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Cytotoxicity assessment of aqueous extract from root barks of *Calotropis procera* (Ait.) R. Br. in human intestinal Caco-2 and mouse neuroblastoma Neuro-2a cell lines

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ABSTRACT

Calotropis procera (Ait.) R. Br (Asclepiadaceae) is a species widely used in traditional medicine for the treatment of various diseases such as sickle cell disease, asthma and cancer. In Burkina Faso, it enter in the composition of FACA® in combination with *Zanthoxylum zanthoxyloides* Lam (Rutaceae), drug used in sickle cell disease treatment. The objective of this study was to evaluate the in vitro cytotoxicity of aqueous extract of root barks of the plant on cell lines to increase the safe use of FACA®. MTT and Neutral Red assays performed on Caco-2 and Neuro-2a cell lines revealed that aqueous extract from root barks of *Calotropis procera* are cytotoxic on these cell lines. DNA fragmentation assay on Caco-2 cell showed DNA smearing reflecting a degradation of nuclear material that indicates a possible genotoxicity. Altogether, it comes out that the most sensitive cell line is the human colorectal carcinoma Caco-2 cells. Comparatively the active compounds of *Calotropis procera* do not affect the mice nervous system cells in the same dramatic extent. Our results strongly suggest that patients under treatment of FACA® must respect doses prescribed in order to avoid adverse side effects on the gastrointestinal tract.

INTRODUCTION

In Burkina Faso, as in many developing countries access to medical care in general and drug in particular, is often difficult. Current drugs are imported, and are not available to most patients because of their high cost (Guissou *et al.*, 1995). In this country more than half of the population lives in rural areas and uses a lot of herbs for their health care. More broadly, according to World Health Organization, it appears that approximately 80% of African people use traditional medicine for primary health care (OMS, 2002). Among the plants used in traditional medicine in Burkina Faso the *Calotropis procera* (Ait.) R. Br, also called "Pomme de Sodome" in French, is a species widely used among treatment of various diseases such as sickle cell disease, asthma and cancer (Nacoulma 1996).

The genus *Calotropis* R.Br. (Asclepiadaceae) is commonly distributed in tropical and subtropical arid regions of Asia, Middle-East and Africa and is represented mainly by two species viz. *Calotropis procera* and *Calotropis gigantean* (Verma *et al.*, 2010). *Calotropis procera* (Ait.) R. Br (*C. procera*) is a shrub belonging to the family of Asclepiadaceae. This wild plant grows typically in arid environments rich in phosphorus and calcium such as cemeteries, in the "wadi" or savannah and appears always verdant. Different parts of this plant are used for different purposes such as treatment of diverse diseases but also widespread in magico-religious practices. The leaves and root barks of *C. procera* are used in traditional medicine and renowned for the treatment of sickle cell disease.

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Numerous investigations conducted by the laboratories of pharmacology and toxicology of the "Unité de Formation et de Recherche en Science de la Santé" (University of Ouagadougou) and the Institute for Research in Health Sciences, Burkina Faso resulted the development of a phytomedicinal drug named FACA® (Nikiema *et al.*, 2010).

FACA® is a mixture of roots barks powder of *Calotropis procera* (Ait.) R. Br (Asclepiadaceae) and *Zanthoxylum zanthoxyloides* Lam (Rutaceae) used in the treatment of sickle cell disease. This phytomedicinal drug revealed some effectiveness in clinical trials to relieve sickle cell crisis in children (Guissou *et al.*, 1995, Nikiema *et al.*, 2010).

The major component of FACA® is *C. procera* well known for its toxicity due to abundant latex. Regardless of the benefit that can be drawn, substances contained in this herbal medicine can be potentially toxic to human health especially during sustained use which can lead to toxicity.

Recent in vitro and in vivo investigations have revealed that many plants in traditional medicine have mutagenic, cytotoxic and genotoxic effects (Plewa andWagner, 1993; Higashimoto *et al.*, 1993; Schimmer et al., 1994; Zin and Chaffin, 1998)

Several studies reported that the root barks extract of *C. procera* possesses cytotoxic properties on cancer cells line (Smith *et al.*, 1995; Van Quaquebeke *et al.*, 2005). Actually, components of the plant including latex appeared to have in vivo anticancer and cytotoxic properties (Choedon *et al.*, 2006).

Considering long-term treatment for sickle cell disease, a better knowledge of potential cytotoxic effects of the part of the plant used in the FACA® proves to be necessary. The aim of this study was therefore to evaluate the in vitro cytotoxic activity of aqueous extract of root barks of the plant on cell lines to contribute to the safe use of FACA® in the treatment of sickle cell disease.

MATERIAL AND METHODS

Plant material and extract preparation

Fresh roots of *C. procera* were collected in Roumtenga located at 25 km north-East of Ouagadougou, capital of Burkina Faso, savannah countries, in January 2012.

A sample of harvested plant was authenticated at the "Herbier National du Burkina (HNBU)" located at "Centre National de Recherche Scientifique et Technologique (CNRST)" where the voucher specimen has been deposited under number 8,716.

The barks were washed with tap water, dried under ventilation in the shade. The dried barks were pulverized using a mechanical grinder (Gladiator Est. 1931 Type BN 1 Mach. 40461 1083).

Aqueous extract was then prepared by maceration of a portion of root bark powder (250g) in 2.5 L distilled water during 48h at room temperature.

The mixture was then filtered through cotton wool, and the filtrate was centrifuged at 2,000 rpm for 5 min. The collected supernatant was then lyophilized using Freeze dryer (CHRIST Alpha 1-2, BIOBLOCK SCIENTIFIC), packaged in a bottle and stored in a desiccator. The lyophilisate obtained was then used for cytotoxicity tests.

Chemicals and culture medium

Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute medium (RPMI-1640), Foetal Calf Serum (FCS), thiazolyl blue tetrazolium bromide (MTT) and neutral red solution were provided from Sigma-Aldrich (France). All other chemicals used were of analytical grade.

Cells and culture condition

Cytotoxic effect of aqueous extract of *Calotropis procera* (Ait.) R. Br was carried out on Caco-2 cells (Human Caucasian colon adenocarcinoma) and Neuro-2a cells (mouse Albino neuroblastoma).

Caco-2 cells were provided from European Collection of Cell Cultures (ECACC) and Neuro-2a cells from American Type Culture Collection (ATCC). These cells were frozen in cryotubes and stored in liquid nitrogen at - 196 ° C before being thawed and cultured.

Caco-2 cells were grown as monolayer cultures in a high glucose (4 g/L) DMEM medium and Neuro-2a cells in RPMI medium. These cells culture medium were supplemented with 10% fetal calf serum (FCS), 8mM L-glutamine, 1% of penicillin (100 IU/mL) and streptomycin (100 g/mL) mixture. Cells were incubated at 37°C in a humidified 5% CO_2 incubator (Jouan, IG 150, France). For cell counting and subculture, the cells were suspended using 0.05% trypsin and 0.02% EDTA for Caco-2 cells and mechanically for Neuro-2a cells.

Cells viability assay by MTT

Cell viability was assessed using MTT colorimetric assay widely used to determine cell growth and cell cytotoxicity, particularly in the development of new drugs (Akhir *et al.*, 2011). It is based on the ability of viable cells to metabolize a tetrazolium salt to formazan blue in the mitochondria (Loveland *et al.*, 1992). The formazan accumulation is proportional to the number of viable cells and inversely proportional to the degree of cytotoxicity (Berridge *et al.*, 2005).

Briefly, the cells are inoculated in 96 well plates at (2 to 3) $x10^5$ cells/mL of complete cell culture medium. Cells were incubated (Jouan IG 150 incubator, France) for 24 hours at 37°C in a humidified-5% CO_2 enriched atmosphere prior to extract exposition. Culture medium was removed and after 48 hours of exposure in the same culture conditions with a concentration range of aqueous extract of plant (ranging from 0.15 to $15x10^3$µg/mL prepared in serum-free medium) or vehicle (cell culture medium) or control (cells with serum-free medium), again culture medium was removed before adding 100 µL of 1 mg/mL solution of thiazolyl blue tetrazolium bromide (MTT). Plates were incubated during 1 hour at 37°C, excess MTT was removed and 100 µL of dimethyl sulfoxide (DMSO) were added to each wells to dissolve formazan crystals, precipitates resulting from the conversion of

MTT by the mitochondrial succinate dehydrogenase. The plates were vortexed 5 min and read at 540 nm with a Microplate Reader LT-4000 (Labtech, France).

The viability was expressed as the optical density value from treated samples versus optical density values from control wells (untreated cells) both corrected by blank measurements of wells without cells.

Each experiment was repeated independently three times in six replicates (n=6) for each concentration point.

Cells viability assay by neutral red

Neutral red (NR) test was also performed to assess cytoxicity. NR assay is a cell viability test based on the ability of living cells to incorporate and bind neutral red. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of NR, thus providing a sensitive, integrated signal of both cell integrity and growth inhibition (Borenfreund and Puerner, 1985). Routinely, the cells were inoculated at a density of (2 to 3) $x10^5$ cells/mL in transparent 96-well plates (100 µL per well) and incubated at 37°C in a humidified incubator with 5% CO_2. After 24 hours, the medium was removed from wells and the cells were exposed to concentration of plant extract ranging from 0.15 to $15x10^3$µg/mL (in serum-free medium) or vehicle (cell culture medium) or control (cells with serum-free medium) for 72 hours. Then, plant extract media are removed and the cell layer washed with 150 µL of phosphate buffered saline solution (PBS). Next 150 µL of a pre-warmed (37°C) solution of neutral red prepared in fresh cell culture medium ($3.3x10^{-2}$ g/L) was added to each well. After 3 hours of incubation at 37°C (5% CO_2) the cells were washed twice with PBS and then lysed with 150 µL of mixture of acetic acid solution / ethanol 50% (1: 99 v/v). The plates were shaken for 15 min with a plate vortex before reading absorbance at 540 nm with Microplate Reader LT-4000 Labtech, France (Kouadio *et al.*, 2005). Cell viability was calculated from relative dye intensity compared with untreated samples.

Each experiment was repeated independently three times in six replicates (n=6) for each concentration point.

Morphological analysis

The morphology of cell was monitored using an inverted microscope. Caco-2 and Neuro-2a cells were checked for morphologic changes after 48 hours exposure to range concentrations of plant extract or vehicle as compared to control (100 µL per well) and photographs were taken (40x).

DNA fragmentation assay

DNA fragmentation assay were performed on Caco-2 cells. Cells were seeded at a density of (2 to 3) $x10^5$ cells/mL in 6-well plates (3 mL per well) and incubated at 37°C in a humidified incubator with 5% CO_2. After 24 hours of culture, the culture medium was removed and the plate was treated with adapted concentrations of plant extract (10, 50 and 100 µg/mL). After 24 hours of exposition at 37°C (5% CO_2), the medium was removed, followed by washing the cell layer with 5 mL PBS. The adherent cells were then lysed with lysis buffer (Tris-EDTA (20:5) pH 8 and 1% SDS). The proteins were digested with proteinase K (100 µg/mL for 1 hour at 37°C) and RNA by RNase A (10 µg/mL at 37°C for 15 min).

DNA extraction was carried out with chloroform isoamyl alcohol (24v: 1v) followed by precipitation with sodium acetate (3M, pH 4.8) and cold absolute ethanol (1v: 2v) and centrifuged at 6,000 g for 45 min at 4°C. The DNA pellet was air dried and re-suspended in ultrapure sterile water. The quantity and purity of DNA was determined by measuring the absorbance at 260 and 280 nm with NanoDrop 2000c Spectrophotometers (Thermo Scientific, USA). DNA fragmentation was analyzed in a 2% agarose gel in the presence of 1 µg/mL ethidium bromide.

Data analysis

The results are express as means ± SEM of three independent experiments in six replicate. Statistical difference between the control and the plant extract with different concentrations to the cell line were analyzed using one-way analysis of variance (ANOVA), followed by Dunett's multiple comparison tests. Differences were considered to be statically significant at $p < 0.05$. Significance levels were reported as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The IC_{50} values were obtained by nonlinear regression using the Graph pad Prism 5.0 program.

RESULTS

Effects on cells viability

Cell Growth Profile with MTT Assay

The effect of increasing concentrations of aqueous extract from root barks of *C. procera* on Caco-2 cells viability using MTT method is shown in Figure 1a. Aqueous plant extract induced cell death in concentration-dependent manner. The effective concentration causing 50% loss of cell viability (IC_{50}) in Caco-2 cell line is about 33 µg/mL after 48 hours exposure to plant extract (Table 1).

Table. 1: IC_{50} Values in MTT and Neutral Red assay.

Cells line	IC_{50} Values ± SEM (µg/mL)	
	MTT	Neutral Red
Caco-2	32.80 ± 12.31	11.39 ± 5.57
Neuro-2a	1802.33± 192.31	230.10 ± 9.5

IC_{50} values were obtained by nonlinear regression using the Graph pad Prism 5.0 program

The effect of increasing concentrations of aqueous extract on Neuro-2a cells using MTT assay is presented in figure 1b. Aqueous extract also causes cell death in concentration-dependent manner but with an IC_{50} of about 1802 µg/mL (Table 1) after 48 hours of exposure of the cells to the plant extract. It can be noticed that mice Neuro- 2a cell are less sensitive (55 time less sensitive) to the *C. procera* aqueous extract than Human Caco-2 cells (Neuro 2a IC_{50}>Caco-2 IC_{50}; Figure 1 and Table 1).

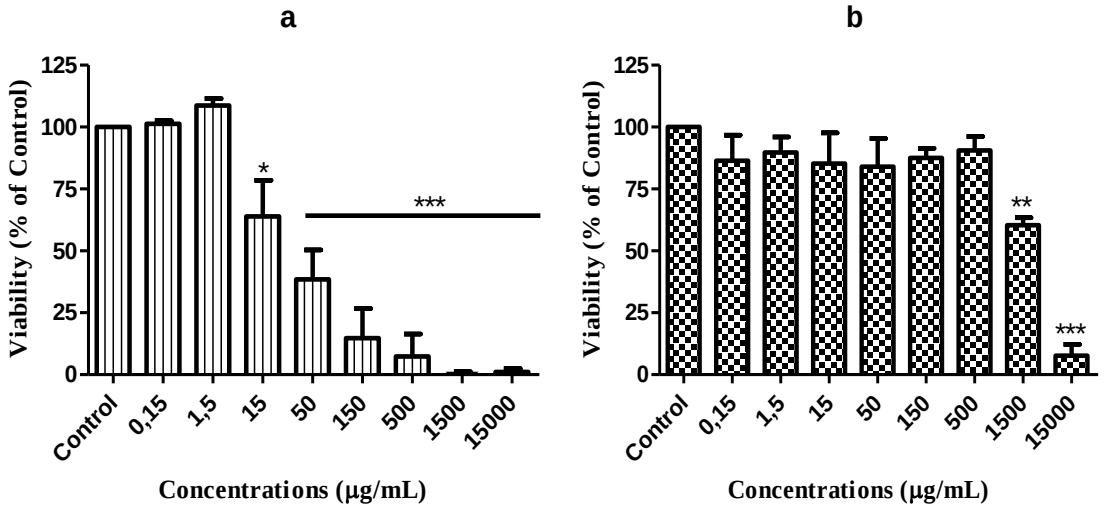


Fig. 1: Cytotoxic effect of aqueous extract of root barks of *Calotropis procera* on Caco-2 (a) and Neuro-2a (b) cells in MTT assay. The Caco-2 and Neuro-2a cells were treated with increasing concentrations of aqueous extract (0.15 to $15x10^3$ µg/mL) for 48 hours. The cell viability was measured in six-replicate using MTT assay. Means ± SEM of three independent experiments are presented. ** p values: * < 0.05; ** < 0.01; *** p < 0.001* as compared to control.

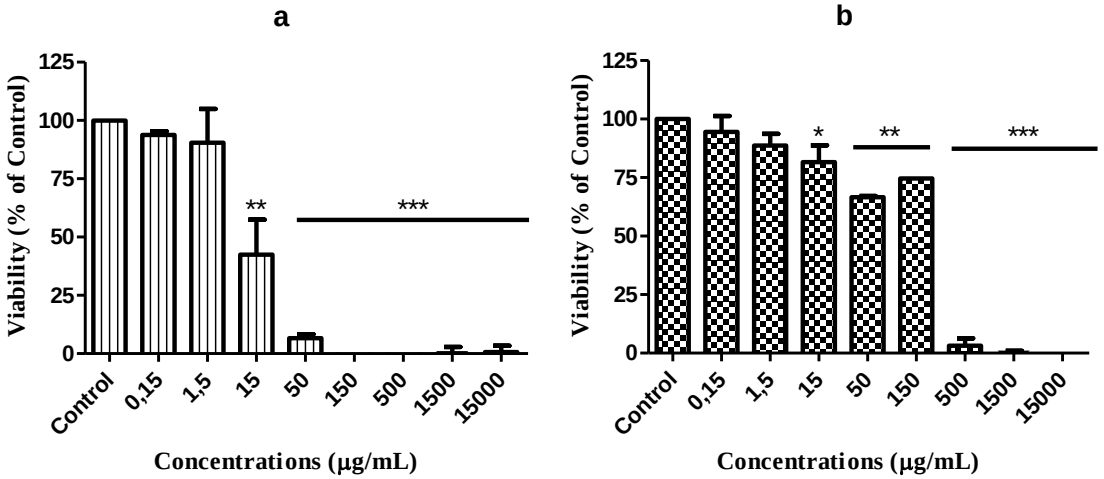
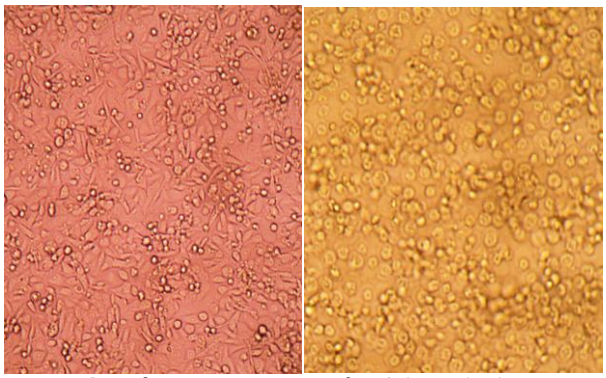
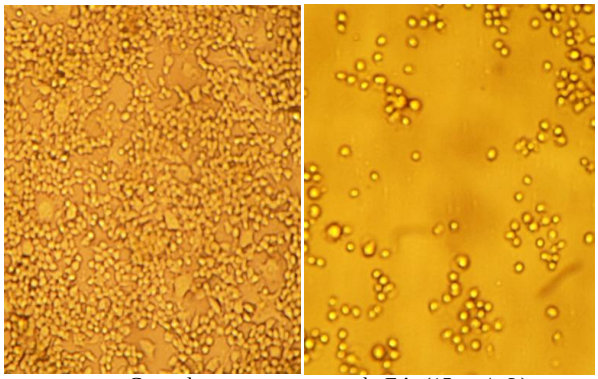


Fig. 2: Cytotoxic effect of aqueous extract of root barks of *Calotropis procera* on Caco-2 (a) and Neuro-2a (b) cells in Neutral Red assay. The Caco-2 and Neuro-2a cells were treated with increasing concentrations of aqueous extract (0.15 to $15x10^3$ µg/mL) for 48 hours. The cell viability was measured in six-replicate using Neutral Red assay. Means ± SEM of three independent experiments are presented. ** p values: * < 0.05; ** < 0.01; *** p < 0.001* as compared to control.



a: Control b: EA (15 mg/mL)

Fig. 3: Comparison of Caco-2 cells morphology under inverted microscope for the control cells (a) and cells treated with 15 mg/mL of aqueous extract (EA) (b) after 48 hours of exposure (x40)



a : Control b: EA, (15 mg/mL)

Fig. 4: Comparison of Neuro-2a cells morphology under inverted microscope for the control cells (a) and cells treated with 15 mg/mL of aqueous extract (b) after 48 hours of exposure (x40)

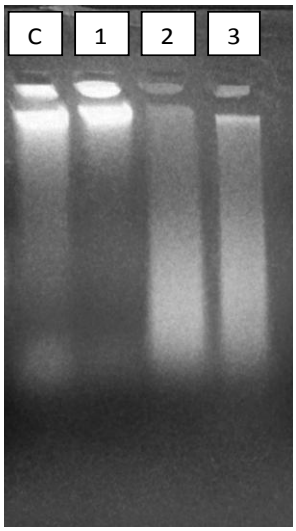


Fig. 5: Effect of aqueous extract of root barks of *Calotropis procera* (Ait.) R. Br on DNA fragmentation in Caco-2 cells. Lane (C): Control; lane (1): EA 10 µg/mL; lane (2): EA 50 µg/mL; lane (3): EA 100 µg/mL

Cell Growth Profile with Neutral red Assay

The effect of *C. procera* root barks aqueous extract on cell viability of Caco-2 and Neuro-2a using neutral red test is shown in figure 2. The plant aqueous extract causes an inhibition of cell growth in a concentration-dependent manner after 48 hours exposure (figure 2). The inhibition was significantly different from control (untreated cells, $p< 0.05$) with 50% inhibitory concentrations (IC_{50}) of about 11 µg/mL for Caco-2 cells and 230 µg/mL for Neuro-2a cells (Table 1). Again with the Neutral Red assay Neuro-2a cells are less sensitive (20 time less) than Caco-2cells (Neuro 2a IC_{50} > Caco-2 IC_{50}; Figure 2 and Table 1).

Morphological aspect of cells

The cytotoxic effect of *C. procera* root barks aqueous extract show morphological changes of Caco-2 cells after 48 hours of exposure (Figure 3). As compared to controls, it can be clearly seen that after 48 hours cells appearance of cell layer is changing (destructuration) strongly suggesting that cell death is occurring in the wells treated with the plant extract (compare Figure 3a and Fig. 3b). The cytotoxic effect of *C. procera* root barks aqueous extract on the morphology of Neuro-2a cells after 48 hours of intoxication is illustrated in Figure 4. The aqueous extract of root bark of plant causes a mortality of Neuro-2a cells in wells at high concentration of extract (15 mg/mL) (Fig. 4b) as compared to the controls (Fig. 4a). It can be seen from Figure 4b that the cell layer is sparser when compared to the control cell layer in Figure 4a.

DNA fragmentation assay

To elucidate the mechanism of cells death, DNA fragmentation assay was performed on Caco-2 cells. As shown in Figure 5, treatment with higher concentrations of aqueous extract of roots barks of *Calotropis procera* (50 and 100 µg/mL) during 24 hours resulted in DNA smearing and not DNA laddering as it can been seen from apoptotic cells. Only DNA from cells treated with 10 µg/mL of the compound remained intact as in the controls (Figure 5 lane C and lane 1). Although mitochondria are affected by the toxic extract as shown with MTT assays, DNA continuous smearing suggests that Caco-2 cell dies due to necrosis-like associated mechanism.

DISCUSSION

Cytotoxicity is seen primarily as the potential of a compound to induce cell death. The in vitro cytotoxicity assays are widely used for chemicals screening for predicting toxicity in animals or Human (Eisenbrand *et al.*, 2002). They allow a rapid assessment of the toxicity of a product and also the reduction of the use of animals for toxicity testing. Some differences in viability measurement can be found depending on the test agent used in cytoxicity test (Weyermann *et al.*, 2005). Consequently, MTT and non-enzymatic Neutral Red assays were performed to evaluate cytotoxic effect of aqueous extract of root barks of *Calotropis procera* (Ait.) R. Br on Caco-2 and Neuro-2a cell lines. The choice of Caco-2 cells was based on the fact that they has been extensively used for studies of intestinal absorption and toxicity of xenobiotics (Meunier *et al.*, 1995; Prueksaritanont *et al.*, 1996; Schmiedlin-Ren *et al.*, 1997). More closely, this cell line model allows the study of presystemic xenobiotic metabolism.

The choice of Neuro-2a was based on fact that they are mouse neural cell line that has been extensively used to study neuronal differentiation, signaling pathways and also neural toxic (Olmsted *et al.*, 1970).

The result obtained show that the aqueous extract of root barks of the plant are cytotoxic on Caco-2 and Neuro-2a cells both in MTT and Neutral Red cytotoxic test. The strongest cytotoxic effect of extract was observed on Caco-2 cells and results are rather similar in both methods. MTT and neutral red assay showing 50% inhibitory concentrations (IC_{50}) of 33 and 11 µg/mL respectively. For Neuro-2a cells, the aqueous extract of root barks of the plant exhibited lower cytotoxic effect with both methods. A significant growth inhibitory effect was obtained at extract concentrations higher than 1.5 mg/mL. MTT assay clearly show that aqueous extract causes cytotoxicity by altering the mitochondrial metabolism through succinate dehydrogenase activities whereas neutral red assay show alteration of cells membranes and transport of neutral red causing an apparent decrease in cell viability. These results are consistent and paralleled with the morphological change and cells erosion observed under inverted microscope and interpreted as cell death.
Several authors have previously reported the cytotoxic effect of parts of *C. procera* on cells lines. The root extract of C. procera has been found to produce a strong cytotoxic effect on COLO 320 tumour cells (Smit *et al.*, 1995). More recently, Van Quaquebeke et al. (2005) have showed that hemi synthetic cardenolides derivate originally isolated from root barks of the plant had a strong in vitro cytotoxic effect on several human cancer cell lines (Van Quaquebeke *et al.*, 2005).

According to the standards of the National Cancer Institute (NCI), an extract is considered to have significant anticancer properties if IC_{50} value is less than 20 µg/mL (Cordell *et al.*, 1993). The IC_{50} obtained in our study on caco-2 cell using

MTT and Neutral red methods is 33 µg/mL and 11 µg/mL respectively meaning that the extract has potential anticancer properties. DNA fragmentation assay carried out with Caco-2 cell show DNA smearing reflecting a degradation of nuclear material indicating potential genotoxicity. Other authors have found that the root extracts of *C. procera* inhibit the proliferation of Hep G-2 cells via apoptotic and cell cycle disruption based mechanisms (Mathur *et al.*, 2009). It has been established that cardiotonic steroid glycosides (bufalin and digoxin, for instance) are capable to kill cancer cells through the activation of apoptotic pathways (McConkey *et al.*, 2000; Kurosawa *et al.*, 2001, Zhang *et al.*, 2008). Caco-2 cells are deficient for both P53 gene copies, this mean that apoptosis in Hep-G2 is most likely due to P53 signaling after alteration of DNA molecules. But the results presented here suggest now that *C. procera* aqueous extract may also triggering cell death via a less safe route causing necrotic damage. Actually, the cytotoxic effect observed in our study is probably due to the presence of cardiotonic glycoside such as cardenolides whose presence in the plant has been reported by Van Quaquebeke *et al.* (2005). Thus, the mechanism of cells death could be apoptosis but it is necessary to clarify them by further study.

The overall results suggests that the active compounds of *C. procera* do not have much negative effect on the nervous system cells but the doses administered should be well adjusted in order to avoid adverse effects in the gastrointestinal tract. Thus patients under treatment FACA® (phytomedicinal drug taken by oral route) must respect doses prescribed to prevent the occurrence of adverse side effects.

CONCLUSION

The cytotoxicity study of the aqueous extract of root barks of *C. procera* on Caco-2 and Neuro-2a cell lines showed that the extract has a potential cytotoxic effect on human colorectal carcinoma Caco-2 cells and much less on Neuro-2a cells, originating from mouse neuroblastoma. However, further studies are required to elucidate the mechanism of cytotoxicity.

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REFERENCES

Akhir NM, Chua LS, Majid FAA, Sarmidi MR. Cytotoxicity of Aqueous and Ethanolic Extracts of *Ficus deltoidea* on Human Ovarian Carcinoma Cell Line. British Journal of Medicine & Medical Research, 2011; 1(4): 397-409.

Berridge MV, Patries MH, Tan AS. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnol Annu Rev, 2005; 11:127-152.

Borenfreund E, Puerner JA. Toxicity determined invitro by morphological alterations and neutral red absorption. Toxicol Lett, 1985; 24:119–124.

Choedon T, Mathan G, Arya S, Kumar VL, Kumar V. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World J Gastroenterol, 2006; 12 (16): 2517-2522.

Cordell GA, Kinghorn AD, Pezzuto JM 1993. Separation, structure elucidation and bioassay of cytotoxic natural products. In: Colegate SM and Molyneux RJ (Eds.): Bioactive Natural Products. Boca Raton, CRC Press, USA. 195-216.

Eisenbrand G, Pool-Zobel B, Baker V, Balls M, Blaauboer BJ, Boobis A, Carere A, Kevekordes S, Lhuguenot JC, Pieters R, Kleiner J. Methods of in vitro toxicology. Food and Chemical Toxicology, 2002; 40:193–236.

Guissou IP, Sawadogo M, Sawadogo A, Ouattara A. Etude de l'efficacité antidrepanocitaire de gelules FACA® chez les enfants en milieu hospitalier de Ouagadougou (chn-yo). Pharm. Méd. trad. afro, 1995; 29-36.

Higashimoto M, Purintrapiban J, Kataoka K, Kinouch T, Vinitketkumnuen U, Akimoto S, Matsumoto H, Ohnishi Y. Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. Mutation Research, 1993; 303 (3):135–142.

Kouadio JH, Mobio TA, Baudrimont I, Moukha S, Danoc SD, Creppy EE. Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. Toxicology, 2005; 213: 56-65.

Kurosawa M, Tani Y, Nishimura S, Numazawa S, Yoshida T. Distinct PKC isozymes regu- late bufalin-induced differentiation and apoptosis in human monocytic cells. Am J Physiol Cell Physiol, 2001; 280: 459-464.

Loveland BE, Johns TG, Mackay IR, Vaillant F, Wana ZX, Hertzog PJ. Validation of the MTT test dye assay for enumeration of cells in proliferative and antiproliferative assay. Biochem. Int., 1992; 27(3): 501–510.

Mathur R, Gupta KS, Mathur SR,Velpandian T. Anti-tumor studies with extracts of *Calotropis procera* (Ait.) R. Br. Root employing Hep2 cells and their possible mechanism of action. Indian Journal of Experimental Biology, 2009; 47: 343-348.

McConkey DJ, Lin Y, Nutt LK, Ozel HZ, New- Man RA. Cardiac glycosides stimulate Ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer Res, 2000; 60: 3807–3812.

Meunier V, Bourrié M, Berger Y, Fabre G. The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications. Cell Biol. Toxicol, 1995;11: 187-194.

Nacoulma OG. 1996. Medicinal plants and their traditional uses in Burkina Faso. PhD Thesis, Biochemistry- Microbiology Department, University of Ouagadougou.

Nikiema JB, Ouattara B, Semde R, Djierro K, Compaore M., Guissou IP, Kasilo OMJ. Promotion de la Médecine Traditionnelle du Burkina Faso: Essai de développement d'un médicament antidrépanocytaire, le FACA. African Health Monitor, 2010; 13 (Special Issue): 52-57.

Olmsted JB, Carlson K, Klebe R, Ruddle F, Rosenbaum J. Isolation of Microtubule Protein from Cultured Mouse Neuroblastoma Cells. Proceedings of the National Academy of Sciences, 1970; 65(1): 129-136.

Organisation Mondiale de la Santé, 2002. Médecine traditionnelle : Rapport du secrétariat. Conseil exécutif. Cent onzième session. *Point 5.7 de l'ordre du jour provisoire. EB111/9 du 12 décembre* 2002.

Plewa MJ, Wagner ED. Activation of promutagens by green plants. Annual Review of Genetics, 1993; 27: 93–113.

Prueksaritanont T, Gorham L, Hochman J, Tran L, Vyas K. Comparative studies of drug-metabolizing enzyme in dog, monkey, and human small intestine, and in Caco-2 cells. DrugMetab. Dispos, 1996; 24: 634-642.

Schimmer O, Kruger A, Paulini H, Haefele F. An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie, 1994; 49 (6): 448–451.

Schmiedlin-Ren P, Thummel K, Fisher J, Paine M, Lown K, Watkins P. Expression of enzymatically active CYP3A4 by Caco-2 cells

grown on extracellular matrix-coated permeable supports in the presence of 1alpha,25-dihydroxyvitamin D3. Mol. Pharmacol, 1997; 51: 741-754.

Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld GJ, Labadie, RP, Zwaving JH. Ayurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol, 1995; 47:75-84.

Van Quaquebeke E, Simon G, Andre A, Dewelle J, Yazidi ME, Bruyneel F, Tuti J, Nacoulma O, Guissou P, Decaestecker C, Braekman JC, Kiss R, Darro F. Identification of a novel cardénolides (2''-oxovoruscharin) from *Calotropis procera* and the hemisynthesis of novel derivatives displaying potent in vitro antitumor activities and high in vivo tolerance: structure acivity relationship analyses. J Med Chem, 2005; 48:849-856.

Verma R, Satsangi GP, Shrivastava JN. Ethno-medicinal profile of different plant parts of *Calotropis procera* (Ait.) R. Br. Ethnobotanical Leaflets, 2010; 14: 721-42.

Weyermann J, Lochmann D, Zimmer A. A practical note on the use of cytotoxicity assays. Int J Pharmaceut, 2005; 288: 369-376.

Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, Dang CV, Liu JO, Semenza GL. Digoxin and other cardiac glycosides inhibit HIF-1 synthesis and block tumor growth. Proc Natl Acad Sci USA, 2008; 105:19579–19586.

Zink T, Chaffin J. Herbal health products: what family physicians need to know. American Family Physician, 1998; 58 (5): 1133–1140.

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