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Revista Cubana de Farmacia, Volúmen 50, Número 4 (2016)

PRODUCTOS NATURALES

Biological activity of fractions from the marine sponge *Iotrochota birotulata* IN mammalian cell lines

Actividad biológica de fracciones de la esponja marina *Iotrochota birotulata* en células de mamíferos

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ABSTRACT

Introduction: Marine sponges are considered an important source of substances with pharmacological potential. They play a key role in the intensive investigation of promising new compounds to treat cancer and other diseases.
Objective: To evaluate in CHO-K1 and Jurkat cell lines, the cytotoxic, genotoxic and antiproliferative effects of two fractions of *I. birotulata* sponge from Colombian Caribbean.
Methods: The cell viability (cytotoxic effect) was determined by Trypan blue exclusion and MTT assays. Genotoxicity was

assessed by single cell gel electrophoresis, the antiproliferative effect was monitored with clonogenic test, sister chromatid exchange proliferative kinetic, and accumulation function. Data was analyzed with lineal regression, one-way ANOVA, and Bonferroni tests.
Results: Both cytotoxic assays showed a similar dose dependent effect for the CHO-K1 and Jurkat cell lines treated with both fractions (F_5 y F_6) of *I. birotulata.* They also revealed an effect on the cell membrane and mitochondrial activity of both cell lines. Fraction F_5 exhibited a greater genotoxic effect on both cell lines, which is consistent with the antiproliferation results obtained by the clonogenic assay. These results are also consistent with the inhibitory effect on the cell cycle, which was evaluated with SCE, proliferative kinetic, and the accumulation function tests. Consequently, the results showed differential sensitivity to the treatment of the Jurkat cells compared to the CHO-K1 cell line.
Conclusions: Together, the results show a differential effect of the two assessed fractions on cell membrane integrity, mitochondrial activity, and antiproliferative effect on both mammalian cell lines.

Key words*:* Marine sponge; Jurkat; CHO-K1; cytotoxic, genotoxic and antiproliferative effects.

RESUMEN

Introducción: Las esponjas marinas son una fuente importante de compuestos con propiedades farmacológicas potenciales. Desempeñan una función esencial en la intensa en la búsqueda de nuevas y prometedores compuestos que brinden tratamiento para el cáncer y otras enfermedades.
Objetivo: Evaluar los efectos citotóxico, genotóxico y antiproliferativo en las líneas celulares CHO-K1 y Jurkat de dos fracciones de la esponja *Iotrochota birotulata* del Caribe colombiano.
Métodos: La viabilidad celular (efecto citotóxico) se determinó mediante ensayos de azul de Tripano y MTT. La genotoxicidad se evaluó mediante la electroforesis en gel de células individuales y el efecto antiproliferativo se monitoreó con pruebas clonogénica, cinética proliferativa por intercambio de cromátides hermanas y la función de acumulación. Los datos se analizaron mediante regresión lineal, ANOVA de una vía, y la prueba de Bonferroni.
Resultados: Ambas pruebas citotóxicas mostraron un efecto similar dependiente de la dosis en las líneas celulares CHO-K1 y Jurkat tratadas con dos fracciones (F_5 y F_6) de *I. birotulata.* Las pruebas citotóxicas también revelaron efectos en la membrana celular y en la actividad mitocondrial en ambas líneas celulares. La fracción F_5 mostró mayor efecto genotóxico en las dos líneas celulares, consistente con los resultados de antiproliferación del ensayo clonogénico y con el efecto inhibitorio en el ciclo celular

evaluado por las pruebas de intercambio de cromátides hermanas, cinética proliferativa y función de acumulación. Por consiguiente, los resultados revelaron mayor sensibilidad diferencial al tratamiento en las células Jurkat comparada con la línea celular CHO-K1.
Conclusiones: Los datos obtenidos demuestran el efecto diferencial de las fracciones F_5 y $F_{6,}$ en la integridad de la membrana celular, la actividad mitocondrial y el efecto antiproliferativo en las dos líneas celulares de mamíferos evaluadas.

Palabras clave: esponja marina; Jurkat; CHO-K1; efectos citotóxicos, genotóxicos y antiproliferativos.

INTRODUCTION

In the last decades, marine natural products have been considered an alternative in the search of new drugs for the treatment of a wide range of diseases, specially cancer. It has been shown that in marine ecosystem there is a greater possibility to find bioactive substances of therapeutic interest than in land ecosystems.[1] Therefore, marine sponges are a source of new pharmacological alternatives due to the comprehensive variety of biological activities and types of structures of the compounds identified on them.[2] Despite, Colombia is surrounded by the Atlantic and the Pacific oceans, the implementation bioprospecting programs are scarce.[3] An abundant diversity of sponges has been reported in the Gulf of Urabá[4] and they have shown to have bioactive potential as antiparasitic, antibacterial, antimycotic, immunomodulator, antitumor, among others.[5,6]

Several studies have identified in Colombian sponge bioactive compounds, fractions, and extracts.[4,5,7] Fractions particularly obtained from*I. birotulata* sponge showed significant cytotoxic activity in three tumor cell lines: lung (A-549), colon (HT29), and breast (MDA-MB-231).[8] Jaspin B, a compound extracted from this sponge, induces apoptosis in melanoma cells by interfering in the ceramide metabolism.[9] The sponge extracts have also shown enzymatic inhibition.[10,11] For example, compounds with cytotoxic activity found in marine sponge is the cytosine arabinoside isolated from the *Cryptotethia cripta,* which is widely used in the treatment of acute myeloid leukemia and its analog fluoride gemcitabine, is used in cancer lung and pancreatic therapy.[6]

Some reports of colombian Caribbean sponges showed the assessed of five fractions of *Topsentia ophiraphidites*. The data revealed that only the T_4 fraction exhibited cytotoxic activity with IC_{50} of 33 µg/mL for Jurkat cells and 58 µg/mL for CHO-K1 cells. The fraction T_4 affected the cell cycle of CHO-K1 cells and caused chronic genotoxic damage in Jurkat cells (Blandon et al., 2013). On the other hand, thirteen fractions of *Amphimedom compressa, Cinachyrella kuekenthali*, *Svenzea zeai* and *Ircinia campana* evaluated by MTT and Trypan blue assays in Jurkat and CHO-K1 cell lines, showed that no single fraction has significant cytotoxic activity (Estrada et al., 2013).

MTT and hemolysis assays of three new triterpene glycosides (Ulososide F and Urabosides A and B) isolated from *Ectyoplasia ferox* evaluated in Jurkat and CHO-K1 cell lines exhibited low cytotoxic effect (Colorado et al., 2014). In addition to, the glycochemical diversity present in *E. ferox* was showed by a liquid chromatography coupled to a tandem mass spectrometry approach, which analyzed a complex polar fraction. Results allowed to identify twenty-five saponins, three of which have been previously reported, other three were found to be composed of known aglycones and twenty-one compounds had never been reported for this species. The cytotoxic activity of polar fraction was about IC_{50} 40 µg/mL on Jurkat and CHO-K1 cell lines without exhibiting hemolysis in human peripheral blood lymphocytes (Colorado et al., 2014).

Recognized natural antitumor compounds can produce an effect in specific phases. For instance, cytarabine affected on S phase, bleomycin causes effect in G2-M phase[14] and the colchicine, vinblastine, vincristine and taxol exert activity in mitotic phase of the cell cycle.[15] In this study, were evaluated the cytotoxic, genotoxic and antiproliferative effects of the fractions F_5 and F_6 of the marine sponge *I. birotulata* on two mammalian cell lines (CHO-K1 and Jurkat cells) with the aim to contribute to the bioprospecting of Colombia.

METHODS

SAMPLE COLLECTION

Samples of *I. birotulata* were collected by autonomous diving at a 15-21 meters depth on March 2010 at geographic coordinates 8°28´N; 77°14´W. Samples were kept frozen until their use. Taxonomic classification was performed by Dr. Sven Zea (Colombia), and two reference samples are in the Laboratorio de Productos Naturales Marinos of the Universidad de Antioquia (Medellín, Colombia) with voucher Nº PNM-21.

FRACTION PREPARATION

Sponge sample was cut in small pieces and dried at a temperature below 40 ºC. The dried sample was subjected to extraction first with dichloromethane and then with ethanol. Each extract was filtered and concentrated until dry at a temperature lower than 40 ºC using constant agitation and reduced pressure. The extract prepared with ethanol was subjected to fractioning by instant column chromatography. Silica gel C_{18} was used as a stationary phase and as mobile phase were used 500 mL of an eluotropic series: water, water/methanol (1:1), water/methanol (1:3), methanol, methanol/dichloromethane (3:1), methanol/dichloromethane (1:1) and dichloromethane. Each fraction was concentrated to dry at a temperature no higher than 40 ºC, using constant agitation and low pressure. Fractions were labeled of F_1 to F_7, in the same order of elution of the solvent. The F_5 and F_6 fractions were active against cultured human cancer cells of A-549 lung carcinoma, HT-29 colon adenocarcinoma and MDA-MB-231 breast carcinoma, from previous research group. Both fractions were dissolved in hexane and RPMI 1640 medium (SIGMA®) for the biological assays.

CELL LINES AND CULTURE CONDITIONS

Experiments were conducted from exponential cultures of CHO-K1 (ATCC HB-K1) and Jurkat (ATCC TIB-152) cells in T-25 containing RPMI 1640 medium (SIGMA), supplemented with 5 % fetal bovine serum (FBS, GIBCO®). Cultures were propagated twice a week and incubated at 37 ºC. Jurkat cells were grown under the same conditions with 5 % CO_2 and in humidified atmosphere > 95 %. Both cell lines were treated with fractions F_5 and F_6 of *I. birotulata* for a period of 14 hours (CHO-K1 cells), and 16 hours (Jurkat cells).

CYTOTOXICITY ASSAYS

CHO-K1 and Jurkat cells (15×10^4 cells) were cultured on 6-well plates in 3 mL of RPMI 1640 medium supplemented with 5 % of SBF per well. After 20 hours, both cell lines were treated during 20 hours additional with 2, 20 and 200 µg/mL of the fractions F_5 and F_6. Based on the preliminary results, 10, 20, 50 and 80 µg/mL concentrations of fractions F_5 and F_6 in CHO-K1 cells were assessed. On the other hand, 10, 15, 20, 25, 30, 35, 50, 80 µg/mL concentrations of fraction F_5 and 5, 20, 35, 45, 55, 60, 70, 75, 80 µg/mL concentrations of the fraction F_6 were assessed in Jurkat cells. For the counting, 20 µL of cell suspension were diluted with 20 µL of Trypan blue (0.4 %) and incubated in humified atmosphere at 5 % CO_2 for 3 minutes at 37 °C. Later,

the number of viable cells and non-viables was visualized in hemocytometer with 10X optic microscope.

Trypan Blue exclusion assay allowed to evaluate cell membrane integrity in viable cells and damaged membrane in non-viable cells by quantifying the number of cells with 10X zoom. The viability percentage[16] was obtained by:

Mitochondrial functionality of the cells was evaluated by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide). The affected cells lose their mitochondrial activity of the succinate dehydrogenase enzyme to reduce yellow tetrazolium dye into purple formazan.[17] To assess cytotoxicity with MTT, cultures of $6x10^3$ and 8×10^3 cells were cultured in 100 µL in 96-well plates for the Jurkat and CHO-K1 cell lines, respectively. After 48 hours, both lines were treated during 20 hours with the fraction F_5 (15 to 60 µg/mL) in Jurkat cells and 30 to 75 /mL in CHO-K1 cells. On the other hand, both cells lines were also treated with fraction F_6 (35 to 80 µg/mL, Jurkat cells) and (55 to 105 µg/mL, CHO-K1 cells). MTT (5 mg/mL) was added four hours before finalized the treatment and then 100µl of acid isopropanol was added to each well. The plates were agitated for three hours to dissolve the formazan crystals and read at 560nm in spectrophotometer (Multiskan, Spectrum, ThermoScientific). Each treatment was repeated three times. The viability percentage[17] was obtained by:

GENOTOXICITY TEST

Clastogenic effect was evaluated by single cell gel electrophoresis (SCGE) which detected single DNA chain damages.[18] Exponential phase cultures were used for the evaluation of the genotoxic effect of fractions F_5 and F_6 of *I. birotulata*. The protocol was as follow: cultures were centrifuged (2,400 rpm during 5 min), then, were counted and the viability with Trypan blue was determined after 6 hour treatment. Later, 1×10^5 and 1×10^6 Jurkat and CHO-K1 cells were added respectively in a final volume of 250 µL, and were treated with the IC_{50} of each of the fractions (F_5, IC_{50}= 50 µg/mL, CHO-K1; IC_{50}= 35 µg/mL, Jurkat; F_6, IC_{50}= 70 µg/mL CHO-K1; IC_{50}= 55 µg/mL, Jurkat). The corresponding controls, $H_2 O_2$ (50 µM) and hexane solvent (< 1 %) were performed. All the cultures were centrifuged (2400 rpm, 5 min), cell suspension were resuspended in PBS and low melting point agarose (LMA, 5 %). Then, 80 µL were deposited on base slides prepared with agarose

of normal melting point (NMA, 1 %). Later, the slides were incubated for cell lysis during 14 and 16 hours for Jurkat and CHO-K1 cells respectively. The denaturated DNA was carried on electrophoresis at 25V and 300 mA during 30 minutes at 4 °C, then stained with Ethidium bromide (0.02 mg/mL) and visualized fluorescence microscope (Carl Zeiss) 40X zoom. The images of hundred cells were photograghed with a Sony DSC-S85 camera, in random fields, and the final analysis was measured the comet total length in the Comet-Score® software.

ANTIPROLIFERATIVE ASSAY BY THE CLONOGENIC ASSAY

The antiproliferative effect was assessed by the clonogenic assay. Briefly, 3×10^4 cells of each cell lines in exponential phase were culture in 12-well plates with 1.5 mL of RPMI 1640 medium with 5 % FBS per well. After 48 hours, CHO-K1 cells were treated during 16 hours with 26, 36 and 46 µg/mL concentrations of F_5 and 20, 30 and 40 µg/mL concentrations of F_6, while Jurkat cells were treated during 18 hours only with the IC_{50} obtained with Trypan blue from fractions F_5 and F_6, respectively, under the conditions described. Only colonies with a number equal or higher to 50 cells were considered for counting, and results were expressed as absolute and relative cloning efficiency percentages (ACE and RCE) for each treatment.[19]

GENERATION TIME BY SISTER CHROMATID EXCHANGE (SCE)

SCE antiproliferative kinetic was used to calculate the generation time (Gt) of CHO-K1 and Jurkat cell lines after treated with F_5 and F_6 fractions of *I. birotulata* with inhibitory concentration lower than IC_{50} obtained by MTT. The CHO-K1 and Jurkat cells (5×10^4 cells/well) were cultured in 6-well plates and incubated during 36 or 48 hours for CHO-K1 and Jurkat, respectively. Twenty hours before harvest, 1 mg/mL BrdU was added and a further treatment with Colcemid (0.2 µg/mL) was applied during 4 hours (CHO-K1 cells) or 2 hours (Jurkat cells) to arrest cells in mitosis. Finally, chromosomal preparations were obtained by conventional cytogenetic technique treating cells with sodium citrate hypotonic solution (0.7 %) and centrifuged at 2,400 rpm by five minutes. Later, they were fixed twice with a methanol/acetic acid solution (3:1) and were dropped on microscope slides. The cells were stained with Giemsa (5 %) during ten minutes and then, they were visualized through stained chromatids and discriminate them between the different cell cycles. The chromosomes were analyzed on 100X objective and classified at ½, 1, 1½, 2, 2½ and ≥ 3 cycles in mitotic cells. The Gt was calculated through the average cell cycle (ACC) and the following equation:[20]

In which Cell # corresponds to the number of cells present in each cycle.

CELL CYCLE BY ACCUMULATION FUNCTION

Assessment of the cell cycle by accumulation function allowed to calculate a mitotic index (MI) and the respective curve slope was used to evaluate the increment or reduction in the Gt.[21] Experiments were conducted twice in exponential cultures treated during 20 hours with F_5 (6 µg/mL), F_6 (32 µg/mL) for Jurkat and F_5 (36 µ/mL), F_6 (30 µg/mL) for CHO-K1 cells in the described conditions. MI was obtained every 2 hours during 16 hours (equivalent to a generation time) for CHO-K1 cells and 18 hours (half a generation time) for Jurkat cells treated both cell lines with Colcemid (0.2 µg/mL). Chromosomal preparations were obtained using a conventional cytogenetic technique and two thousand cells were counted with a 40X objective, in random fields for each treatment. The MI was estimated with the following relation:

Finally, to determine the antiproliferative effect of the fractions, the Log (1 + IM) function versus the treatment time with colcemid was graphed.

STATISTICAL ANALYSIS

Data obtained from viability assays: cell proliferation, clonogenic assay, mitotic index, and single cell gel electrophoresis (SCGE) were analyzed using a lineal regression test and one-way ANOVA. The SCGE data were analyzed using the Bonferroni test with Statgraphics Centurion XV software version 15.2.05 and $p< 0.05$.

RESULTS

TRYPAN BLUE AND MTT VIABILITY ASSAYS

Assessed concentrations of F_5 and F_6 fractions of *I. birotulata* with Trypan blue were selected based on guidelines established by the USNCI which considers promissory fractions with IC_{50} below 100 µg/mL.[22] The IC_{50} obtained with Trypan blue from both cell lines allowed to evaluate by MTT ten different concentrations which differ in 5 units each. Results obtained by Trypan blue (Figure 1a) showed that the F_5 (IC_{50} of 50 µg/mL) and F_6 (IC_{50} of 70 µg/mL) fractions of *I. birotulata* decreased the viability CHO-K1 cells, while Jurkat cells treated with the same fractions showed a reduction in viability at 35 µg/mL and 55 µg/mL of IC_{50}.

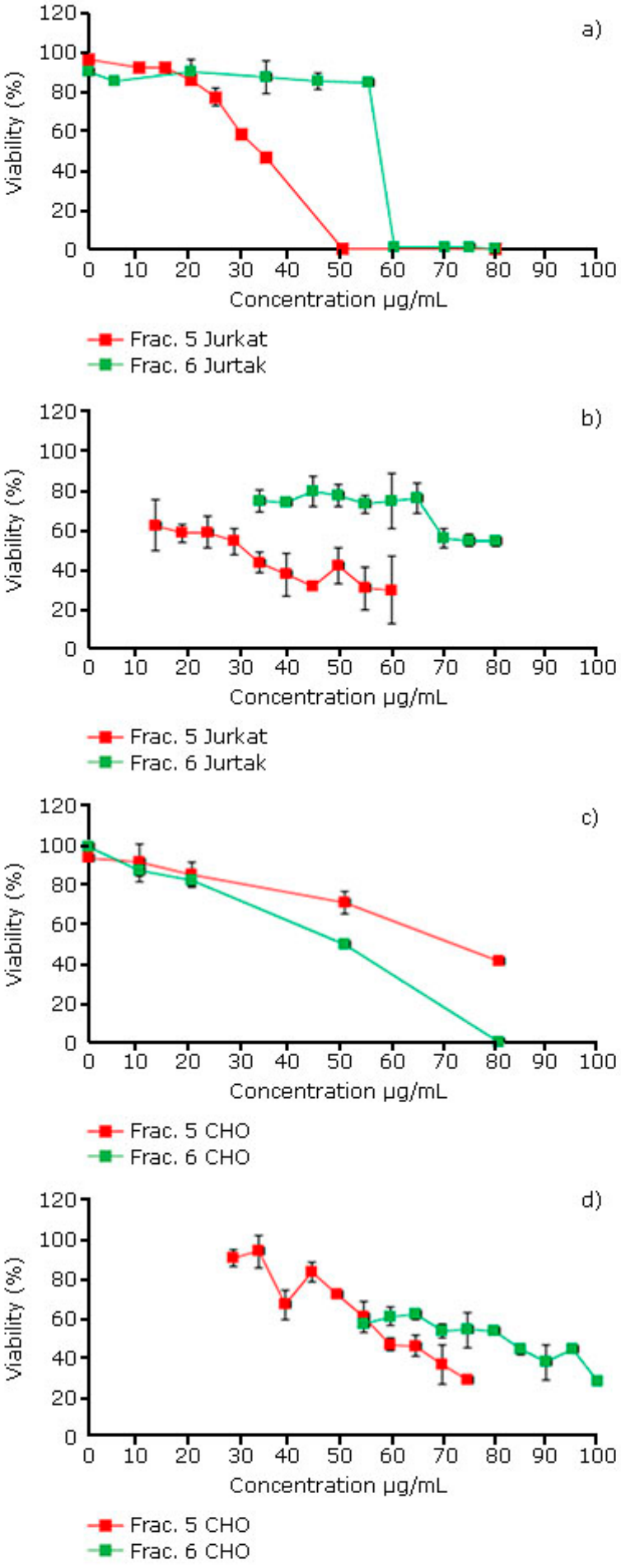

Fig. 1. Viability assays. (a) and (c) data of Trypan blue; (b) and (d) viability curves by MTT i

Fig. 1. Viability assays. (a) and (c) data of Trypan blue; (b) and (d) viability curves by MTT in CHO-K1 and Jurkat cell lines treated with F_5 and $F_{6\ fractions}$ of *I. birotulata*. Each point on the curve represent the mean ± SD of three replicate experiments. Data obtained from viability assays were analyzed using a lineal regression test and one-way ANOVA with $p<0.05$

In summary, viability curves obtained with MTT revealed concentration effect of the fractions F_5 and F_6 of *I. birotulata* on CHO-K1 and Jurkat cell lines (Figure 1b). The IC_{50} were equal to 73 µg/mL and 12 µg/mL for F_5 fraction, and 61 µg/mL and 65 µg/mL for F_6 fraction in CHO-K1 and Jurkat cells respectively. Together, the results of both fractions evaluated with the Trypan blue and MTT assay in the two cell lines, showed a similar cytotoxic effect for both assays despite that the obtained values are different.

GENOTOXICITY EFFECT

The SCGE results of CHO-K1 and Jurkat cells showed that F_5 and F_6 produced an acute genotoxic effect in the IC_{50} concentrations found with Trypan blue in both cell lines with respect to the control. Besides, F_5 fraction was more genotoxic than F_6 fraction (Fig. 2).

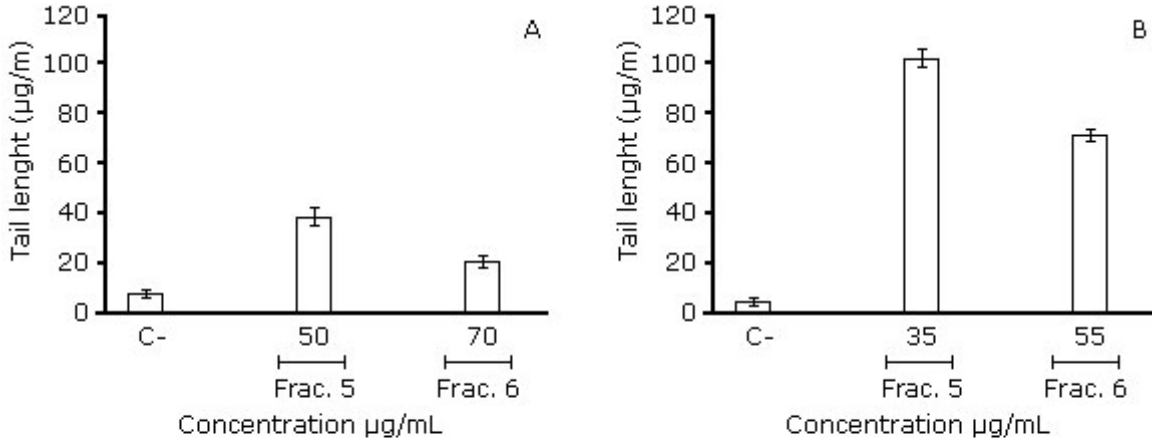

Fig. 2. Genotoxic effect of F_5 (50 y 35 µg/mL) and F_6 (70 and 55 µg/mL) fractions of I. birotulata on CHO-K1 (a) and Jurkat (b) cell lines by comet assay. C- (control PBS). Bars represent the mean ± SE of 50 nucleus. Analysis was performed using one-way ANOVA with $p<0.05$ and Bonferroni test to compare treatments with the corresponding control.

ANTIPROLIFERATIVE EFFECT BY CLONOGENIC ASSAY

Figure 3 shows the data of the RCE test obtained in CHO-K1 cells treated during 16 hours and Jurkat cells treated during 18 hours with the different concentrations of F_5 and F_6 fractions of *I. birotulata*. The results showed a great dose-dependent reduction in the RCE (88.3 %, 62.4 %, and 33.7 %) of CHO-K1 cells treated with concentrations of 26, 36 and 46 µg/mL of F_5

fraction, while the F_6 fraction showed a RCE (84.9 %, 82.3 %, and 78.4 %) at concentrations of 20, 30 and 40 µg/mL, compared with the corresponding control. On the other hand, the Jurkat cells treated with F_5 (35 µg/mL) fraction, showed a total inhibition (0 %) in the RCE, while an evident reduction of the RCE (8.4 %) was observed with F_6 (55 µg/mL) fraction (Fig. 3). Together, these data revealed a differential effect between cell lines, being the Jurkat cells more susceptible.

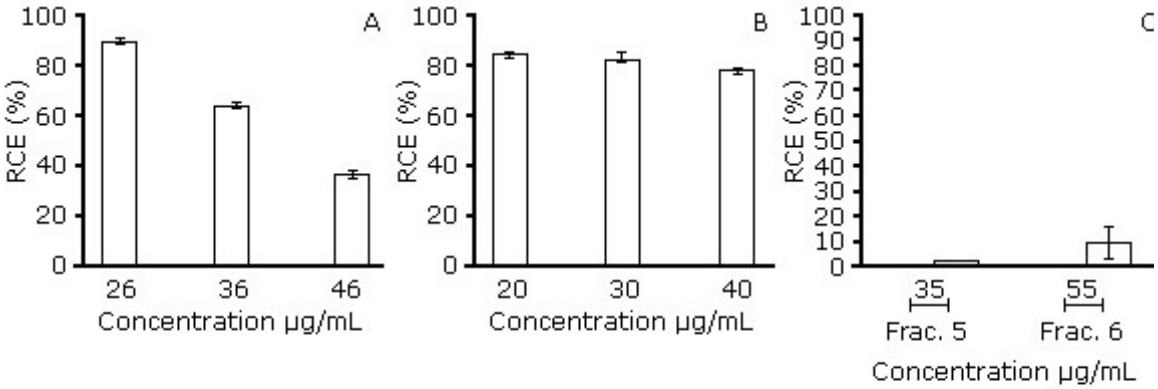

Figure 3. Relative Clonation Efficiency (RCE) of the cell line CHO-K1 treated with the fraction (a) F_5 (26, 36 and 4635µg/mL) and (b) F_6 (20, 30 y 40 35µg/mL).Chart (c) RCE of Jurkat ce line treated with F_5 (35µg/mL) and F_6 (55µg/mL) . Bars represent the mean ± SD of thre replicate experiments.

GENERATION TIME (GT) BY SISTER CHROMATID EXCHANGE (SCE)

Table 1 shows the distribution of three cell cycles of CHO-K1 and Jurkat cells treated with F_5 and F_6 fractions of *I. birotulata*. The Gt of CHO-K1 cells treated with F_5 (36 µg/mL) was not calculated due to the absence of mitotic cells, which, did were not visualized probably by the cell cycle arrest, which prevented the arrival of cells to mitosis. In contrast, CHO-K1 cells treated with F_6 (40 µg/mL) fraction showed a low significant reduction in the Gt compared with the corresponding control. In other hand, Jurkat cells treated with F_5 (6 µg/mL) fraction showed a Gt equivalent to one and a half of the cell cycle with respect to the control, while Jurkat cells treated with F_6 (32 µg/mL) fraction showed an increase of only half of the cell cycle with respect to the untreated control.

CYCLE CELL EFFECT BY ACCUMULATION FUNCTION CURVE

Figure 4 shows the proliferation curves of CHO-K1 and Jurkat cells treated with the half of concentration of the IC_{50} obtained of F_5 and F_6 fractions of *I. birotulata* by MTT test. The data showed a significant difference in the slopes for CHO-K1 and Jurkat cells treated with the two fractions, compared with the control. The fractions F_5 and F_6 caused a reduction of the slope

in the cell cycle time compared to the control in both cell lines, which is more evident in Jurkat cells.

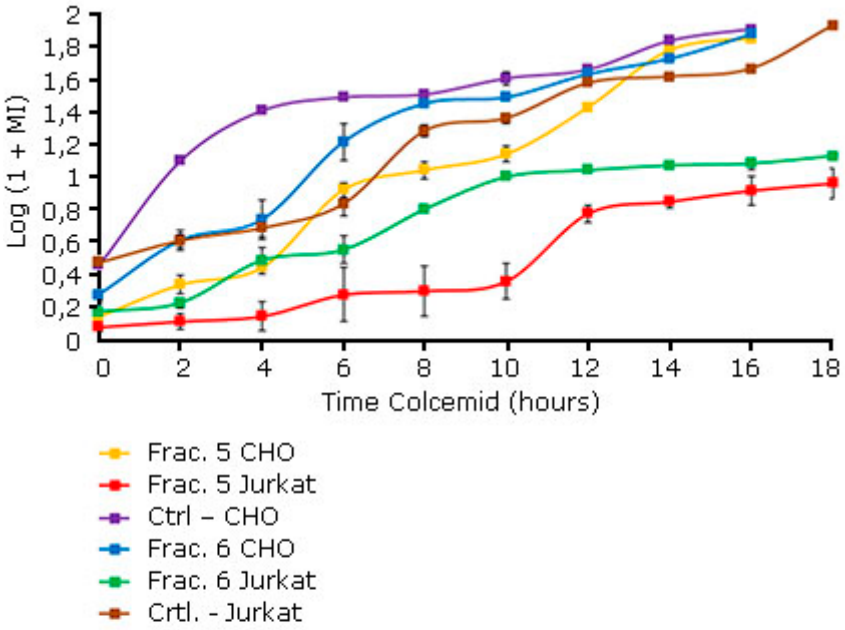

Fig. 4. Accumulation function of CHO-K1 and Jurkat cell lines treated with the fractions F_5 (36 and 6µg/ mL) and F_6 (30 and 32 µg/mL) respectively. The graphic represents the function Log (1+MI) versus the Colcemid exposure time. The MI is the estimated percentage between mitotic cells and total cells. Each point on the curve represent the mean± SD of two replicate experiments and it is Log (1+MI). The data were analyzed using a lineal regression test and one-way ANOVA with $p<0.05$.

DISCUSSION

These results reflect a differential effect between evaluated fractions and the cell lines. For example, F_5 showed an increase in the antiproliferative effect on both cell lines and the highest inhibitory effect in Jurkat cells, while F_6 showed significant antiproliferative effect in Jurkat cells but not in CHO-K1 cells. These differential effects were compared to the respective controls and the effect caused in Jurkat cells is similar to results obtained with other tumor cell lines derivated of lung A549 (1 µg/mL; F_5: IC_{50}= 15 µg/mL), colon HT29 (5 µg/mL; F_5: IC_{50}< 1 µg/mL) and breast MDA-MB-231 (F_5: IC_{50}= 19 µg/mL) cell lines treated with this same fractions.[8]

On the other hand, the results of 0 % RCE in Jurkat cells treated with 35 µg/mL of the F_5 fraction, and the value of 8.4 % of RCE in cells treated with 55 µg/mL of the F_6 fraction, corroborate the differential clastogenic and cytotoxic effects of the two fractions in both cell lines. Additionally, the proliferative kinetic analysis obtained by SCE did not showed a delay in the cell cycle of CHO-K1 cells, but showed a delay of the cell cycle of Jurkat cell,

equivalent to half the cell cycle, compared to the corresponding controls.

Summarizing, F_5 and F_6 fractions *I. birotulata* showed a differential effect on CHO-K1 and Jurkat cell lines which could be explained by the genetic origin of both cell lines. CHO-K1 cells possess a p53 tumor suppressor gene (normal phenotype). This gene is highly conserved among mammals and acts as a negative regulator of cell proliferation. The inactivation of p53 gene has an important role in cell tumor origin and progression, while Jurkat cells derived from tumor leukemic T cells, exhibit different mutations in this gene.[22] The genetic difference in the genes p53 could explain the low cytotoxic and genotoxic damage, and the minimal delay in the cell cycle in CHO-K1 cell line, reflected in the clonogenic assay and accumulation function tests.

Furthermore, the absence of mitotic cells in the cell cultures treated with F_5, suggested, an apoptotic process which could be evaluated in future studies. On the other hand, in Jurkat cells the P53 gene is mutated, causing little or no DNA damage repair, therefore, the cells were able to move to the next phase of the cell cycle despite the delay of the half of the cell cycle described above. This results are consistent with the observed clastogenic and cytotoxic effects.

Finally, the genotoxic, cytotoxic, and antiproliferative assays showed a differential effect of F_5 and F_6 fractions of *I. birotulata* on Jurkat cells, compared to the CHO-K1 cells. However, F_5 fraction showed more dose-dependent activity compared to the F_6 fraction. To elucidate others effects of these fractions in other human cell lines, it can be used others approaches such as flow cytometry and to explore in different systems other biological potential such as antimicrobial, antimycotic and antiparasitic activities.

AKNOWLEDGMENTS

We thank to Estrategia de Sostenibilidad 2014-2015 of the Universidad de Antioquia and Colciencias for the financial support (11150520268). This work fulfills what is stated in the agreement access N° 28 between the Ministerio de Ambiente, Vivienda y Desarrollo Territorial of Colombia and the professor Alejandro Martínez from Universidad de Antioquia. We also thank to Juan M. Díaz, Jaime Garzón-Ferreira, Guillermo Díaz, and Juan A. Sánchez for the sample collection.

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Recibido: 13 de marzo de 2015.
Aprobado: 31 de marzo de 2016.

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