In vivo and *in vitro* effects of *Curcuma longa* in normal and cancerous cell line

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Abstract

This study aims to determine the active components in the *Curcuma longa* rhizome's ethanolic crude extract and evaluate the ethanolic extract's cytotoxic capabilities against the L20B and AMN-3 murine mammary cancer cell lines, two malignant cell lines. lengths of time that cell lines are exposed In a microtitration plate, different concentrations ranging from 1 to 800 µg/ml were generated and measured after 24, 48, and 72 hours under strict sterile conditions.

Chemical analysis of the extract revealed that it contained flavonoids, saponins, glycosids, alkaloids, and cumarines; phenols, resins, volatile oils, tannins, terpins, and steroids produced undesirable outcomes. After 48 hours, all doses of the ethanolic extract of *Curcuma longa* showed an inhibitory effect on cancer cell lines.

Curcuma longa crude extract in ethanolic form which, while alkaloids, glycosids, and cumarines produced unfavorable outcomes, contain flavonoids steroids, tannins, terpins, volatile oils, resins, phenols, and saponins. Strong cytotoxic effects have been observed by the ethanolic crude extract of *C. longa* against.

Keywords: cell lines, curcumin extract, in vitro and in vivo

Introduction

Turmeric (*Curcuma longa*), also referred to as the "golden spice," is a widely used flavoring, coloring, and spice. Turmeric extract has been demonstrated to exhibit biological activities that include antibacterial, antifungal, antiprotozoal, antiviral, anti-fibrotic, anti-carcinogenic, anticoagulant, anti-fertility, and anti-diabetic effects. It is also antivenum, antiulcer, hypotensive, and hypocholesteremic [1]. [1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-dione], another name for curcumin, is one of the main active components of turmeric. Due to its lack of water solubility, curcumin has a poor bioavailability, which is one of its main limitations. Because curcumin is a polar molecule, it is also not extremely lipophilic. Another reason for curcumin's limited medicinal use is that it is unstable under physiological settings [2-7]. Curcumin (77%), demethoxy (17%), and bisdemethoxy (3%), together make up commercial curcumin. Curcumin and two of its derivatives, di-glutaryl curcumin (DGC) (Figure 1) and di-acetyl curcumin (DAC) [8], were selected due to their relative advantages over curcumin in terms of lipophilicity (DAC) and hydrophilicity (DGC). Curcumin's two polar phenolic groups are shielded by acetyl groups in DAC, which makes DAC less polar and more lipophilic. DGC is significantly more hydrophilic Due to the presence It is more (polar) than curcumin because it has two free carboxylic groups on the side chain esters bound to the phenolic groups of curcumin. In the present investigation, we compared aspirin to curcumin, DAC, and DGC's anti-inflammatory and analgesic qualities in vivo. Additionally, we investigated these compounds' in vitro inhibitory qualities on PC-3, SKBR-3, and PANC-1 cancer cell lines are compared with the impact on WI38. The evaluation of the literature and an animal pilot research were used to determine the drug dosages for the in vivo investigations. Medication was taken orally along with food.

Numerous therapeutic plants' phytochemicals have been investigated for their potential to prevent cancer [9]. It has been documented that natural phytoconstituents can prevent cancer development and slow the growth of cancers in models that are both in vivo and in vitro [10]. Immunomodulation, cell cycle growth arrest, apoptosis induction, suppression of oncogenic pathways such as NFkb and kinases, inhibition of DNA synthesis and other signal transduction pathways' regulation are all possible explanations for these chemopreventive actions. Natural product chemoprevention targets changing cells exclusively, sparing normal cells from harm. A few clinical trials are being conducted to use dietary supplements, micronutrients, and other substances in the chemoprevention of cervical cancer. however these are not always successful or the chemicals may have unacceptably high adverse effects. Known by many as haldi or turmeric, haridra (*Curcuma longa* Linn) is an herb that has been used traditionally to treat a variety of conditions, including dyspepsia, anemia, wound healing, blood disorders, diabetes, hemorrhoids, and skin ailments. Early Ayurvedic literature describes it. Curcuminoids, a group of several bioactive compounds present in turmeric, including curcumin (diferuloylmethane), demethoxycurcumin, monodemethoxycurcumin, cyclocurcumin, bisdemethoxycurcumin, and curcumene, are the primary active ingredients of *C. longa*. Additionally, there is pharmacological activity in volatile oils such as cineol, borneol, sabinene, zingiberene, aphellandrene, and sesquiterpines [9].

Objectives of the study

1. *Curcuma longa*'s effects on damage DNA in white mice as determined using the comet test method.
2. Identification of the active ingredient in *Curcuma longa* rhizome crude extract.
3. Investigating the effects of an ethanolic crude extract of *Curcuma longa* rhizome on AMN-3 and L20B cancer cell lines' in vitro proliferation.

Materials and methods

Two cell culture NB cell lines, AMN-3 and L20B, are kept at 37°C in RPMI1640 or DMEM media (Invitrogen, Carlsbad, CA, USA), with 5% CO2 humidity and 10% FCS (PAA, Pasching, Germany) added. In addition, the cells are cultured with 100 IU/mL of penicillin-streptomycin (Gibco, Grand Island, NE, USA). One of the ingredients used to dissolve curcumin and TQ was dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA).

Cytotoxicity assay for curcumin

The colorimetric assay made use of the tetrazolium salt MTT (Sigma). In summary, a monolayer of 1 × 104 B16-R cells were grown for a whole 12-hour period. They were then incubated for 24 to 48 hours in 96-multiwell plates with 200 µL of RPMI, 10% FBS, and curcumin (Sigma) at final concentrations ranging from 1 to 100 µM. Following these incubations, 500 µl of fresh culture media containing MTT (0.3 mg/mL) was added for the colorimetric experiment, and the cells were then twice rinsed in PBS. A curcumin-based test for cell survival was also investigated on B16-R spheroids. Spheroids that were three days old were cultivated in 24-multiwell plates using 1 mL of RPMI-FBS supplemented with curcumin at final concentrations varying between 1 and 200 µM over a period of 24 to 48 hours. The spheroids were then manually separated using pipetting, and 500 µL of fresh culture media containing 0.3 mg/mL of MTT were added after two PBS washes. In both cases, crystals (200 µL for monolayer cultures and 750 µL for spheroids) were solubilized with DMSO following a three-hour incubation at 37°C with MTT. Using a model 550 microplate reader (Bio-Rad, Marnes la Coquette, France), optical density fluctuations between 550 and 650 nm were assessed to determine the viability of the cells. By dividing the mean absorbance values of treated samples by the mean absorbance of untreated control samples, the surviving fraction of cells was calculated.

Cell line

(AMN-3) cell line

Al-Shamery (2003) originally described and traced the development of this cell line to a female mouse carrier of the balb/c species' AMN-3 cell line in vivo (mouse breast cancer). 186 extracts in total were employed in the research. preserved in 10% cow calf serum-containing RPMI-1640 medium.

Natural body line L20B

It came from the Cancer Department of the Iraqi Center for Cancer Research and Medical Genetics and was used at progression (26). This lineage is from mouse L fibroblast-producing cells that are immune to human poliovirus. Hela (human) poliovirus DNA cells have been created from the genome of mouse L cells infected with poliovirus since these cells lack a poliovirus receptor. The L20B cells were prepared for use in the laboratories of the Global Polio Virus Network of the World Health Organization (WHO).

Result

In vivo

Estimation of DNA damage in bone marrow utilizing the Comet Assay method in mice:

The comet assay in vivo is useful in determining a substance's potential for genotoxicity, evaluating dose-response, and comprehending its mode of action.

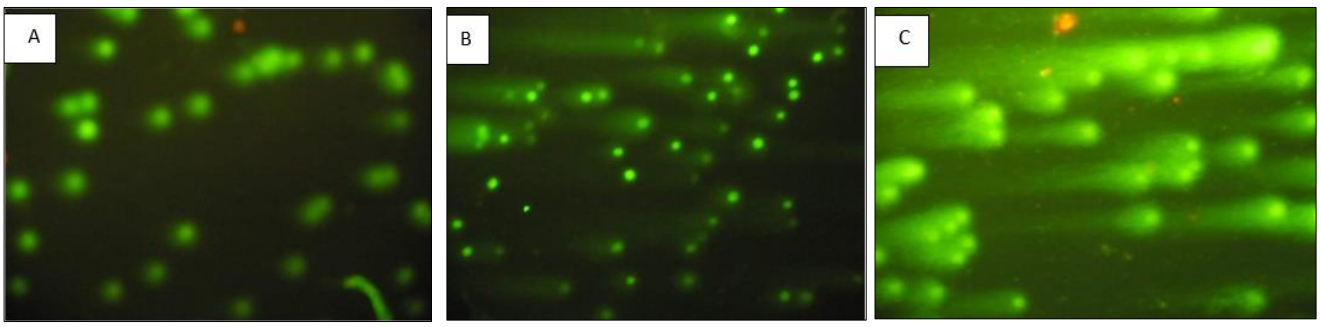
In this study, mouse bone marrow DNA movement was assessed. The comet assay results with negative control demonstrated a highly significant increase in BM damaged cell in treatment groups (50, 100, 200, 400, and 800 µg.kg-1).

Table 1 displays the total amount of damage to the DNA in the bone marrow cells of white mice given varying doses of *Curcuma longa*.

Table 1: Damage to DNA in white mouse bone marrow cells

Groups	DNA-damaged cells (M±S.E.)	Total DNA damage (M±S.E.)
Negative	0.81 ± 3.80	0.79 ± 4.20
50	4.4 ± 14.51*	5.8 ± 3.2*
100	9.0 ± 14.51*	11.0 ± 3.2*
200	13.8 ± 14.51*	18.0 ± 3.2*
400	25.4 ± 14.51*	35.6 ±3.2*
800	42.4 ± 14.51*	20.8 ± 3.2*
Positive	60.8 ± 14.51*	110.4 ± 3.2*

Significant at $p<0.05$



Normal cells of negative control Cells contain DNA damage +1 Cells contain DNA damage +2

Fig 2: Different level of DNA damage in normal group and treatment group

In vitro

When *Curcuma longa* ethanolic extracts were examined in vitro, the findings showed that the incidence was cytotoxic.

The results indicate that Chemical analysis was used to identify the active ingredients in the ethanolic crude extract of *C. longa*. steroids, tannins, terpins, volatile oils, flavonoids, saponins, and phenols were found to be positive; alkaloids, glycosids, and cumarines were found to be negative. The extract's pH was 4.2, indicating that it was acidic due to the absence of alkaloids.

Table 2: Effect of *Curcuma longa* on AMN-3and L20B cell line

Concentration µg mL-1	Mean viability (%) ± SD	
	AMN-3	L20B
800	59.80± 5.3	20.09±2.3
400	64.07±1.40	26.10±1.01
200	74.01±1.04	36.95±5.02
100	84.90±1.30	52.24±2.323
50	92.99±0.78	60.05±2.102

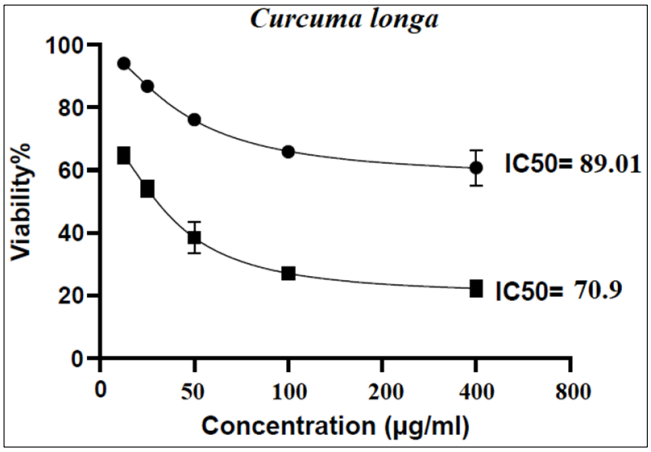


Fig 2: Effect of *Curcuma longa* on AMN-3and L20B cell line

The assay for cytotoxicity on AMN-3 cell lines is shown for 24, 48, and 72 hours in Table (1) and Figure (1). For all employed concentrations, it was discovered that the extract decreased the viability of cell culture after three exposure times. Following a controversial exposure, this inhibitory effect decreased until 72 hours had passed, at which point there were extremely When compared to the L20B control, there were significant changes at the level ($p<0.05$) across the board (50, 100, 200, 400, and 800 µg/ml). The greatest inhibition was 70.9% when the control was 89.01.

Discussion

In vivo

In contrast Comparing the mean values to the negative control group of bone marrow cells with damaged DNA (50, 100, 200, 400, 800 Positive control and Negative control), respectively, the results demonstrated statistically significant differences table (1).

When human lymphocytes were isolated, their DNA was analyzed, and they were exposed to non-steroidal antigens. The results of the study showed Thus, in comparison to cells exposed to the lowest dosage levels of the medications, the cells exposed to the highest dose levels of the drugs had the largest proportion of DNA damage. This suggests that the damage to the DNA is continuing and that cell death is beginning [10], Conversely, other research showed that long-term treatment of non-steroidal anti-inflammatory medicines (NSAIDs) to mice resulted in DNA damage and the stimulation of liver cell tumors. Including the impact on covalent binding in DNA, causing single-strand DNA breaks, and blocking the DNA repair machinery [11], Because the Comet assay methodology, also known as SCEG, is sensitive enough to detect DNA damage in interphase cells, it provides a direct method of determining When compared to the conventional methods of identifying DNA damage, which involve demonstrating DNA damage in single cells, SCEG has a great advantage on single-cell healing sites, basal visible damage, and single-strand breaks. It is thought to be a simple and uncomplicated method utilized in genotoxic scientific research. The quantification technique, which is performed in baseline circumstances, identifies single-strand fractions and tags base locations in DNA, is currently widely utilized to identify DNA damage in human lymphocytes and is used to analyze the DNA of all separated cells [12]. This is because the method offers a suitable test system to track harmful effects in living organisms, and damaged cells' DNA shows an increase in migration from the nucleus, generating a comet-like structure [13]. Because DNA migration is dependent on the size and quantity of fractures due to a particular stimulus, this technique necessitates the use of cells with a high viability level, often between 70 and 95 percent.

This method provides quantitative analysis that is suitable for various applications. It includes five types damage, almost all of the DNA in the tail (3 form) from having no tail (0 shape). Olive *et al*. (2006) introduced the tail moment, a different approach to comet assessment that is computed as tail length X tail DNA measurement. The investigator's resources and the study design determine the approach that is selected [14].

In vitro

One of the most well-known areas of cancer control is cancer chemotherapy using food and herbal medicine approaches.While alkaloids, glycosids, and cumarines had unfavorable outcomes, these natural compounds include flavonoids, saponins, volatile oils, tannins, terpins, phenols, resins, and steroids. Terpenes and volatile oils in *C. longa* are responsible for the plant's strong flavor and aromatic aroma.

Extraction

The extraction method produced a crude product yield that matched the findings of Al-Khazraji's study.Numerous factors, such as the type of study plant and the solvent and extraction method employed, affected the proportionate amount of plant utilized for extraction and the crude product [15].

Assay for cell growth (in vitro research)

The findings demonstrated that suppression of growth advanced noticeably with increasing extract concentrations and exposure times. However, the kind of extract and the duration of exposure had different effects on the different cell lines. The

extract's cytotoxic effects on the two cell lines under research was dependent on both time and dose, which was a clear outcome of the growth inhibitory experiment.The current findings aligned with the findings presented in the Campbell study, which produced dose-response curves for a number of the strongest crude extracts. According to flow cytometric studies, In the G2/M phase of the cell cycle, the herb inhibits the growth of breast cancer cells. It was claimed that numerous cell lines are subjected to cytotoxic effects by the plant extracts from their research, including AMN-3 and L20B, in a manner that is dependent on time and dose.

Depending on the origin and kind of cell, different AMN-3 cell types have different plasma membrane receptor binding capacities. The cell surface is made up of several molecules that are extremely specialized receptors. These are receptive locations for cognition that are frequently transported into cells by chemical signals. These signals have the ability to start a range of chemical reactions and eventually turn off particular genes [16]. The anti-carcinogenic effect of non-nutritive constituents in the diet is mediated by a number of mechanisms, including their antioxidant properties, deactivation of carcinogens, and increased levels of protective enzymes in the body's tissues. The diet's nutritive and non-nutritive elements are important in inhibiting the carcinogenic process [17]. The immune system of the body detoxifies toxic byproducts of dangerous medications and substances. Certain species of vegetables, such as mustard, turmeric, and allium, contain phytochemicals that can act in multiple ways to give their advantageous effects [18].

The extract's ability to limit the growth of L20B and AMN-3 cells was observed over an extended period of time at all concentrations, but its suppression of the growth of the L20B cell line was observed during a brief period of time at low concentrations. This may be explained by the cytotoxic action of the extract's active ingredients throughout exposure times, or it may be related to the sensitivity of L20B and AMN-3 cells. The variations in L20B response to various treatments may be due to the different ways that the cells interact even at the same dose depending on if particular cellular receptors are found in every kind of cell line or not. Additionally, each line's metabolic pathways responded differently to each therapy. This fact was brought up in many research that looked into the effectiveness of various plant extracts in treating various cell line types [19]. Bioactive phenolic compounds found in *C. longa* have strong chemopreventive and antioxidant effects. The primary cause of phenolic compounds' antioxidant activity is their ability to absorb and neutralize free radicals, quench singlet and triple oxygen, and break down peroxides. Many plants and vegetables, including curcumin, possess phenolic compounds, which might substantiate their antioxidant qualities directly.

When 1.0 g of polyphenolic compounds from a diet high in fruits and vegetables is consumed daily, it has been claimed that these compounds have inhibitory effects on human mutagenesis and carcinogenesis [20]. Among the primary forms of cell death, apoptosis is necessary for both homeostasis maintenance and appropriate development. Furthermore, the apoptotic inclinations of cells are expected to influence the effects of current antineoplastic therapy, chemotherapy, and radiation therapy; so, this process has clear therapeutic implications. Plant extracts' capacity to either promote or prevent apoptosis depends on a number of variables, including cell type, extract concentration, and the coordinated action of various micronutrients [21]. Recent research has demonstrated that curcumin root and its primary polyphenolic constituents, turmeric and zerumbone, suppress the transcription-related factor nuclear transcription factor Kappa-α. Because it regulates the expression and activity of several genes involved in persistent angiogenesis, cell proliferation, and apoptosis evasion, NF-κB is a key player in cancer. It has been demonstrated that ovarian cancer and other tumor types have increased constitutive nuclear factor-kappa-B (NF-κB) activity [22].

Additionally, it has been demonstrated that curcumin modifies the transcriptional and posttranslational levels of COX2 and 5-LOX expression and function. Therefore, rather than being the result of direct interactions with curcumin, It's likely that many of the cellular and molecular alterations seen in cells treated with curcumin are the result of side effects. Curcumin inhibits the growth of the cell cycle and causes apoptosis in rat aortic smooth muscle cells, two actions that are essential in halting the growth of malignant cells. While the antiproliferative impact partially mediates the expression of c-myc mRNA and protein tyrosine kinase, it partially mediates the expression of protein kinase, protein kinase C, bcl-2 mRNA, and the apoptotic effect. Topoisomerase II toxin curcumin causes apoptotic cell death in human cancer cell lines TK-10, MCF-7, and UACC-62 by damaging DNA. Recent research has demonstrated that curcumin inhibits the ubiquitin–proteasome system via the mitochondrial pathway, killing mouse CNS 2a cells [23]. Increased expression of glutathione S-transferase (protein 1) P1 (GSTP1) has been linked to cancer. It has been demonstrated that curcumin inhibits the transcriptional level of (GSTP1) expression, which results in the apoptosis of K562 leukaemia cells. Curcumin induces apoptosis in Caki cells by downregulating Bcl-XL and protein A1 (IAP), which releases cytochrome c. This action is significantly inhibited by acetylcysteine, suggesting a possible involvement of ROS in curcumin-induced cell death. Curcumin causes apoptosis in LNCaP prostate cancer cells by upregulating tumor necrosis factor-related apoptosis inducing ligand (TRAIL). After the cell is treated with curcumin and TRAIL, procaspase 3, 8, and 9 are cleaved, Bid is truncated, and cytochrome c is released from the mitochondria. These results suggest that these cells go through apoptosis mediated by both external and internal chemicals. Curcumin inhibits apoptosis and ends the cell cycle in the G1 phase in colorectal cancer cell lines. Furthermore, curcumin inhibits P53 gene production, which coincides with the first depletion of intracellular Ca2+ stimulating the HSP-70 gene (98). Additionally, curcumin inhibits the proliferation of nontransformed hematopoietic progenitor cells, leukemia cell lines, and fibroblast cell lines in a nonselective manner. Curcumin prevents the activation, proliferation, and secretion of chemokines that is triggered by isopentenyl pyrophosphate,

which causes large-scale DNA breakage and the induction of death in Vg9Vd2+ T cells. On different cell lines, however, curcumin exhibits different effects. Apoptosis occurs in leukemia, breast, colon, hepatocellular, and ovarian cancer cells. when exposed to curcumin, melanoma cells and malignancies of the lung, prostate, kidney, cervix, and central nervous system show resistance to curcumin's cytotoxic activity [24].

The results of this investigation enable the following conclusions to be taken into consideration: Flavonoids, saponin, phenols, resins, volatile oils, tannins, terpins, and steroids are present in the ethanolic crude extract of *Curcuma longa*, but alkaloids, glycosids, and cumarines had unfavorable outcomes. In the in vitro experiment, the cell lines AMN-3 were particularly vulnerable to the inhibitory effects of the ethanolic extract of *C. longa* rhizomes. After 48 hours of treatment, the extract exhibited a cytotoxic impact at the maximum concentration, but the cell line L20B responded less to low concentrations. In vitro, *C. longa*'s ethanolic crude extract has strong cytotoxic effects on the L20B and AMN-3 cell lines that are dependent on time and concentration.

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