ORIGINAL ARTICLE

In vitro CYTOTOXIC AND GENOTOXIC EFFECT OF THE CRUDE AND ETHANOLIC EXTRACT FROM THE RHIZOME OF *Curcuma longa* L.

Martha F. Cosquillo-Rafael [1,a], Maritza D. Placencia-Medina [2,b], Tomás Y. Miranda-Tomasevich [3,c], Miriam Moreno-Hinojosa [3,d], Mónica G. Retuerto-Figueroa [1,e]

[1] Grupo de Investigación «Farmacognosia y Medicina Tradicional», Universidad Nacional Mayor de San Marcos, Lima, Perú.
[2] Centro de Investigaciones Tecnológicas, Biomédicas y Medioambientales, Universidad Nacional Mayor de San Marcos, Lima, Perú.
[3] Centro de Investigación en Biología Molecular y Bioinformática, Universidad Nacional de San Cristóbal de Huamanga, Ayacucho, Perú.
[a] Bachelor in Pharmacy and Biochemistry; [b] Doctor of Pharmacy and Biochemistry; [c] Bachelor in Biology; [d] Master of Public Health; [e] Master in Environment and Sustainable Development.

The article is part of the thesis of Cosquillo-Rafael M.: "Efecto antioxidante, antitumoral y genotóxico del extracto crudo y etanólico del rizoma de *Curcuma longa* L. "palillo" [Master's Thesis]. Lima: Facultad de Medicina, Universidad Nacional Mayor de San Marcos; 2019.

ABSTRACT

Objectives: To determine the *in vitro* cytotoxic and genotoxic effect of the crude and ethanolic extract from the *Curcuma longa* L. rhizome. **Materials and methods:** The cytotoxic effect was evaluated using DU-145, HT-29, 3T3 BALB/c cell lines. The growth percentages in 48 hours; and the half maximal inhibitory concentration (IC_{50}) were determined. The genotoxic effect on human genomic DNA was determined using the Tomasevich method. **Results:** Crude extract produced an IC_{50} of 12.98 ± 0.21 µg/mL for the HT-29 tumor cell line, which is lower than the value obtained for DU-145, with an IC_{50} of 36.77 ± 9.12 µg/mL. The ethanolic extract presented an IC50 of 13.24 ± 0.77 and 20.54 ± 2.58 µg/mL for both cell lines, respectively; the curcumin standard compound presented an IC_{50} of 3.96 ± 0.60 and 13.94 ± 2.79 µg/mL, respectively. Crude extract concentrations of 50 and 100 mg/mL fragmented between 40% to 95% of human genomic DNA; while at 200 mg/mL, fragmentation was greater than 95%. The ethanolic extract at all concentrations did not fragment the DNA. Curcumin at 200 mg/mL fragmented less than 5% of human genomic DNA. **Conclusions:** The crude and ethanolic extracts of *Curcuma longa* L. demonstrate different *in vitro* cytotoxic effects for the human tumor cell lines DU-145 and HT-29; similar to the standard curcumin compound. The crude extract of *Curcuma longa* L. shows a potent genotoxic in vitro activity against human genomic DNA; this type of effect is not produced by the ethanolic extract.

Keywords: Genotoxic; Cytotoxins Agents; Curcumine, Genomic DNA; Cell Line; HT29 Cells; BALB 3T3 Cells; Gel Electrophoresis (Source: MeSH NLM).

Cite as: Cosquillo-Rafael MF, Placencia-Medina MD, Miranda-Tomasevich TY, Moreno-Hinojosa M, Retuerto-Figueroa MG. *In vitro* cytotoxic and genotoxic effect of the crude and ethanolic extract from the rhizome of *Curcuma longa* L. Rev Peru Med Exp Salud Publica. 2020;37(3)454-61. doi: https://doi.org/10.17843/rpmesp.2020.373.4817.

Correspondence to: Martha Francisca Cosquillo Rafael; Av. La Cantuta 294, Zárate, San Juan de Lurigancho, Lima, Perú; martha.cosquillo@unmsm.edu.pe

Received: 19/09/2019
Approved: 27/05/2020
Online: 25/08/2020

INTRODUCTION

Cancer is a public health problem in Peru. During 2017, 10,650 cancer cases were registered; of which 7,537 (70.8%) were new as per the consolidated figures from 47 national health establishments [1]. Cancer incidence is 20% higher in men than in women, while the cancer mortality rate is 40% higher [2]. Colon cancer therapy in men and women, as well as prostate cancer, is considered to have a high economic impact.

In the human body, chronic inflammation can affect homeostasis and metabolism of normal cells, causing susceptibility to genomic instability, which can lead to uncontrolled cell growth and tumorigenesis [3,4]. During this inflammation process, a variety of cytotoxic mediators are produced, such as free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS), which play an important role in the damage to deoxyribonucleic acid (DNA) [5].

https://doi.org/10.17843/rpmesp.2020.373.4817

Several studies established the preventive effect of consuming fruits, vegetables, spices, and aromatic herbs. These products have bioactive phytochemicals that have anticarcinogenic, antimutagenic [6,7], and antioxidant properties; which prevent, neutralize, or repair in a direct or indirect way the cellular damage caused by free radicals, such as the oxidation to lipids, proteins, and nucleic acids [8].

The turmeric, *Curcuma longa* L., is a spice used in traditional Chinese medicine to treat inflammatory conditions [9]. According to the Indian Ayurvedic pharmacopoeia and the Chinese pharmacopoeia, it is used as a tonic, a stomach carminative, and may relieve pain, eliminate blood stasis, and stimulate menstrual flow. The main polyphenolic phytochemical constituents of *Curcuma longa* L. include three curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), whose main component, polyphenol curcumin, has great antioxidant capacity [10]. The crude (CE) and ethanolic (EE) 96% extracts of *Curcuma longa* L. rhizome have *in vitro* antioxidant capacity determined by DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2-Azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) methods (11,12). Also, polyphenol curcumin has anti-inflammatory and anti-cancer properties, modulating the epigenetic alterations typically associated with cancer [13].

Genotoxicity is the ability to trigger damage in a part or in the whole genetic material of a cell, ultimately on the DNA molecule [14,15]. There are experimental investigations on genotoxic potential about curcumin isolated from *Curcuma longa* L. that do not report cytotoxic or genotoxic potential [16], and others where the supplementation of this isolated principle significantly antagonizes genotoxic effects [17].

Therefore, the aim of this research was to determine the cytotoxic and genotoxic *in vitro* effect of the crude and ethanolic extract of *Curcuma longa* L.'s rhizome.

KEY MESSAGES

Motivation for the study: It is necessary to look for alternative cancer treatments. Some people use the crude extract of *Curcuma longa* L. as an alternative treatment for colon and prostate cancer.

Main findings: The crude and ethanolic extract of *Curcuma longa* L. has differentiated cytotoxic activity for prostate and colon cancer cell lines. The crude extract and curcumin were found to be genotoxic, and the ethanolic extract, non-genotoxic.

Implications: The use of the ethanolic extract of *Curcuma longa* L. could be an alternative for developing an affordable phytomedicine for the treatment of colon and prostate cancers.

MATERIALS AND METHODS

This is a quantitative, analytical study with an experimental design. The experimental units were cells belonging to the human cell lines DU-145 (prostate carcinoma), HT-29 (colon adenocarcinoma), and 3T3 (normal mouse fibroblasts) provided by the Research and Development Laboratory of the Universidad Peruana Cayetano Heredia (LID-UPCH). The human genomic DNA was provided by the Research Center for Molecular Biology and Bioinformatics of the Universidad Nacional de San Cristobal de Huamanga. The samples of the complete specimen and rhizomes of *Curcuma longa* L. were collected in the province of Chanchamayo, Junin, Peru. The taxonomic identification was carried out by a taxonomist/curator from the Universidad de la Amazonía.

Obtention of raw extract

The fresh, clean, peeled and weighed rhizome was processed in a Philips brand extractor, and the CE was obtained. It was evaporated to dryness in a stove with circulating air at 40 °C. The dry extract obtained was stored in a suitable container, a labeled amber bottle, to protect it from light and humidity.

Obtention of ethanolic extract

The rhizome was peeled and dried in an oven with circulating air (Memmert) at 40 °C. Afterwards, it was milled in a Willey Mill grinder with Arthur H. Thomas CO blades, and a dry and homogeneous powder was obtained. It was then weighed and mixed with 96% ethanol in a (2:1) ratio of solvent: dry rhizome powder, in an amber flask; it was then macerated for 28 days at room temperature with rotary movements for 15 minutes each day; it was filtered with gauze layers and the filtered solution was evaporated to dryness in an oven at 40 °C.

Evaluation of cytotoxic activity

The experiment was developed in the Cell Biology and Virology Laboratory of the LID-UPCH. The DU-145 cell line was cultured and maintained in Minimum Essential Medium (MEM) culture medium supplemented with 10% fetal bovine serum and 50 µg/mL gentamicin; the HT-29 cell line, in Roswell Park Memorial Institute (RPMI)-1640 culture

medium supplemented with 7.5% fetal bovine serum and 50 µg/mL gentamicin; and the 3T3 BALB/c cell line, in Dulbecco's Modified Eagle medium (DMEM) culture medium supplemented with 10% fetal bovine serum and 50 µg/mL gentamicin.

To activate each cell line, the cell monolayer was washed twice with 5 mL Ca- and Mg-free Hanks solution, then 1 mL of the trypsin-ethylenediaminetetraacetic acid (EDTA) solution was added and removed after 10 seconds. It was then incubated for 10 minutes at 37 °C, after which the activated cells were suspended with 3 mL of the corresponding culture medium.

The cells were inoculated into 96-well cell culture plates and incubated at 37 °C in a humid atmosphere of 5% CO_2 and 95% air for 24 hours to fix the cells in the plate wells. Each plate containing each of the cell lines was fixed *in situ* with trichloroacetic acid (TCA) to obtain the cell values at zero-time before adding the extracts.

Then, each well received 40 µL of each serial dilution at increasing concentrations from 3.9 µg/mL to 62.5 µg/mL of the CE and EE from *Curcuma longa* L. and curcumin, and from 0.03 µg/mL to 1.95 µg/mL of 5 fluorouracil (5-FU); the zero plate wells received 40 µL from MEM. It was homogenized with Heidolph plate agitator for 30 seconds and incubated for an additional 48 hours under the same conditions. Then, 100 µl of 20% TCA were added to stop the assay and it was refrigerated at 4 °C for 1 hour. The TCA was removed, and each plate was washed 5 times with 500 mL of water, drained and dried. The anti-tumor drug 5-FU was used as a positive control.

Sulforhodamine B assay

Determination of cell growth inhibition was performed by the sulforhodamine B (SRB) cytotoxicity assay described by Skehan *et al.* [18], which allows to indirectly estimate the number of viable cells, since the SRB dye can stain the total cell protein.

Then, 50 µl of the 0.2% SRB solution in 1% acetic acid were added to the cells fixed with TCA in each well and left to stand at room temperature for 30 minutes. The excess of non-protein bound SRB dye was removed by rapid washing for 5 times with 1% acetic acid, then the culture plates were allowed to dry. The SRB dye bound to proteins was eluted with a 10 mM solution of Tris base (Tris hydroxymethyl aminomethane) at a 10.5 pH. Finally, the optical density was determined, which is directly proportional to the number of cells, using an Ibo-Rad model 450 to 510 nm wavelength microplates reader. The anti-tumor drug 5-FU was used as a positive control. The inhibitory concentration 50 (IC_{50}) is the extract concentration that inhibits 50% of the cell growth. All the assays were carried out in triplicate.

Genotoxic activity evaluation using the «Tomasevich method»

The "Tomasevich method" [19] is a modification of the "comet assay" [20,21] and is used to evaluate the in vitro genotoxic effect of medicinal plants and their extracts or phytotherapeutic products against genomic DNA. After an incubation period, the fragmentation degree of the DNA strands is measured by subjecting it to agarose gel electrophoresis and staining it with ethidium bromide in order to visualize it in an ultra-violet (UV) light transilluminator and to record images with a digital camera [19].

For this purpose, we had a stock of human genomic DNA at a concentration of 1,500 ng/µL in a final volume of 200 µL for each test, then we proceeded to prepare the solutions of *Curcuma longa* L. rhizome extract at concentrations of 5, 10, 25, 50, 100 and 200 mg/mL, respectively, using sterile bi-distilled water as solvent. A battery of nine 500 µL tubes was labeled with numbers (1 to 9) and the assay components were discharged as indicated in Table 1, immediately incubated at 37 °C for one hour to enable the action of the extract on the genomic DNA. It should be noted that the preparation of the extract at different concentrations was independent for each assay, i.e. with CE, EE, and curcumin [19,22-24].

After the incubation period, contents from each of the tubes were seeded into the respective wells of 1% agarose gel immersed in Triz-EDTA (TE) run buffer with a 1X concentration, installed in the Biometra® brand electrophoresis chamber. The 10 µL loading volume is the result of 8 µL of the sample plus 2 µL of run dye (bromophenol blue plus xylene). In the first well, the molecular size marker of 100 base pairs (bp) was seeded; in the following six wells, the treated products were seeded with the extracts at their respective concentrations; the next well contained the extract at the concentration of 100 mg/mL as a target; the next well only contained DNA at the concentration of 1,500 ng/µL as a control; and the last well was seeded with the treatment product with extract of 100 mg/mL plus the enzyme proteinase K. The electrophoretic run was performed at 30 volts for 180 minutes [19,22-24].

Once the electrophoretic run was finished, the agarose gel was removed, then immersed for 15 minutes in 1% ethidium bromide contained in an exclusive tray, later it was rinsed twice with running water, it was installed in an Ultra Lum brand UV transilluminator and the photographs were taken with a 12.1-megapixel full HD Canon 20X brand digital camera to interpret the results. Each test was repeated four times with each of the different extracts of *Curcuma longa* L. and curcumin [19,22-24].

https://doi.org/10.17843/rpmesp.2020.373.4817

Table 1. Preparation of compounds for in vitro genotoxicity testing of crude and ethanolic extract of *Curcuma longa* L. rhizome and curcumin, respectively, at concentrations of 5, 10, 25, 50, 100 and 200 mg/mL against human genomic DNA.

Conditions	Compounds for in vitro genotoxicity testing								
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7 Target	Tube 8 Control	Tube 9 PK
ADN stock (1500 ng/µL) (µL)	14	14	14	14	14	14	NI	14	14
CE, EE or curcumin (mg/ml)	5	10	25	50	100	200	100	NI	100
EC, EE or curcumin (mg/ml)	6	6	6	6	6	6	20	NI	3
Proteinase K (µL)	NI	NI	NI	NI	NI	NI	NI	NI	3
Sterile bi-distilled water (µL)	NI	NI	NI	NI	NI	NI	NI	6	3
Total volume (µL)	20	20	20	20	20	20	20	20	23
Incubation in bain-marie at 37 °C	1 hour								

PK: proteinase K; Target: extract or curcumin, respectively, used as a target; Control: only human genomic DNA, used as a control; CE: crude extract; EE: ethanolic extract; NI: not included.

Data analysis

To analyze the *in vitro* cytotoxic activity, the data were grouped and presented Excel tables; and the statistical analysis, in SPSS 21. The IC_{50} was determined through the analysis of linear regression with a 95% confidence interval. Results are expressed as the mean and standard deviation of the IC_{50} values, obtained in triplicate. The ANOVA test was carried out according to each sample type, comparing the respective averages in the cell line groups and considering a 95% confidence interval.

To study the genotoxic effect, numerical values were assigned to the different degrees of DNA fragmentation, considering the color intensity of the corresponding lane band, shown in the photographic records. To evaluate the genotoxicity due to the degree of DNA fragmentation, the non-parametric Kruskal Wallis test was used, because the data do not show normal distribution. This test allows us to determine if at least one treatment differs significantly in its effect from the rest of the treatments.

RESULTS

The growth percentages of the DU-145 cell line varied from 56.7% to 93.6% for CE and from 11% to 104.9% for EE; for the HT-29 cell line, the variation was from 12.1% to 96.2% for CE and from –0.2% to 79% for EE; and for the 3Q3 control cell line, the range was from 34.8% to 100% for CE and from –1.1% to 92.2% for EE. For the 5-FU positive control, the lowest percentage of growth (18.5%) was observed in the 3Q3 cell line, while the highest percentage of growth (114.4%) was seen in the DU-145 cell line, shown in Table 2.

Table 3 shows the IC_{50} values for the CE and EE of *Curcuma longa* L., as well as the 5-FU control in the studied tumor cell lines. The behavior of the extracts showed differentiated cytotoxic capacity in all the tumor cell lines.

The EE of *Curcuma longa* L. showed a lower percentage of cell growth in the DU-145, HT-29 and 3T3 cell lines (Figure 1A, 1B and 1C) compared to the CE.

The photographic records in Figure 2 reveal the results of the *in vitro* genotoxicity of CE and EE of the rhizome of *Curcuma longa* L. and curcumin against human genomic DNA. CE at a concentration of 25 mg/mL fragmented between 5% and 20% of the DNA; at 50 mg/mL and 100 mg/mL the fragmentation was 40% to 95% of the DNA, while at a concentration of 200 mg/mL the fragmentation was greater than 95%. EE did not fragment DNA at any of the tested concentrations from 5 mg/mL to 200 mg/mL, which shows no genotoxic effect. Meanwhile, at a concentration of 200 mg/mL, curcumin fragmented 5% to 20% of human genomic DNA.

At a concentration of 100 mg/mL, plus the enzyme proteinase K, CE fragmented 40% to 95% of human genomic DNA; at 100 mg/mL, plus proteinase K, EE did not fragment DNA; likewise, at 100 mg/mL, plus the enzyme proteinase K, curcumin did not fragment human genomic DNA.

DISCUSSION

The results found show that the crude and ethanolic extract of *Curcuma longa* L. rhizome have cytotoxic effect and potential anti-tumor activity against HT-29 and DU-145 cells, by revealing a decrease in cell survival when the concentrations of *Curcuma longa* L. and curcumin extracts are increased. We used three concentrations closest to the IC_{50} to build a line [25].

Table 2. Inhibitory concentration 50 of crude and ethanolic extract of *Curcuma longa* L. rhizome, curcumin, and 5 fluorouracil with cytotoxic effect according to tumor cell lines DU-145 and HT-29 and 3T3.

Cytotoxic substance	Inhibitory concentration 50 with cytotoxic effect (µg/mL)			p value [b]
	Human Tumor Cell DU-145 [a]	Human Tumor Cell HT-29 a	Normal mouse cell 3T3 [a]	
Crude extract of *Curcuma longa* L.				0.004
Ethanolic extract of *Curcuma longa* L.	20.54 ± 2.58	13.24 ± 0.76	14.39 ± 1.10	0.000
Curcumin	13.94 ± 2.78	3.96 ± 0.60	7.65 ± 0.68	0.001
5- fluorouracil	1.72	1.27	0.59	Np

[a] Mean and standard deviation; [b] Anova test.

NP: not performed.

The cytotoxic activity of the extracts and curcumin is different between the treated cells. However, the best activity was exhibited by the ethanolic extract, which presented a lower IC_{50}. The CE and EE with curcumin inhibited the growth of HT-29 cells more effectively than of the DU-145 cell line.

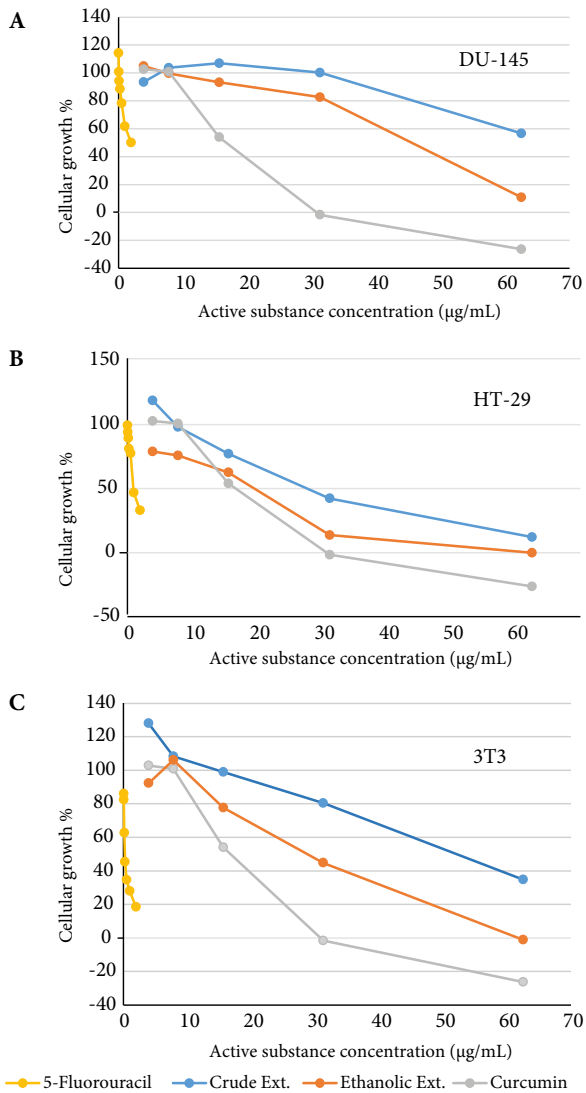


Figure 1. Growth percentage curves of cell lines (A) DU-145, (B) HT-29 and (C) 3T3 at different concentrations of Curcuma longa L., curcumin and 5 fluorouracil extracts.

Using human murine colon 26 cells, human HT-29 and HCT 116 from colorectal cancer (CRC), Kuete et al. (26) demonstrated the *in vitro* cytotoxic activity of CE (18.8% g/g) in all tests, particularly by finding an IC_{50} of 15 µg/mL for CRC after been incubated between 48 and 72 hours; while the IC50 for curcumin was 5 µg/mL. These results are similar to those from our study and are encouraging because of their cytotoxic effect against HT-29.

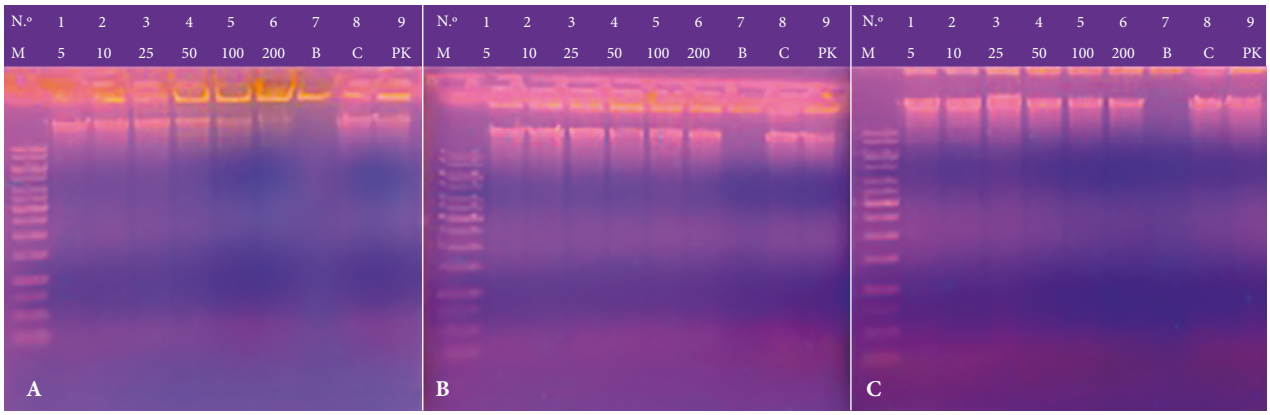
Yue *et al.* [27] conducted an *in vitro* study on the cytotoxic activity of the isolated components α/β-turmerone, ar-turmerone, curcumin and the ethanolic extract of *Curcuma longa* L, which inhibited the growth of colon cancer cells at a time-dependent dose. The ethanolic extract had the highest lC_{50} at 11.67 µg/mL in the HT-29 cell line, a similar result to ours. These findings would provide scientific evidence on the use of turmeric as an adjuvant therapy for colorectal cancer.

Cao *et al.* [28] have reported that curcumin inhibits the growth of HT-29 cells (colon carcinoma) with a IC_{50} of 40.7 ± 0.5 mM in *in vitro* culture for 24 hours. This cytotoxic activity seems to be mediated by the induction of apoptosis in these cells; our results corroborate this antiproliferative activity that shows therapeutic potential.

Hong *et al.* [29] state that curcumin inhibits the cellular proliferation of the DU-145 cell line (prostate cancer) depending on the dose, starting with 10 g up to 30 g where a IC_{50} is obtained, without showing any major effect after 50 g. Our results behaved in a similar way, indicating an antiproliferative and antimetastatic effect in the prostatic tissue.

Calaf *et al.* [30] point out the ability of curcumin to induce apoptosis in tumor cells through various methods, such as assays for caspase-3/7 activity, adnexin V, poly (ADPribosa) polymerase-1 activation and caspase-3 protein expression, nuclear factor (NF) - transcription factor κ B and prolife-

https://doi.org/10.17843/rpmesp.2020.373.4817



M: 100 bp molecular size marker; No. 1: with 5 mg/mL; No. 2: with 10 mg/mL; No. 3: with 25 mg/mL; No. 4: with 50 mg/mL; No. 5: with 100 mg/mL; No. 6: with 200 mg/mL; No. 7: with 100 mg/mL extract (white); No. 8: with 100% genomic DNA (control); No. 9: with 100 mg/mL extract + proteinase K.

Figure 2. Photographic record of the *in vitro* genotoxic assay of the crude (A), ethanolic (B) extract of *Curcuma longa* L. rhizome and curcumin (C) at concentrations of 5, 10, 25, 50, 100 and 200 mg/mL against human genomic DNA at 1,500 ng/µL, incubated at 37 °C for one hour. In Figure A, No. 4 and No. 5 correspond to concentrations of 50 and 100 mg/mL, respectively, and show DNA fragmentation between 40% and 95%, while No. 6, which corresponds to 200 mg/mL, shows DNA fragmentation > 95%, all compared to No. 8, which is the DNA band that received no treatment and serves as a control. In Figure C, No. 6, which corresponds to 200 mg/mL shows DNA fragmentation < 5% compared to No. 8

rating cellular nuclear antigen; or to enhance the induction of apoptosis by classical chemotherapeutic drugs, such as placlitaxel in cell lines MCF7 and MDAMB231, supporting their potential use as anti-cancer therapies. These quantitative and explanatory results of the molecular mechanisms could be inferred from the results of our research.

Marca *et al.* [23], who used the same method as in this study, evaluated the *in vitro* genotoxicity of ethanolic extract and *Allium sativum* L. (garlic) at concentrations of 5, 10, 50, 100, 200, 300, 400 and 500 mg/mL, found no genotoxic effect against genomic DNA of S*taphylococcus sp*, while with the garlic bulb juice, at concentrations of 5, 10, 50 and 100%, did show a powerful genotoxic effect, fragmenting 100% of the genomic DNA of *Staphylococcus sp.* They concluded that the juice of the bulb of Allium sativum L. presents a powerful genotoxic activity against the genomic DNA of *Staphylococcus* sp. In this sense, the turmeric has also presented genotoxic activity only with the CE, starting at a concentration of 25 mg/mL, but it did not present genotoxic activity with the EE, perhaps because the secondary metabolites present in the CE responsible for the genotoxic activity are not found in the EE, for not having been carried by the solvent or because they could minimally change its chemical structure and lose its function. Meanwhile, curcumin, a chemically pure compound, the main secondary metabolite of turmeric, did show moderate genotoxic activity, but at a high concentration of 200 mg/mL.

Studies conducted on medicinal plants from other families report that the genotoxic effect is attributed to the presence of secondary metabolites, mainly phenolic com-

Table 3. Numerical values of *in vitro* genotoxicity tests of crude and ethanolic extract of *Curcuma longa* L. rhizome and curcumin at concentrations of 5, 10, 25, 50, 100 and 200 mg/mL, against human genomic DNA.

Crude extract of the rhizome from *Curcuma longa* L. (mg/mL)						**Ethanolic extract of rhizome from *Curcuma longa L.* (mg/mL)**						**Curcumin (mg/mL)**					
5	**10**	**25**	**50**	**100**	**200**	**5**	**10**	**25**	**50**	**100**	**200**	**5**	**10**	**25**	**50**	**100**	**200**
0	0	1	3	3	4	0	0	0	0	0	0	0	0	0	0	0	1
0	0	1	3	3	4	0	0	0	0	0	0	0	0	0	0	0	1
0	0	1	3	3	4	0	0	0	0	0	0	0	0	0	0	0	1
0	0	1	3	3	4	0	0	0	0	0	0	0	0	0	0	0	1

0: DNA Fragmentation < 5%; 1: DNA fragmentation 5 to 20%; 2: DNA fragmentation 20 to 40%; 3: DNA fragmentation 40 to 95%; 4: DNA fragmentation > 95%.

Source: Colins *et al.* [21]

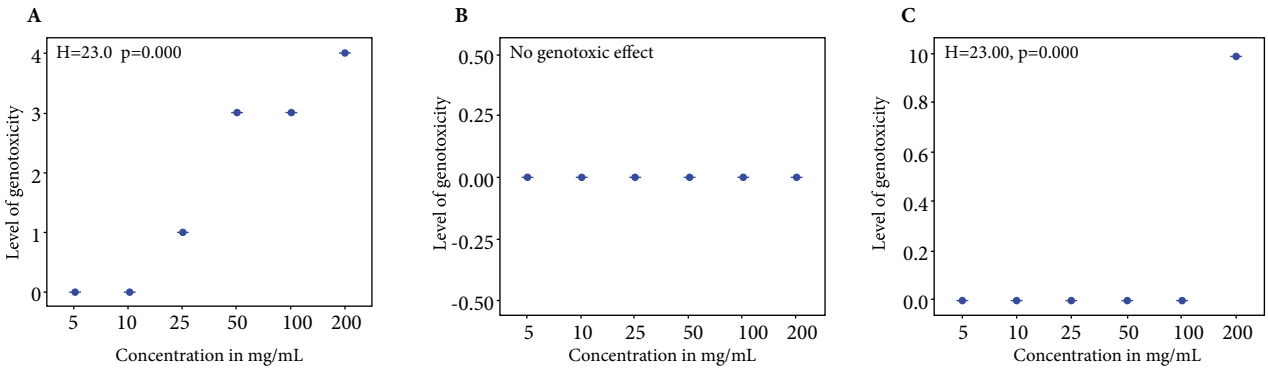


Figure 3. Kruskal Wallis test to determine the level of *in vitro* genotoxicity of the crude (A), ethanolic (B) extract of *Curcuma longa* L. rhizome and curcumin (C) at concentrations of 5, 10, 25, 50, 100 and 200 mg/mL, against human genomic DNA at 1500 ng/µL, incubated at 37 °C for one hour.

pounds, tannins, and alkaloids. Likewise, the metabolites present a synergistic effect, showing allelopathic activity, causing damage to the cell and, particularly, to the DNA, leading to cell death [30]. However, the nuclease enzymes of *Curcuma longa* L. have the property of fragmenting the DNA, and these enzymes could be present in the extracts, exerting such a function. For this reason, to confirm that DNA fragmentation is due to the action of secondary metabolites, in the Tomasevich method used, a tube was prepared with 100 mg/mL of the extract plus the proteinase K enzyme and the DNA, and it was incubated at 37 °C. If after the electrophoresis it is observed that the DNA has been fragmented, it is by the action of the secondary metabolites and not by the nuclease enzymes, since these enzymes of protein constitution would be degraded by the action of the proteinase K enzyme during the incubation period. In our study, the two treatments of the crude rhizome extract of *Curcuma longa* L. at 100 mg/mL with and without proteinase K reveal similar behavior, so it can be concluded that the fragmentation of DNA is due to the effect of the secondary metabolites present in the extracte los metabolitos secundarios presentes en el extracto.

It should be mentioned that there were limitations related to economic financing to get the cellular lines DU 145, HT-29, 3T3 BALB/c, which was finally provided by professionals and institutions that collaborated in the research. Even though materials and reagents had to be optimized, the results were not affected.

In conclusion, the *Curcuma longa* L. CE and EE show different *in vitro* cytotoxic effects for the human tumor cell line DU-145 and HT-29 similar to the standard compound curcumin. The *Curcuma longa* L. CE presents a genotoxic effect according to its concentration, while the EE does not. These results will allow the scientific community to carry out studies to develop a phytomedicine that guarantees its therapeutic use.

Acknowledgements: To Dr. Abraham J. Vaisberg Wolach, main professor of the Department of Microbiology and researcher of the Cellular Biology and Virology Unit of the Universidad Peruana Cayetano Heredia, for his valuable collaboration.

Contributions of the authors: MFCR, MDPM, TYMT, MMH and MGRF designed the article, collected the data, carried out the statistical analysis, wrote the manuscript and approved the final version.

Funding: Self-funded.

Conflicts of Interest: The authors declare no conflict of interest with the publication of this article.

REFERENCES

1. Ministerio de Salud. Situación epidemiológica del cáncer de acuerdo a la vigilancia epidemiológica de cáncer basada en registros hospitalarios. Enero-diciembre 2017. Boletín epidemiológico del Perú Volumen 27 - SE 31. [Internet]. 2017 [cited on June 19, 2018]. Available at: http://www.dge. gob.pe/portal/docs/vigilancia/boletines/2018/05.pdf.
2. Siegel R, Miller K, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017;67(1):7-30. doi: 10.3322/caac.21387.
3. Sciarra A, Gentilucci A, Salciccia S, Pierella F, Del Bianco F, Gentile V, *et al*. Prognostic value of inflammation in prostate cancer progression and response to therapeutic: a critical review. J Inflamm (Lond). 2016;13:35. doi: 10.1186/s12950-016-0143-2.
4. Morgillo F, Dallio M, DellaCorte C, Gravina A, Viscardi G, Loguercio C, *et al*. Carcinogenesis as a Result of Multiple Inflammatory and Oxidative Hits: a Comprehensive Review from Tumor Microenviron-

 https://doi.org/10.17843/rpmesp.2020.373.4817

ment to Gut Microbiota. Neoplasia. 2018;20(7):721-33. doi: 10.1016/j.neo.2018.05.002.
5. Garcia B, Saldaña A, Saldaña L. El estrés oxidativo y los antioxidantes en la prevención del cáncer. Rev Haban Cienc Méd. 2013;12(2):187-196. Available at: http://scielo.sld.cu/scielo.php?pid=S1729-519X2013000200005&script=sci_arttext&tlng=pt.
6. Aune D, Giovannucci E, Boffetta P, Fadnes L, Keum N, Norat T, *et al.* Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and allcause mortality–a systematic review and doseresponse meta-analysis of prospective studies. Int J Epidemiol. 2017; 46(3): 1029- 56. doi: 10.1093/ije/dyw319.
7. Kapinova A, Kubatka P, Golubnitschaja O, Kello M, Zubor P, Solar P. Dietary phytochemicals in breast cancer research: anticancer effects and potential utility for effective chemoprevention. Environ Health Prev Med. 2018;23(1):36. doi: 10.1186/s12199-018-0724-1.
8. He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, Inflammation, and Chronic Diseases: How Are They Linked? Molecules. 2015;20(5):9183-9213. doi: 10.3390/molecules20059183.
9. Li M, Gar-Lee G, Kwok-Wing S, Kwok-Pui F, Bik-San C. Turmeric extract, with absorbable curcumin, has potent anti-metastatic effect in vitro and in vivo. Phytomedicine. 2018;(46):131-141. doi: 10.1016/j.phymed.2018.03.065.
10. González-Albadalejo J, Sanz D, Claramunt R, Lavandera J, Alkorta I, Elguero J. Curcumina y curcuminoides: química, estudios estructurales y propiedades biológicas. An R Acad Nac Farm. 2015;81(4):278-310. Available at: https://analesranf.com/wp-content/uploads/2015/81_04/8104_02.pdf.
11. Cosquillo M, Placencia M, Retuerto-Figueroa M, Gorriti A, Huamaní J. Caracterización físico-química y capacidad antioxidante de extractos del rizoma de Curcuma longa L. Rev Per Med Int. 2018;3(4):160-6. doi: 10.26722/rpmi.2018.34.97.
12. Vamanu E, Gatea F, Sârbu L, Pelinescu D. An In Vitro Study of the Influence of Curcuma longa Extracts on the Microbiota Modulation Process, In Patients with Hypertension. Pharmaceutics. 2019;11(4):191. doi: 10.3390/ pharmaceutics11040191.
13. Mazidi M, Karimi E, Meydani M, Ghayour-Mobarhan M, Ferns G. Potential effects of curcumin on peroxisome proliferator activated receptor -γ in vitro and in vivo. World J Methodol. 2016; 6(1):112-117. doi: 10.5662/ wjm.v6.i1.112.
14. Araki A, Quispe V, Pereira da Silva V, Rangel S, Estadella D, Barros M, *et al.* Putative mechanisms of genotoxicity induced by fluoride: a comprehensive review. Environ Sci Pollut Res. 2017; 24:15254–59. doi: 10.1007/s11356- 017-9105-3.
15. Guerreiro N, Dinis-Oliveira R. Drugs of abuse from a different toxicological perspective: an updated review of cocaine genotoxicity. Arch Toxicol. 2018;92(10):2987-3006. doi: 10.1007/s00204-018-2281-1.
16. Silva de Sá I, Paula A, Leimann F, Vitoeia F, Bressan G, Krum, Nunes B, *et al.* In vitro and in vivo evaluation of enzymatic and antioxidant activity, cytotoxicity and genotoxicity of curcumin-loaded solid dispersions. Food Chem Toxicol. 2019; 125: 29-37. doi: 10.1016/j.fct.2018.12.037.
17. Verma R, Awasthi K, Rajawat N, Soni I, John P. Curcumin modulates oxidative stress andgenotoxicity induced by a type II fluorinated pyrethroid, beta-cyfluthrin. Food Chem Toxicol. 2016; 97: 168-176. doi: 10.1016/j.fct.2016.09.014.
18. Skejan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. J Natl Cancer Inst. 1990;82(13):1107-12. doi: 10.1093/jnci/82.13.1107.
19. Miranda T. Método Tomasevich: para determinar el efecto genotóxico in vitro de plantas medicinales y/o productos fitoterapéuticos. En: 2° Congreso Latinoamericano de Farmacogenómica y Medicina Personalizada: 25 al 28 de octubre del 2017. Durando: Dgo., México; 2017.
20. Gajskia G, Žegurab B, Ladeirac K, Novakb M, Sramkovae M, Pourrutf B, *et al.* The comet assay in animal models: From bugs to whales – (Part 2 Vertebrates). Mutat Res. 2019;781:130-164. doi: 10.1016/j.mrrev.2019.04.002.
21. Collins AR. The Comet Assay for DNA Damage and Repair. Mol Biotech. 2004;(26):249-261. doi: 10.1385/MB:26:3:249.
22. Moreno M, Miranda T, Quispe C, Rivera J, Ango H. Evaluación de la genotoxicidad in vitro de látex fresco y cristalizado de Carica papaya L. «papaya» frente a ADN genómico humano. En: 2° Congreso Latinoamericano de Farmacogenómica y Medicina Personalizada. 25 al 28 de octubre de 2017. Durando, Dgo., México; 2017.
23. Marca P, Miranda T, Moreno M, Galindo I, Arenas J. Preliminary evaluation of the in vitro genotoxicity of the ethanolic extract and allium sativum l. «garlic» juice against the DNA of Staphylococcus sp. En: 5to Congreso Internacional sobre Farmacología de Productos Naturales. 25 al 30 de mayo de 2018. Topes de Collantes, Cuba; 2018.
24. Miranda T, Moreno M, Alarcón G, Aguilar E, Infante G. Genotoxicidad in vitro de la planta medicinal Xanthium catharticum HBK «amor seco» frente a DNA genómico humano. En: 5to Congreso Internacional sobre Farmacología de Productos Naturales. Principios farmacológicos y evidencias científicas en la Medicina Tradicional y Natural. 25 al 30 de mayo de 2018. Topes de Collantes, Cuba; 2018.
25. Coico León AY, Juárez Pimentel AEY, Laurente Sánchez DI, Mantari Ochante FE, Alvarado Novoa AC, Gonzales Palomino M. Actividad citotóxica del extracto etanólico de Alternanthera mexicana en las líneas celulares 3T3 Y HUTU 80. Rev Per Med Int. 2016;1(2):5-11. Available at: http://www.ojs.rpmi.pe/index.php/RPMI/article/viewFile/12/16.
26. Kuete V, Seo E, Krusche B, Oswald M, Wiench B, Schröder S, *et al.* Cytotoxicity and Pharmacogenomics of Medicinal Plants from Traditional Korean Medicine. Evid Based Complement Alternat Med. 2013;2013:341724. doi: 10.1155/2013/341724.
27. Yue G, Jiang L, Kwok H, Lee J, Chan K, Fung K, *et al.* Turmeric ethanolic extract possesses stronger inhibitory activities on colon tumour growth than curcumin – The importance of turmerones. J Functional Foods. 2016; 22:565-77. doi: 10.1016/j.jff.2016.02.011.
28. Cao A, Li Q, Yin P, Dong Y, Shi H, Wang L, *et al.* Curcumin induces apoptosis in human gastric carcinoma AGS cells and colon carcinoma HT-29 cells through mitochondrial dysfunction and endoplasmic reticulum stress. Apoptosis. 2013;18(11):1391–1402. doi: 10.1007/s10495-013-0871-1.
29. Hong J, Ahn K, Bae E, Jeon S, Choi H. The effects of curcumin on the invasiveness of prostate cancer in vitro and in vivo. Prostate Cancer Prostatic Dis. 2006; 9(2):147-52. doi: 10.1038/sj.pcan.4500856.
30. Calaf G, Ponce-Cusi R, Carrión F. Curcumin and paclitaxel induce cell death in breast cancer cell lines. Oncol Rep. 2018;40:2381-88. doi: 10.3892/or.2018.6603.