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Cytotoxic effects of *Curcuma longa* leaves on MCF-7 and HepG2 cells

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Abstract

Curcuma longa (tumeric) is listed in several therapeutic regimens used in the management of cancer. The present study investigated the potential cytotoxic effects of ethanol extract of *Curcuma longa* (turmeric) leaves on HepG2 (a human hepatocellular carcinoma cell line), and MCF-7 (a human mammary gland adenocarcinoma cell line) cells. The HepG2 and MCF-7 cells were exposed to different concentrations; 2, 0.2, 0.02, 0.002, 0.0002, 0.00002, 0.000002 µg/ml, of the ethanol extract of *C. longa* leaves. The Water-Soluble tetrazolium salt (WST-1) assay was used to estimate the cell viability of the cells after which the percentage proliferation was computed. The results of this study indicated that *C. longa* had cytotoxic effect on the different cell lines, caused a decrease in proliferation and it is more selective to breast cancer cell lines based on the results of WST-1 analysis on the HepG2 and MCF-7 cells. These results suggest that *C. longa* leaf possesses cytotoxic properties, which can be exploited to develop novel cytotoxic drugs for cancer management.

Keywords: *Curcuma longa*, turmeric, HepG2, MCF-7, WST-1, proliferation, cytotoxic, cancer

Introduction

Cancer is the second leading cause of death globally that killed 10 million people in 2020 (Ferlay *et al*., 2020). It is characterised by uncontrolled proliferation and growth of abnormal cells consequently, forming tumours of malignant cells with the potential to be metastatic (WHO, 2021). Cancer is a deadly disease that results in the uncontrolled growth of tumor cell cycle (Hassanpour & Dehghani, 2017). Despite the advances in drug development, it is still necessary to develop new plant-derived medicines to achieve selective efficacy and reduce their cost (Greenwell & Rahman, 2015). Phytochemicals, in plants with potent antioxidant, anti-inflammatory and immunomodulatory activities have been reported to reduce the growth and spread of cancer (Lawal *et al.*, 2016, Kapinova *et al.,* 2018, Lawal *et al*., 2019).

Curcuma longa L., is a tuber-like, herbaceous perennial plant with wide leaves and yellow flowers (Akpolat *et al.,* 2010). According to Deogade & Ghate (2015), it is a member of the ginger family and grows well in tropical climate. *C. longa* is a plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, *C. longa* is used not only as a prin-

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cipal spice but also as a component in religious ceremonies (Prasad & Aggarwal, 2011). It has shown potent antibacterial activity against *Staphylococcus aureus* (Usamah *et al*., 2012). The rhizome of *C. longa* (turmeric) inhibited the growth of cancer cells and also caused the extermination of lymphoma cells and lymphocytes (Kewitz *et al*., 2013). The anticancer activity of *C. longa* was attributed to curcumin, a polyphenol, present in the rhizome (Devassy *et al.,* 2015). Curcumin has been reported to be effective for use in the treatment of various inflammatory diseases such as diabetes, obesity, cardiovascular diseases, neurological disorders, and inflammatory bowel diseases (Shehzad *et al.,* 2013). It was also reported to suppress the actions of tumor necrosis factor (TNF) in cell lines (Gupta *et al.,* 2013). However, not much has been reported about the anti-cancer potential of *C. longa* leaves extract. Hence, this study investigated potential cytotoxic activity of ethanol extract of *Curcuma longa* leaves on HepG2 (a human hepatocellular carcinoma cell line), and MCF-7 (a human mammary gland adenocarcinoma cell line) using the WST-1 assay.

Materials and Methods

Cell Lines and Chemicals

The cell lines (HepG2 and MCF-7) were obtained from the Department of Biochemistry, Nigerian Institute of Industrial Research, Lagos, Nigeria. Dulbecco's Modified Eagle's Medium (DMEM), Viral transport Medium (VTM) and Dimethyl sulfoxide (DMSO), WST-1 assay kit, used for cytotoxicity assay, were obtained from Sigma-Aldrich (St. Louis, US). All other chemicals used were of analytical grade available locally.

Plant Materials

Fresh samples of *C. longa* leaves were collected fresh from forest areas in Osogbo, Osun State, Nigeria in August, 2021.

Identification and Authentication of Plant Materials

The plant leaves were authenticated by Dr George Nodza, a taxonomist in the Department of Botany, University of Lagos, Lagos, Nigeria. Voucher specimen of the leaf samples was deposited in the University Herbarium, University of Lagos, Lagos, Nigeria with the voucher number LUH8804.

Preparation of plant extracts

Briefly, the leaves were air dried, for 7 days, until a constant weight was obtained and the dried material was ground to fine powder using a local pulveriser. One hundred grammes of the powdered plant material were extracted in 1000 ml of ethanol (1:1) for 48 h, after which the liquid was decanted and filtered twice, using Whatman paper (size 14) attached to a filter funnel, to obtain a filtrate. The residue from the process was re-macerated, twice, in same volume of ethanol to ensure exhaustive extraction. The filtrate from each extraction process was combined and evaporated to dryness at -4^{o}C under reduced pressure (Rotavapor® R-300, BUCHI, Switzerland) and further concentrated to constant weight *in vacuo* using a lyophilizer (Lyotrap, LTE, England).

Maintenance of Human hepatic cell carcinoma (HepG2) and Breast cancer cell lines (MCF-7)

Briefly, the cells (MCF-7 and HepG2) were washed in phosphate buffered saline (PBS) to remove debris and dead cells. Exactly 500 µL Trypsin was added after which cells were incubated in a CO_2 incubator at 37^0C for 5 min. A volume of 2 ml of 5% growth media was added to inactivate the trypsin after which the mixture was centrifuged at 400 × *g* for 10 min. Exactly 1ml of 5% growth media was added to the pelleted cells and vortexed to generate a homogenous cell suspension. Cells were stained with trypan blue and counted using a haemocytometer. The cells were thereafter maintained in Dulbecco's Modified Eagle's Medium. About 1×10^6 viable cells were added into each well plate in a 96-well plate (Vantangoli *et al*., 2015).

Cell viability assays

WST-1 test

The WST-1 assay described by Baysan *et al*. (2020) was used for the determination of cell viability. The WST-1 reagent reacts with the mitochondrial succinate-tetrazolium reductase with the formation of the formazan dye. The amount of

formazan dye formed in the WST-1 assay is directly related to the metabolic activity of cells. Briefly, cells were seeded in transparent 96-well plates and exposed immediately to different concentrations; 2, 0.2, 0.02, 0.002, 0.0002, 0.00002, 0.000002µg/ml of ethanol extract of *C. longa* leaves for 24h. A blank control was also prepared that was not exposed to extract. Cells were then washed twice with phosphate buffered saline and incubated in fresh medium with 10% WST-1 reagent for 2h. Absorbance was read at 450 nm (690 nm was used as reference wavelength and subtracted) in a multiplate reader (Varioskan Multimodal plate reader, Thermofisher Scientific USA). Results were reported as relative WST-1 activity, where 1.0 corresponds to the absorbance measured in control cultures.

Statistical Analysis

Values are represented as means absorbance values ± SD of experiments conducted in triplicates. Data were analysed with GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) and Microsoft Excel.

Results and Discussion

This study evaluated the cytotoxic activity of ethanol extract of *C. longa* (turmeric) leaves on HepG2 (a human hepatocellular carcinoma cell line), and MCF-7 (a human mammary gland adenocarcinoma cell line) using the WST-1 assay. The WST-1 based cell cytotoxicity assay is regarded as a good substitute for the MTT-based assay. The MTT assay, though widely accepted for the quantification of cytotoxicity and anti-proliferation, takes relatively much more time than the WST assay (Ngamwongsatit *et al.*, 2008). The WST-1 assay is similar to MTT which reacts with the mitochondrial succinate-tetrazolium reductase with the formation of the formazan dye. However, the WST-1 reagent produces a water-soluble formazan rather than the water-insoluble product of the MTT assay. Equally, the WST-1 reaction product, unlike the MTT product, can be quantified within 4 h without an additional solubilization step (Baysan *et al*., 2020). The different absorbance values (an indication of the extent of cell cytotoxicity) recorded from WST-1 assay on exposure of HepG2 and MCF-7 cells to various concentrations of ethanol extract of *C. longa* leaves are as shown in Table 1 and Table 2. The percent decrease in proliferation of MCF-7 cells exposed to ethanol leaf extract of *C. longa* is as shown in Table 1. The results in Table 1 indicate that as the extract concentration increased, the percentage decrease in proliferation increased. The exposure to the extract caused a high percentage decrease in proliferation of MCF-7 cells as shown in Table 1. This means that the extract was more cytotoxic at higher concentrations. The results of this study indicate that the ethanol leaf extract of *C. longa* showed cytotoxic activity against MCF-7 cells *in vitro*. The percent decrease in proliferation of HepG2 cells exposed to ethanol leaf extract of *C. longa* is as shown in Table 2. However, the results in Table 2 indicate that as the extract concentration increased, there were negative values of percentage decrease in proliferation calculated. However, the HepG2 cells were relatively unaffected by the exposure to ethanol leaf extract of *C. longa* as shown by these negative values (Table 2). This indicates that ethanol leaf extract of *C. longa* did not adversely affect the HepG2 cells.

Table 1: Percentage decrease in proliferation of MCF-7 cells exposed to various concentrations of ethanol extract of *Curcuma longa* leaves*

Concentration of extract (µg/ml)	WST-1 Average Absorbance @ 450 nm	% Decrease in proliferation
2.0	0.4237 ± 0.0277	29.4304
0.2000	0.4305 ± 0.0541	28.2978
0.0200	0.4247 ± 0.0098	29.2638
0.0020	0.4278 ± 0.0744	28.7475
0.0002	0.4312 ± 0.0282	28.1812
0.00002	0.4374 ± 0.0666	27.1486
0.000002	0.4845 ± 0.0541	19.3038
Negative control	0.6004 ± 0.1146	0.0000

*Results were expressed as Mean of 3 values ± Standard deviation.

Table 2: Percentage decrease in proliferation of HepG2 cells exposed to various concentrations of ethanol extract of *Curcuma longa* leaves

Concentration of extract (µg/ml)	WST-1 Average Absorbance @ 450 nm	% Decrease in proliferation
2.0	0.4418 ± 0.0114	-1.2374
0.2000	0.4745 ± 0.0693	-8.7305
0.0200	0.4589 ± 0.0402	-5.1558
0.0020	0.4428 ± 0.0481	-1.4665
0.0002	0.4695 ± 0.0408	-7.5848
0.00002	0.4505 ± 0.0470	-3.2310
0.000002	0.4587 ± 0.0346	-5.1100
Negative control	0.4364 ± 0.0154	0.0000

*Results were expressed as Mean of 3 values ± Standard deviation.

The findings of this study suggest that the ethanol leaf extract of *Curcuma longa* was selectively cytotoxic to some cancer cells and this indicates the importance of the extract in treatment of cancer (Ayo-Lawal *et al*., 2020). The study involved the seeding of equal quantities of cells in the different treatment plates but results indicate that the reduction in proliferation was more pronounced in the MCF-7 cells than the HepG2 cells. It has been reported that non-selectivity of medicines and plants used in the treatment of cancer can cause a high percentage of healthy cells to be lost with cancer cells (Kooti *et al*., 2017). Hence, safe drugs that can decipher between cancer cells and non-cancer cells need to be developed. Exposing cancer cell lines like MCF-7 together with other cell lines such as HepG2 cells to the same extract, under same environmental conditions affords us the opportunity to compare the selectivity of the plant as an agent that can be used safely to manage cancer. This study has demonstrated the use of cancer cell lines in the development of safe and effective drugs for management of cancer.

Conclusion

This study has shown that ethanol extract of *C. longa* leaves was cytotoxic against cell lines. However, the ethanol extract of *C. longa* leaves was more cytotoxic and selective against MCF-7 cells than the HepG2 cells.

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