtered with hydrophilic cotton before being filtered on Whatman N° 1 filter paper under vacuum pumping. The solvent was evaporated with Rotavapor and the total hydro ethanolic extracts obtained were used to prepare solutions of concentration 100 mg/ml which were sterilized by filtration under vacuum on 0.45 µm millipore membrane. The recovered extracts were stored at 4°C in a refrigerator before testing.

Cell culture

Isolation of peripheral blood mononuclear cells (PBMCs): PBMCs were isolated by the Ficoll gradient centrifugation technique.20 ml of whole blood was previously diluted with 15 ml of DPBS. Then the mixture of whole blood and PBS (35ml) was gently poured into 15 ml of Ficoll reagent using a graduated pipette (Paestum pipette). The flask containing the mixture is then centrifuged at 2000 rpm for 20 min. After centrifugation, the cell interphase was recovered and washed with RPMI 1640 supplemented with 2mM/ml L-Glutamine 50µg/ml gentamicin to 100µg/ml Penicillin - Streptomycin (RPMI1640+++). Finally, cell viability was evaluated by the Trypan Blue exclusion technique and the count was done under the microscope using the Neubauer hematimeter.

Placing in culture : 2x105 cells were cultured in the presence or absence of different concentrations of hydroethanol extract of *Bauhinia rufescens*, *Euphorbia hirta*, Phytohaemagglutin (PHA) and DMSO 100%. The cell culture medium was RPMI 1640++/10% FCS. The set was incubated for 24 h at 37 0 C under 5% CO2 and in a humid atmosphere.

Evaluation of cytotoxicity

After 24 h of culture, the cell pellet was recovered and labeled with propiduim iodide for cytotoxicity evaluation using flow cytometer.

Evaluation of immunostimulatory activities

Cytokine assay: The cytokine TNFα was assayed on the supernatant of cell cultures collected after 24 h by Sandwich ELISA. Huma Reader HS plate reader was used for reading.

Data processing and analysis

Computer and statistical analysis was done on a microcomputer using Epi Info 3.5.1. and Microsoft office Excel 2007. The Chi-square test was used for comparisons. The difference was significant for p <0.05.

RESULTS

Cytoxicity of hydroethanol extracts of *Bauhinia rufescens*

The data showed a dose-dependent cytotoxicity. The highest concentrations of the extract (500 µg/mL to 12,5 µg/mL) were found to be highly toxic compared to 100% DMSO.

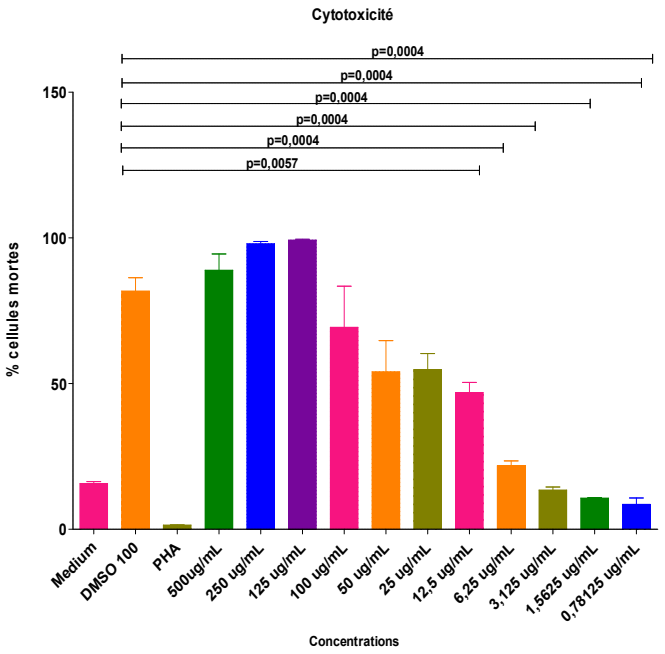

Figure 1. Cytotoxicity of *Bauhinia rufescens.*

Cytotoxicity of hydroethanolic extracts *of Euphorbia hirta*

The cytotoxicity data of the hydroethanolic extract of *Euphorbia hirta* do not show a dose-dependent relationship. This relationship is established only at the concentration 12.5 µg/mL. Only the concentrations of 50 µg/mL, 25 µg/mL, 1.5625 µg/mL and 0.78125 µg/mL are significantly less toxic than 100% DMSO.

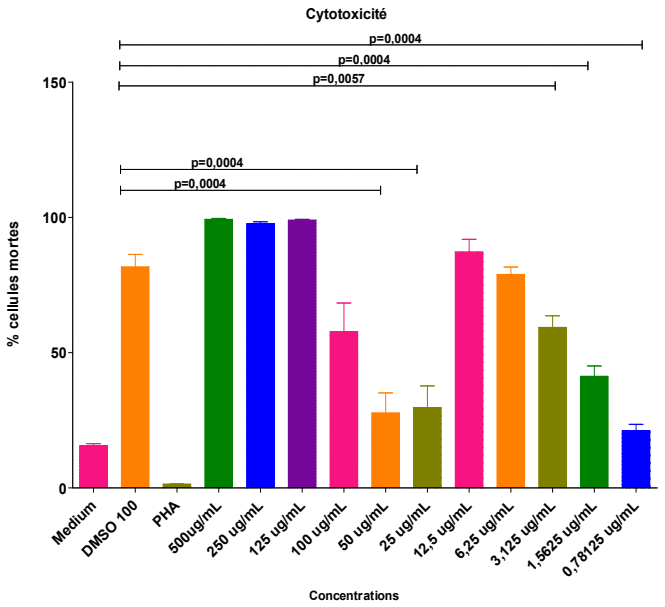

Figure 2. Cytotoxicity of *Euphorbia hirta*

Immunostimulatory capacity of *Bauhinia rufescens*

Peripheral blood mononuclear cells were stimulated with the different concentrations of *Bauhinia rufescens.* The significantly immunostimulatory activity was also observed for concentrations of 1,5625 µg/mL and 0.78125 µg/mL.). this plant significantly stimulated the production of the proinflammatory cytokine, TNF, compared to phytohemagglutin (PHA).

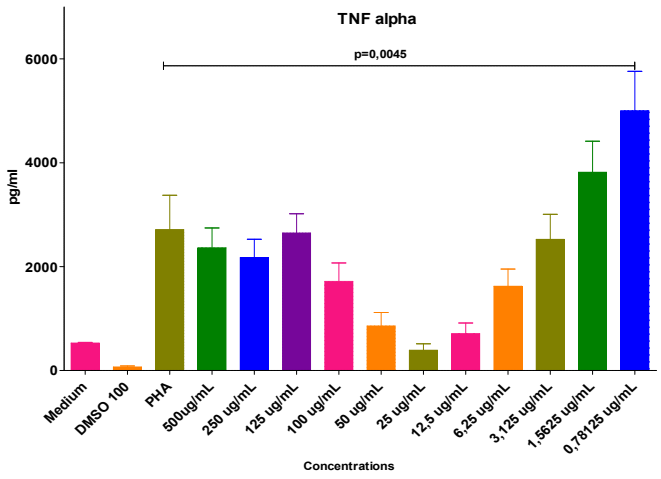

Figure 3. Immunostimulatory properties of *Bauhinia rufescens*

Immunostimulatory capacity of *Euphorbia hirta*

Peripheral blood mononuclear cells were stimulated with the different concentrations of *Euphorbia hirta* . A hight production of TNF α was observed at a concentration 500 µg/mL with a significant value compared to phytohemagglutin (PHA).

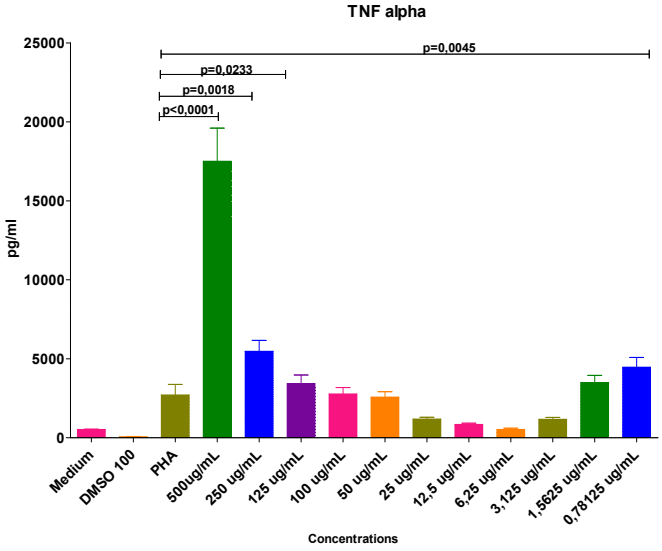

Figure 4. Immunostimulatory properties of *Euphorbia hirta*

DISCUSSION

Peripheral blood mononuclear cells were cultured in the presence of different concentrations of the hydroethanol extract of *Bauhinia rufescens* (500µg/mL to 0.78125µg/mL). and 100% DMSO as a positive control and phytohemagglutin (PHA) as a negative control. The highest concentrations of the extract (500µg/mL to 12,5 µg/mL) were found to be highly toxic compared to 100% DMSO. From 12.5µg/mL, the extracts showed significantly low toxicity compared to 100% DMSO. On the other hand, our results corroborate with those of a study on the cytoxicity of *Bauhinia rufescens* on vero cells in Sudan (Mohammed *et al.,* 2015). Cytotoxicity data of hydroethanol extracts of *Euphorbia hirta* do not show a dose-dependent relationship. This relationship is only established from the concentration of 12.5µg/mL . Only the concentrations of 50µg/mL, 25µg/mL, 3.125µg/mL, 1.5625µg/mL and 0.7812µg/mL are significantly less toxic than 100% DMSO . A cytotoxicity study using vero cells was conducted in Malaysia. According to the results of this study, the hydroethanol extract of *Euphorbia hirta* was not toxic with an IC50 of 126 µg/ml (Shanmugapriya *et al.,* 2012). An analysis of blood cells of rats given ethanolic extracts of *Euphorbia hirta* showed no effect on the cells which confirms that the extracts of *Euphorbia hirta* are not cytotoxic (Ogbulie1, 2007).

Immunostimulant activities

Immunostimulating capacity of *Bauhinia rufescens*: Different concentrations of the hydroethanol extract of *Bauhinia rufescens* in culture with peripheral blood mononuclear cells .This significant immunostimulatory activity was also observed for concentrations of 1,5625 µg/mL and 0.78125 µg/mL.

Immunostimulatory capacity of *Euphorbia hirta:* Different concentrations of the hydroethanol extract of *Euphorbia hirta* in culture with peripheral blood mononuclear cells showed a dose-dependent immunostimulatory capacity. Thus, a concentration of 500 µg/mL induced a high production of TNF α compared to the positive control (PHA).

Conclusion

Bauhinia rufescens and *Euphorbia hirta*, two plants used in traditional Chadian medicine have biological activities that justify their use. However, studies on cytoxicity show that at high concentrations the hydroethanol extracts of these plants can be cytotoxic. *Bauhinia rufescens* has an immunostimulant action at low concentration while *Euphorbia hirta* has a high production of TNF αat high concentration.

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