Annals of West University of Timişoara, ser. Biology, 2020, vol. 23 (2), pp.251-256

EVALUATION OF ANTIPROLIFERATIVE POTENTIAL OF *CURCUMA LONGA* EXTRACTS

Molka BAMBIA[1], Ion Valeriu CARABA[2]*, Doina VERDES[3], Alexandru NAN[2], Rodica TOROK OANCE[1], Adrian SINITEAN[1], Gabriela VLASE[1], Gabi DUMITRESCU[2], Marioara Nicoleta FILIMON[1]

[1]Department Biology-Chemistry, Faculty Chemistry-Biology-Geography, West University of Timisoara, Timisoara

[2] Faculty of Bioengineering of Animal Resources, Banat University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania" from Timisoara, Timisoara

[3]Department of Morphologic microscopy, University of Medicine and Pharmacy "Victor Babes", Timisoara

**Corresponding author's e-mail: caraba_i@animalsci-tm.ro*

Received 25 November 2020; accepted 22 December 2020

ABSTRACT

Colorectal cancer is one of the leading causes of death worldwide, it is ranked the third most common cancer in the world and the fourth leading cause of death. In this study, we aimed to assess the antiproliferative potential of the aqueous and hydro-alcoholic extracts of Curcuma longa influence on therapy-resistant colon cancer cells. HCT cells were seeded in the presence of cisplatin in order to develop the cell line resistant to this drug. Subsequently, the cells were exposed for 24, 48 and 72 hours to different concentrations of aqueous and hydro-alcoholic extracts of C. longa. After the study it can be concluded that the plant extracts effects vary depending on the solvent used for the extraction and the incubation times of the cancer cells with the extracts.

KEY WORDS: *curcuma longa extracts, colon cancer cells, antiproliferative.*

INTRODUCTION

Neoplazic diseases are included among the most frequent causes of chronic pathologies. Colorectal cancer (CRC) is a malignant disease with high morbidity and mortality, being one of the most common types of cancer worldwide, as well as the fourth leading cause of cancer death (Selvam *et al.*, 2019). Although there is currently different methods of diagnosis and treatment, cancer mortality is still very high. Standard drugs for cancer treatment are limited and in the most cases do not give the expected results. In addition, it's accompanied by the appearance of resistance to therapy phenomenon and side effects onset. On the other hand, it is known that in patients with neoplasia the immune system is affected.

The chemotherapy used currently is represented by chemical synthesis compounds. These synthetic therapies development have as starting point the discovery of compounds with cancer therapeutic effects: antioxidant, immunity booster and organism resistance. Therefore, recent scientific community and pharmaceutical industries concerns is to find new herbal therapies formulations which are non-toxic to the body and allow the disease treatment improvement

(Giordano & Tommonaro, 2019). These natural compounds have a chemo-preventive or anticancer potential with minimal side effects (Guilford & Pezzuto, 2008). *Curcuma longa* is a plant known for its antioxidant, immunological and antibacterial effects. Therapeutic effects of biologically active compounds of *C. longa* (such as curcumin) exerts therapeutic effects in many cancers, including colorectal cancer (Wong *et al.*, 2019).

In the current study, we investigated the antiproliferative potentials of *C. longa* (turmeric) extracts. This study followed the in vitro analysis of *C. longa* aqueous and hydro-alcoholic extracts on chemotherapy resistant human colon cancer cell lines. Cancer cells were exposed to 5 different extracts concentrations for 24, 48 and 72 hours. The study found that *C. longa* extracts, especially those obtained in a hydro-alcoholic medium reduce the rate of human adenocarcinoma cells multiplication.

MATERIAL AND METHOD

Extraction protocol:

In the 50 ml bottle, 5 g of plant material was dissolved in 50 ml of different solvents: water and hydro-alcoholic solvent (50% water + 50% ethanol). The extraction was carried out with an orbital shaker 24 h at 30°C in the dark. Then, the stirring was performed at a speed of 100 rpm. The organic plant product was filtered from the solution and the raw extract was obtained after the solvent evaporation from the solution. The extracts were weighed in an analytical balance and diluted in culture medium, then sterilized with sterile membranes.

Cell culture:

After cell adhesion and monolayer formation, cells were multiplied for a week in DMEM medium, to which 10% FBS and 1% penicillin/streptomycin were added at 37° C and 5% CO2. The cells were grown in the presence of cisplatin in the culture medium for 3 months. Cell extraction was performed by treatment with Trypsin /EDTA after a preliminary wash with PBS. Then, after counting, the cells were put back suspended in the medium to perform the assays.

Extracts cytotoxic effects analysis:

Cells were grown at 2 x 10^5 cells/well in 96 well plates. 24 hours after cultivation, turmeric extracts were added in 10 µl to 90 µl of medium. Turmeric extracts were resuspended in culture medium at the following final concentrations: 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml. In parallel, a control culture was used where no turmeric extract has been added. After applying the extracts, the cell proliferation test was performed at 24, 48 and 72 hours.

RESULTS AND DISCUSSIONS

The HCT8 cells exposed to aqueous extracts for 24, 48 and 72 hours showed reduced viability, while the cell proliferation decrease was low and dose-dependent (fig. 1). Cells incubation with hydro-alcoholic extracts for 24, 48 and 72 hours revealed a decrease in HCT8 cells population with a time-dependent correlation. For the cells incubated with hydro-alcoholic extracts, a non-dose-dependent proliferation inhibition was observed (fig. 2).

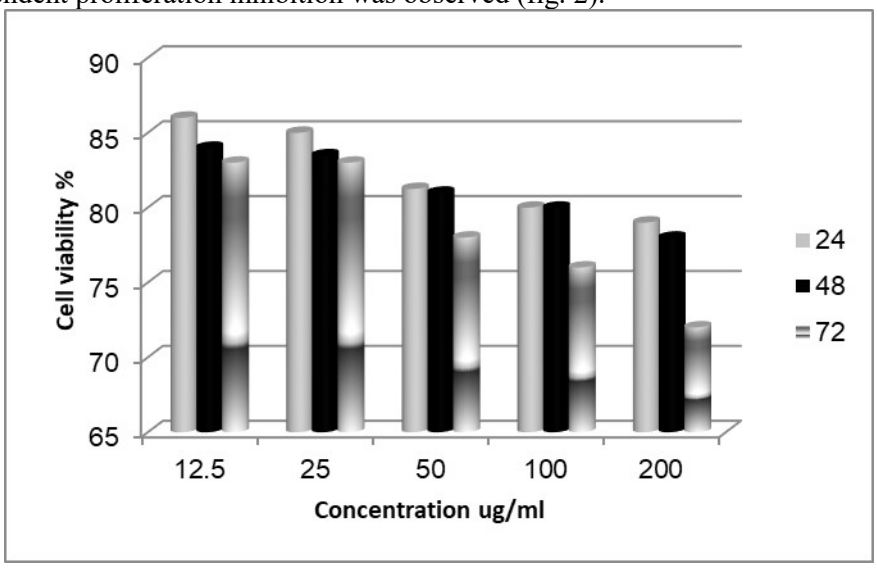

FIG. 1. Effects of *C. longa* aqueous extracts on HCT cell proliferations.

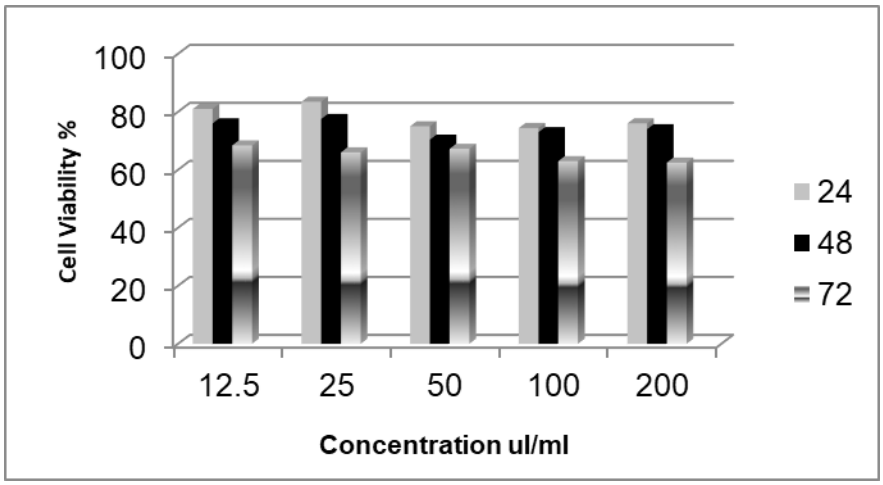

FIG. 2. Effects of *C. longa* hydro-alcoholic extracts on HCT cell proliferations.

Regarding the cell phenotype microscopic analysis, we evaluated the cells morphology in culture under the MCF microscope. We did not reveal any cellular morphological alterations control cells (fig. 3). In the case of aqueous extracts (fig. 4) and cells exposed to 24h and 48h hydro-alcoholic extracts, microscopic evaluation revealed reduced cell death lesions (fig. 5). Apoptotic and necrotic lesions were more severe in cells incubated 72 hours with hydro-alcoholic extracts.

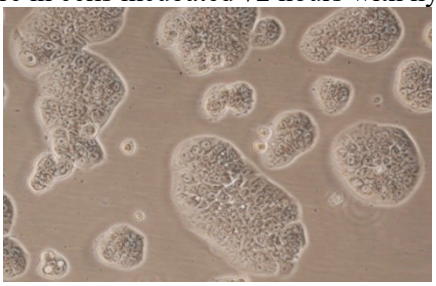

FIG. 3. HCT cell morphology - control - 72h.

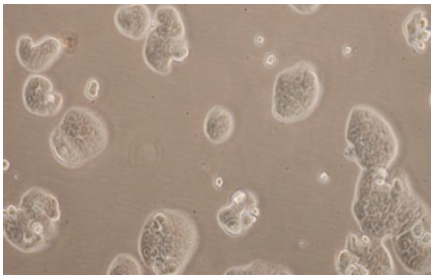

FIG. 4. HCT cell morphology - aqueous extracts 72h.

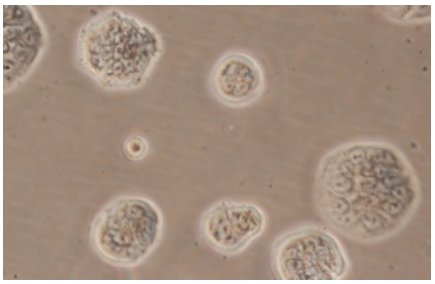

FIG. 5. HCT cell morphology - hydro-alcoholic extracts 48h.

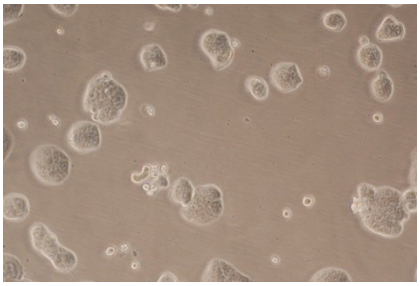

FIG. 6. HCT cell morphology – hydro-alcoholic extracts 72h.

Although the current therapeutic agents available against cancer have a remarkable effect, resistance to anti-cancer drugs has already appeared in the cancer chemotherapy starting and it still hinders the treatment success. Many anti-cancer drugs developed kill proliferative cells, whether they are malignant or not. This approach causes only modest tumor specificity, and normal non-tumor proliferative tissues are also affected. Therefore, there based drugs doses application to kill all tumor cells including less sensitive tumor subpopulations, can not be applied without causing serious side effects in patients with cancer. Currently, new strategies with natural products are used aiming to replace or supplement current therapies which have the advantage of being non-offensive, inexpensive and readily available (Wang *et al.*, 2015).

Turmeric is one of the most studied medicinal plants that has been discovered about two centuries ago by researchers, numerous *in vitro* and *in vivo* studies have been performed on turmeric extracts or pure active curcumin. The anticancer effect was reported in a few clinical trials, mainly as a chemo-preventive agent in the colon and pancreas cancer (Mansouri *et al.*, 2020).

Kunnumakkara *et al.* (2017) injected the HCT116 cells into mice, divided into four groups, and treated with vehicle (corn oil), curcumin, curcumin combined with g-radiation. Their studies showed that curcumin significantly increases the fractional radiotherapy effectiveness by extending the tumor regrowth delay and reducing the proliferation index. In addition, curcumin inhibited NF-kB activity and the gene products regulated by NF-kB expression. Many of these were induced by radiation therapy and induce radio-resistance. The curcumin and radiotherapy combination also suppressed angiogenesis, through a decrease in vascular endothelial growth factor and micro-vessel density (Kunnumakkara *et al.*, 2017).

Chemotherapy with natural antioxidants, like curcumin, could be a new approach to improve therapeutic strategies in malignant tumors (Zhijun *et al.*, 2018). In an attempt to redefine treatment for colorectal cancer, recent research has analyzed cancer stem cells (CSCs) from colorectal cancer that are assumed to be responsible for recurrence, relapse, metastasis, and resistance to treatment (Sordillo

et al., 2015). *In vitro* administration of curcumin and 5-FU (fluorouracil) has been shown to result in a significant decrease in CSC (Ramasamy *et al.*, 2015). FOLFOX and curcumin administrated to chemotherapy-resistant SCCs have been shown to decrease the colorectal stem cells number, demonstrating the curcumin ability to increase response to standard chemotherapy. Another study showed that colorectal cancer cells treated with FOLFOX significantly decreased after treatment, but SCCs survived and multiplied, demonstrating SCC chemotherapy resistance. However, treatment with curcumin alone or concomitantly with FOLFOX has demonstrated a significant reduction in SCC (Yu *et al.*, 2009).

CONCLUSION

The aqueous extracts *C. longa* causes light cytotoxicity. The *C. longa* hydro-alcoholic extracts possess time dependent HCT8 cells antiproliferative and cytotoxic potential.

REFERENCES

- Selvam C., Lakshmana Prabu S., Jordan B.C., Purushothaman Y., Umamaheswari A., Sadat Hosseini Zare M, Thilagavathi R. 2019. Molecular mechanisms of curcumin and its analogs in colon cancer prevention and treatment. *Life Sciences* 239: 117032.
- Giordano A., Tommonaro G. 2019. Curcumin and Cancer. *Nutrients* 11(10): 2376.
- Guilford J.M., Pezzuto J.M. 2008. Natural products as inhibitors of carcinogenesis. *Expert Opin. Investig. Drugs* 17: 1341-1352.
- Wong K.E., Ngai S.C., Chan K.G., Lee L.H., Goh B.H. Chuah L.H. 2019. Curcumin Nanoformulations for Colorectal Cancer: A Review. *Front. Pharmacol.* 10: 152, doi.org/10.3389/fphar.2019.00152.
- Wang P., Yang H.L., Yang Y.J., Wang L., Lee S.C. 2015. Overcome Cancer Cell Drug Resistance Using Natural Products. *Evidence-Based Complementary and Alternative Medicine* Article ID767136.
- Mansouri K., Rasoulpoor S., Daneshkhah A., Abolfathi S., Salari N., Mohammadi M., Rasoulpoor S., Shabani S. 2020. Clinical effects of curcumin in enhancing cancer therapy: A systematic review. *BMC Cancer* 20: 791.
- Kunnumakkara A.B., Bordoloi D., Padmavathi G., Monisha J., Roy N.K., Prasad S., Aggarwal B.B. 2017. Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *Br J Pharmacol.* 174(11): 1325-1348.
- Zhijun L., Pengyun H., Siukan L., Haiyan T., Wingnang L., Chuanshan X. 2018. Preventive Effect of Curcumin Against Chemotherapy-Induced Side-Effects. *Front Pharmacol.* 9: 1374.
- Sordillo P.P., Helson L. 2015. Curcumin and Cancer Stem Cells: Curcumin Has Asymmetrical Effects on Cancer and Normal Stem Cells. *Anticancer Research.* 35(2): 599-614.
- Ramasamy T.S., Ayob A.Z., Myint H.H.L, Thiagarajah S., Amini F. 2015. Targeting colorectal cancer stem cells using curcumin and curcumin analogues: insights into the mechanism of the therapeutic efficacy. *Cancer Cell Int.* 15: 96.
- Yu Y., Kanwar S.S., Patel B.B., Nautiyal J., Sarkar F.H., Majumdar A.P. 2009. Elimination of colon cancer stem-like cells by the combination of curcumin and OLFOX. *Translat Oncol.* 2(4):321-328.