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Original Article

Assessment of the cytotoxicity activity of aqueous and methanolic leaf extracts of *Alchornea cordifolia* (Euphorbiaceae)

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ABSTRACT

Alchornea cordifolia is commonly used by African populations for its healing properties. However, the cell toxicity of this plant for optimal safe use is still a concern. The objective of this study was to assess the cytotoxicity activity of aqueous and methanolic leaf extracts of *Alchornea cordifolia*. The cytotoxicity activity of *Alchornea cordifolia* leaf extracts was investigated on healthy human umbilical endothelial cells (HUVEC), human malignant liver cancer cells (HEP-G2) and on human malignant lung cancer cells, alveolar epithelium (A549). The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium-bromide (MTT) colorimetric method was used to evaluate the ability of living cells to reduce the yellow-coloured MTT to its metabolite, the blue-coloured formazan. The number of living cells after 72 hours of incubation was proportional to the intensity of purple staining measured by flow cytometry. On the living cells, the methanolic and aqueous extracts were not toxic. The viability rates were 53.56 ± 2.17% and 55.60 ± 0.30%, respectively and with doxorubicin it was 19 ± 1% (p = 0.0021). On malignant cells, the methanolic and aqueous extracts did not show any toxicity either. The viability rates of HEP-G2 malignant cells were 71.35 ± 0.23% and 81.44 ± 3.95%, respectively, while that obtained with doxorubicin was 39 ± 1% (p = 0.05). The viability rates of A549 malignant cells were 51.07 ± 1.97% and 44.84 ± 6.22%, respectively whereas that of doxorubicin was 25 ± 3% (p = 0.05). The methanolic and aqueous leaf extracts of *A. cordifolia* were not toxic for healthy cells. The safe use of this plant in traditional medicine has now been demonstrated. Also, this plant has not been shown to be toxic to cancer cells, and therefore should not be used in the treatment of cancer.

KEYWORDS: cytotoxicity, aqueous extract, methanolic extract, *Alchornea cordifolia*.

INTRODUCTION

In African countries, the population relies on medicinal plant for their healthcare on account of their socio-cultural habits and their low income level [1]. The therapeutic properties of plants have been kept secret by traditional healers for several generations [2]. Among these varieties of plants one can point out *Alchornea cordifolia* (Euphorbiaceae), a plant which has been deeply studied by our research team [3-6]. *Alchornea cordifolia* is a widely used plant in traditional African medicine. Several pharmacological properties of this plant have been demonstrated, such as its antimicrobial [7, 8], antiplasmodial [9], anti-inflammatory [10, 11], antispasmodic [12], antioxidant [6, 13], antidiarrhoeal [14], antipyretic [4] and hepatoprotective properties [5]. Other studies have meanwhile emphasized on

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users safety by assessing its *in vivo* acute toxicity [4, 15], sub-acute toxicity [16, 17], sub-chronic toxicity [18] and chronic toxicity [19]. However, the cell toxicity of *A. cordifolia* for optimal safe use still remains a concern. Thus, the aim of this study was to investigate on the cytotoxicity activity of an aqueous and methanolic leaf extracts of *Alchornea cordifolia*.

MATERIALS

Plant material

This study was carried out on *Alchornea cordifolia* (Schum. and Thonn.) leaves (Euphorbiaceae), collected from Yakassemé in the department of Adzopé, 75 kilometer far from Abidjan, (Côte d'Ivoire). A plant voucher specimen (herbarium N° AC 2016) was deposited at the laboratory of Pharmacology. The plant was identified and authenticated by the National Floristic center of Abidjan (Côte d'Ivoire). Fresh leaves weighing 4,204 g were washed and dried for one week in a shaded room at 18 °C at the laboratory of Pharmacology, faculty of pharmaceutical and biological sciences (University of Félix Houphouët-Boigny). Then, dried leaves weighing 1,703 g were ground to powder.

Biological material

The following cell lines were used for cytotoxicity tests at the laboratory of biology of Chatenay Malabry (Paris-France):

- Healthy human cell line of umbilical endothelial (HUVEC)
- Human liver cancer malignant cell line (HEP-G2)
- Human Lung cancer malignant cell line (alveolar epithelium) A549.

METHODS

Preparation of extracts

Dried leaf powder of plant (100 g) was macerated for 24 hours in 1 L of distilled water for the aqueous extract or in 1 L mixture of methanol-water (70:30) for the methanolic extract. The mixture was filtered on cotton and then on filter paper (WHATMAN). Filtrate was dried in an oven at 60 °C for 72 hours for the aqueous extract, and evaporated using a rotary evaporator for the methanolic extract. Dry residue obtained was scraped off with a spatula and collected in clean flasks and kept at 4 °C for experiments.

Cell line culture

The culture was carried out at the laboratory of biology of Chatenay Malabry (Paris-France). HUVEC, HEP-G2, and A549 cells were cultured in specific media supplemented with 10% Foetal Veal Serum (FVS) and 1% penicillin streptomycin (PS).

Cytotoxicity study

The method used was that described by Price and Mc Millan [20]. It is a colorimetric method consisting in investigating the capability of living cells to reduce 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium-bromide (MTT), a yellow tetrazolium salt, to its formazan metabolite which turns to blue colour after reduction by cell enzymes of the respiratory chain of living cells. The number of living cells after 72 hours of incubation with or without tested compounds or standard drug is directly proportional to the intensity of the purple staining measured.

Preparation of stock solutions

A solution of 10 mM using dimethylsulfoxide (DMSO) was prepared for each sample to be tested. The positive control compound was Doxorubicin (hydrochloride, 5 µM); the white control was the solvent (0.1%). An untreated control was also prepared, a medium with endothelial cells alone, with 100% viability.

Assay

- Day 1: three (3) wells received 5,000 cells + 100 µl of each sample (microplate of 100 wells); they were then incubated for 24 hours;
- Day 2: cultures were treated with 100 µl of the sample twofold concentrated, meaning 20 mM for a final volume of 10 mm^3;
- Day 3: an autotoxicity test was performed with MTT after 48 hours of incubation and then analysed using flow cytometry. The optical density (OD) was measured at 517 nm thrice for each sample as well as controls.

Percentage of survival or inhibition

The results were expressed as a percentage of survival compared to controls and calculated according to the following formula:

$$\% \text{ of survival} = \frac{\text{OD trial}}{\text{OD control}}$$

OD trial: Optical Density (OD) of tested compound

OD control: Optical Density (OD) of untreated control

Data analysis

The values were expressed as means ± standard of deviation. The comparison of means was performed by the Pearson test. The graphical representation was made by GraphPad Prism 8.0.2 software.

RESULTS

Percentage of survival of healthy HUVEC cells

The percentage of survival of healthy HUVEC cells is shown in Figure 1. This figure shows that in the presence of the methanolic leaf extract of *A. cordifolia* 53.56 ± 2.17% of the living cells remained viable. This viability rate was 55.60 ± 0.30% with the aqueous leaf extract. These values were not significantly different from those obtained with the white control (55 ± 5% survival) ($p > 0.05$), but were significantly different from those obtained in the presence of doxorubicin which spared19 ± 1% of the living cells ($p = 0.0021$).

Percentage of survival of HEP-G2 malignant cells

Percentage of survival of HEP-G2 malignant cells is shown in Figure 2, which demonstrates that 71.35 ± 0.23% of HEP-G2 malignant cells continued to survive in the presence of the methanolic leaf extract of *A. cordifolia*. This survival rate was 81.44 ± 3.95% in the presence of the aqueous leaf extract. These values are not significantly different from those obtained with the white control (77 ± 9% survival) ($p > 0.05$), but are significantly different from those obtained in the presence of doxorubicin where only 39 ± 1% of the malignant cells ($p = 0.05$) survived.

Percentage of survival of malignant cells A549

The percentage of survival of A549 malignant cells is shown in Figure 3. This figure shows that A549 malignant cells remained viable at 51.07 ± 1.97% in the presence of the methanolic leaf extract of *A. cordifolia*. In the presence of the aqueous leaf extract, the viability of these cells was 44.84 ± 6.22%. These values are not significantly different from those obtained with

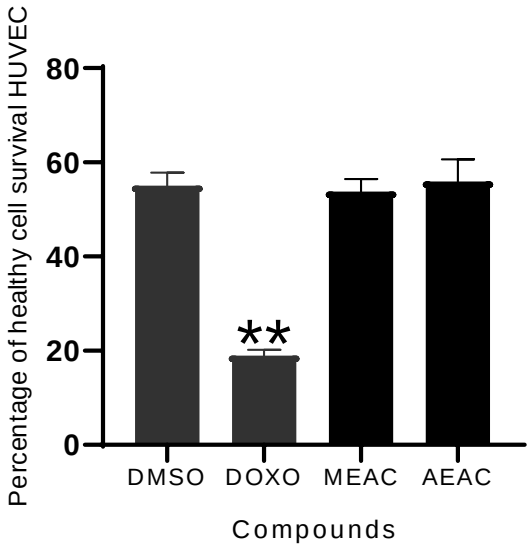

Figure 1. Percentage of survival of healthy HUVEC cells.
Values were measured 3 times and expressed as means ± standard of deviation.
**: $p < 0.002$, Pearson's test compared to control (DMSO). MEAC: Methanolic extract of *Alchornea cordifolia*; AEAC: Aqueous extract of *Alchornea cordifolia;* DOXO: Doxorubicin.

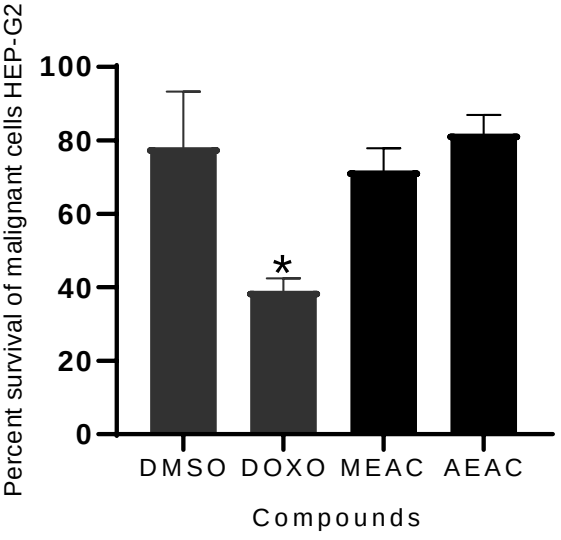

Figure 2. Percentage of survival of HEP-G2 malignant cells.
Values were measured 3 times and expressed as means ± standard of deviation.
*: $p < 0.05$, Pearson's test compared to control (DMSO). MEAC: Methanolic extract of *Alchornea cordifolia*; AEAC: Aqueous extract of *Alchornea cordifolia*; DOXO: Doxorubicin

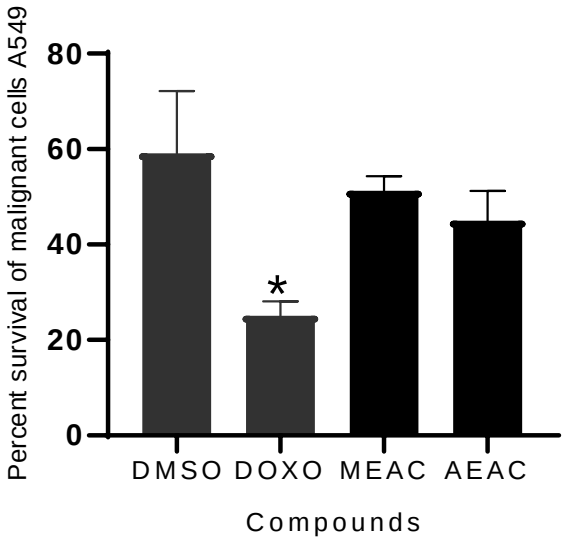

Figure 3. Percentage of survival of malignant cells A549. Values were measured 3 times and expressed as means ± standard of deviation.
*: $p < 0.05$, Pearson's test compared to control (DMSO); MEAC: Methanolic extract of *Alchornea cordifolia*; AEAC: Aqueous extract of *Alchornea cordifolia*; DOXO: Doxorubicin

the white control (59 ± 15% survival) ($p > 0.05$), but are significantly different from those obtained in the presence of doxorubicin which spared only 25 ± 3% of the malignant cells ($p = 0.05$).

DISCUSSION

The objective of this study was to investigate the cytotoxicity activity of an aqueous extract and a methanolic leaf extract of *Alchornea cordifolia*. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium-bromide) cytotoxicity test was used. This test assesses the viability of living cells by means of mitochondrial dehydrogenase activity. At cell death the mitochondrial activity disappears with its potential for tetrazolium salt reduction. A living cell therefore allows the reduction of yellow MTT into its metabolite, blue formazan [20].

This study showed that the methanolic leaf extract as well as the aqueous leaf extract of *A. cordifolia* were not toxic for healthy human umbilical endothelial cells (HUVEC). *Alchornea cordifolia* does not represent a significant risk of cell toxicity when used in traditional medicine. This absence of cytotoxicity has been confirmed by various *in vivo* toxicity studies on *A. cordifolia*. Indeed, studies showed that in laboratory animals *A. cordifolia* could be used at doses up to 5000 mg/kg without any sign of toxicity [4, 21].

In malignant human liver cancer cell (HEP-G2) and human lung cancer cell (alveolar epithelium) (A549), methanolic and aqueous leaf extracts of *A. cordifolia* were also not cytotoxic. *A. cordifolia* leaf extracts could therefore have no antiproliferative effect on these cancer cells. However, studies showed that some phytochemical groups found in plants such as phenolic compounds, could confer an anticancerous activity. That is the case of gallotanins (pentagalloylglucose) which have antitumour activities on various prostate [22], lung [23], breast [24], melanoma [25], liver [26] and sarcoma cancers [27]. As for ellagitanins, they are against the mutagenicity of some carcinogens [28].

Our previous studies have indeed demonstrated that the aqueous and the methanolic leaf extracts of *A. cordifolia* were particularly rich in phenolic compounds such as flavonoids and tannins [5, 6]. However, flavonoids are basically cytotoxic, but this property depends on their glycolysation and their degree of methoxylation. Glycosylated flavonoids reduce anticancer activity [29]. The flavonoids found in the aqueous and methanolic leaf extracts of *A. cordifolia* could then be at some degree of glycosylation.

CONCLUSION

The methanolic and aqueous leaf extracts of *A. cordifolia* had no effect on the viability of healthy cells. The safety of using this plant in traditional medicine has therefore been demonstrated. Also, this plant should not be used in the treatment of cancer as it had no effect on the malignant cells used in our study.

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AUTHOR CONTRIBUTIONS

All the authors contributed to the study.

CONFLICT OF INTEREST STATEMENT

We declare no conflicts of interest.

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