Effect of CurcumineExtract (Curcuma Longa) On Cancer Cell Lines inVitro

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الخلاصة:

الأهداف : تهدف هذه الدراسة **للتحري عن المركبات الفعالة الموجودة في المستخلص** الايثانول يلرايزومات نبات **الكركم**Curcuma longa بوساطة الاختبارات الكيميائية النوعية،**واختبرت** التأثيرات السمية للمستخلص الكحولي الخام في اثنين من الخطوط الخلوية السرطانية (**خط سرطان الحنجرة البشري** (Hep-2) وخط خلايا سرطان الثدي الفاري(AMN-3) وخط الخلايا الجنينية الليفية الفاري المتحولة (REF-3)

المواد وطرق العمل:المنهجية: دراسة تحليلية **للفترة من**(1 / 1 /2011 **الى** 1/1 / 2012) **أجريت** هذه الدراسة في مركز بحوث السرطان\ الجامعة المستنصرية. **بغداد** .

طريقة العمل: تعريض الخلايا السرطانية إلى تراكيز مختلفة من المستخلص الكحولي الخام تراوحت بين (1– 1000) مايكروغرام/ مليليتر في أوقات تعريض مختلفة (24، 48، و72) ساعة في المزرعة الخلوية بواسطة الاختبارات السمية بمعدل ثلاث مكررات لكل تركيز وتحت**ظروف معقمة.**

النتائج:أظهر الكشف الكيميائي احتواء المستخلص الكحولي الخام على فلافونيدات، وصابونيات، ، و فينولات، و زيوت طيارة، و تانينات، وتربينات، وستيرويدات وخلوهُ من القلويدات و كلايكوسيداتوالكومارينات.اوضحت بأن المستخلص الايثانولي الخام لرايزومات الكركم Curcuma longa أظهر تأثيرات مثبطة لمدة التعريض (48) ساعة لكل التراكيز على الخلايا السرطانية والخلايا الطبيعية المتحولة . **ولقد بلغت اقصى نسبة** تثبيط لنمو خلايا AMN-3(88.3%) عند المعاملة بتركيز(6) مايكروغرام/ مليليتر بعد (24)**ساعة من مدة** التعريض ، والتأثير السام للمستخلص الخام بدأ عند التراكيز الواطئة على خط خلايا Hep-2 **بعد**(48) ساعة من مدة التعريض والذي اعطى نسبة تثبيط بلغت(77.1%) عند المعاملة بتركيز(2000) ميكروغرام/ مليليتر **بعد** (48) ساعة من مدة تعريض الخلايا للمستخلص الخام . واظهر **المستخلص الكحولي الخام** سمية على خط خلايا REF المتحولة والذي اعطى اعلى نسبة تثبيط والتي بلغت (93%) عند المعاملة بتركيز(100) مايكروغرام/ مليليتر**من مدة** التعريض(48)**ساعة** .

الاستنتاجات: احتواء المستخلص الكحولي الخام لنبات الكركم على فلافونيدات، وصابونيات، ، و فينولات، و زيوت طيارة، و تانينات، وتربينات، وستيرويدات وخلوهُ من القلويدات و كلايكوسيداتوالكومارينات، االدراسة الخارج الخلوي وجد ان المستخلص الكحولي الخام ذات فعالية ضد خلايا الجنين **ألفأري** المتحولة وذات فعالية على الخط AMN3 **والخط** HEP2 بالاعتماد على التركيز والوقت، الخط الخلوي لجنين الفأر المتحولة **و**AMN3 ذو حساسية وذات تأثير مثبط على الخلايا السرطانية، ولقد بينت النتائج المستخلص الكحولي الخام لرايزومات الكركم ذو حساسية اقل عند التراكيز الواطئة وذات تأثير اعلى عند زيادة التركيز بعد 48 ساعة من وقت التعريض.

التوصيات:استخدام مستخلص الكركم على خطوط خلوية سرطانية طبيعية وعلى خطوط خلوية سرطانية **أخرى مثل سرطان المعدة وسرطان القولون وسرطان الكبد**، استخدام تقنيةcomet test لمعرفة فعالية مستخلص الكركم على المادة الوراثية للخلايا السرطانية، استخدام المذيبات العضوية الاخرى مثل الميثانول لاستخلاص المركبات الفعالة لنبات الكركم.

Abstract

Objectives: This study aims to detect the active compounds found in ethanolic crude extract of Curcuma longa rhizome and evaluate the cytotoxic effect of ethanolic extract on two malignant cell lines; Human laryngeal Carcinoma (HEP-2) cell line, murine mammary adenocarcinoma (AMN-3) cell line and one transformed cell line of Rat Emberyogenic Fibroblast (REF-3).

Material and methodology:

Administrative Arrangement: This study was conducted in Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR), Baghdad, Iraq to period (1 / 1 / 2011 to 1 / 1 / 2012), periods of exposure of cell lines Different concentrations were prepared from ethanolic extract starting from (1 to 10000) µg/ml to were measured at (24 , 48 , and 72)hrs in a microtitration plate under complete sterile conditions.

Results: Chemical detection of extract appeared that the ethanolic extract contain flavonoid, saponin, phenols , resins , volatile oils , tannins , terpins and steroids while glycosids, alkaloids and cumarines gave negative results appeared the ethanolic extract of curcuma longa showed inhibition effect at 48 hr. period for all concentration on cancer cell lines. And amount the inhibition percentage of (AMN-3) cell line (88.3%) for treatment in concentration(6µg/ml) after (24hr). from exposure time, and inhibition percentage begin in lower concentration on (Hep-2) cancer cell line and gave inhibition percentage (77.1%)after treatment in (2000 µg/ml) after (48hr). from exposure time and appeared the ethanolic extract on REF transformed cell line and gave higher inhibition percentage(93%) after treatment in (100µg/ml) from exposure time.

Conclusion: The ethanolic crude extract of Curcuma longa Which contain flavenoid, saponin, phenols, resins, Volatile Oils, tannins, terpins and steroids while alkaloids, glycosids, and cumarines gave negative results, In the in vitro study,Theethanolic crude extract of C. longa has a potent cytotoxic activity against REF transformed cell line, AMN-3 and Hep-2 cell lines in a concentration and time dependent manners (in vitro), The cell lines REF and AMN-3 were the most sensitive to the inhibitory effects of ethanolic extract of C. longa rhizomes,The cell line Hep-2 showed less response at low concentration while the extract had cytotoxic effect at highest concentration after exposure 48 hr.

Recommendations: Using further different cancer cell lines as well as normal cell lines to study the cytotoxicity of Curcuma longa extract such as oral , stomach and colon cell lines. Using the comet test technique to detect the effects of plants extracts on DNA, use different solvent to extract ofCurcuma longa for example methanol.

Key word: curcumin extract, cell lines, in vitro.

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INTRODUCTION:

Usage of medical plant and herb are very old that it begin with human civilization at Summaries time since 2500 BC. The idea of use the herbal medicine appears clearly at the 17th BC when there was found tangible indication and manuscript about treatment by herbal medicine. The usage have flourished at Saurian time and many Islamic philosophical were famous at this aspect like Abu-Baker Al Razy (925-864) AC, Avicenna (980-1038) AC and Abno- Al Bettar (1228) AC (AL-Rawi and Chakravarty, 1998). Curcumin (Curcuma longa.) is one of the most widely used herbs and food flavouring. Its neutraceutical properties have been interesting in food processing and the pharmaceutical industries (Balachandran et al., 2006).Many patients that do not response to conventional therapies and all oncologists often seek help in complementary alternative medicine treatment currently, complement alternative medicine cancer prevention strategies employ, herbs, foods, spices and specific nutrition's toward specific physiological pathway(Balachandran et al., 2006).Curcumin, or diferuloylmethane, is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*. It is better known as the yellow pigment in the widely used Asian spice turmeric. Recently, curcumin gained attention as an anti-cancer agent because of its chemopreventive and chemotherapeutic potential while having no discernable side effects,Curcumin induces apoptosis in various tumor cells and can prevent tumor initiation and growth in carcinogen-induced rodent models as well as in subcutaneous and orthotopictumorxeno grafts; although it is still not known why curcumin preferentially kills tumor cells, it has been identified as one of the major natural agents that inhibit tumor initiation and tumor promotion**(**Seung et al 2012)**.**Curcumin inhibits the proliferation of a wide variety of cancer cells including breast, blood, colon, liver, pancreas, kidney, prostate, and skin,we and others have shown that it induces cell death in medulloblastoma, the most common pediatric brain tumor and inhibits tumor growth in *in vivo* medulloblastoma models. Curcumin has been suggested to selectively target tumour cells by affecting signaling pathways that regulate cell growth and survival, and thus preferably induces apoptosis in highly proliferating cells . Accumulating evidence suggests that curcumin-induced cell death is mediated both by the activation of cell death pathways and by the inhibition of growth/proliferation pathways(Lee *et al* 2011). Cell cycle regulatory proteins and checkpoints are downstream elements of cellular signaling cascades crucial for cell proliferation. Curcumin exerts various effects on cell cycle.Proteins and checkpoints, including protein 53(p53), cyclin D1, cyclin dependent kinases (CDK), and CDK inhibitors (CDKi) such as $p16^{INK4a}$, $p21^{WAF1/CIP1}$, and $p27^{KIP1}$, It most often induces G2/M arrest, although gap1 and gap0(G0/G1) arrest has been found in some cells. It is well accepted that a prolonged arrest in G2/M phase leads to apoptotic cell death;however, how curcumin induces G2/M arrest is not well understood (Karunagaran and Joseph, 2007). In USA proofing **Seung and Sigid**(2012) effect of curcuma curcumin-induced cell cycle arrest and apoptosis. In England proofing Stacey*et al* (2011), effect of curcumininduced apoptosis in osteosarcoma cell lines.In India Proofing Ishita (2010), effect the curcumin inhibits proliferation of cancer cell line and in Iraq mention Hussien*et al(*2008) effect curcumin on growth inhibitory effect of Cabbage extracts on Hep-2 cell line, refers to have an antitumour effect.Turmeric has different biological activities and pharmacological actions, like antitumour effect that inhibit the *in vitro* proliferation of cancer cell line (Al- Taee*et al*., 2008).

It has been used in traditional medicine as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism andsinusitis. For the last few decades, extensive work has been done to establish the biological activities and pharmacological actions of turmeric and its extracts. Curcumin (diferuloyl methane), the main yellow bioactive component of turmeric, has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory ,antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenin, antiulcer, hypotensive and hypocholesteremic activities. Its anticancer effect is mainly mediated through induction of apoptosis. Its anti-inflammatory, anticancer and antioxidant roles may be clinically exploited to control rheumatism, carcinogenesis and oxidative stress-related pathogenesis **(**Stacey *et al* 2011).Clinically, curcuminhas already been used to reduce post-operative inflammation. Safety evaluation studies indicate that bothturmeric and curcumin are well tolerated at a very high dose without any toxic effects. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases. Uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. The colouring principle of turmeric is the main component of this plant and is responsible for the anti-inflammatory property (Zhang et al 2011).Its derivatives, demethoxy curcumin and bis-demethoxy curcumin also have antioxidant effect and Curcumin exerts powerful inhibitory effect against H2O2-induced damage in human keratinocytes and fibroblasts and in NG 108-15 cells80 (Song, 2001).

OBJECTIVES OF THE STUDY:

1. Detection of the active compound in crude extract of rhizome of *Curcuma longa* .
2. Studying the effect of ethanolic crude extract of rhizome of *Curcuma longa*on the growth of two cancer cell lines (AMN-3 and Hep-2) and one transformed cell line REF (*in vitro*).

Materialsand methodology:

Administrative Arrangement: This study was conducted in ICCMGR, Baghdad, Iraq to period (1 – 1 2011 to 1 – 1 2012), periods of exposure of cell lines were measured at (24 , 48 , and 72) hrs. in a microtitration plate under complete sterile conditions.

Setting of the study: The study was carried out inICCMGR, Baghdad, Iraq to period(1 / 1 / 2011 to 1 / 1 /2012), periods of exposure of cell lines were measured at(24 , 48 , and 72) hrs.

Study Design: Analytical study was carried out to to detect the active compounds found in ethanolic crude extract of *Curcuma longa* rhizome, Different concentrations were prepared from ethanolic extract starting from (1 to 10000) µg/ml and to evaluate the cytotoxic effect of ethanolic extract on two malignant cell lines Human laryngeal Carcinoma (HEP-2) cell line and murine mammary adenocarcinoma (AMN-3) cell line and one transformed cell line of Rat Emberyogenic Fibroblast (REF-3).

Tool of the study:

Rhizome collection:

*C.longa*rhizome**s** were collected from local markets**.** Therhizomes were washed several times with distilled water and air dried at room tempreture then grinded in to coarse powder by electrical grinder and kept in clean plastic bottle (Harborn, 1984).

Preparation of ethanolic extract:

Fifty grams of *C. longa*rhizome powder were extracted with ethanol 250 ml by soxhlet for 6 hours at 45-50 ºC, the ethanolic extract evaporated under

vaccum to dryness by rotary evaporator at 40ºC and kept at 4 ºC until use (AL-Jeboory, 1994).

Detection of alkaloids:

Myer's test

Myer's reagent was prepared by:

1. Solution 1: 1.35 g of mercuric chloride was dissolved in 60 ml distilled water.
2. Solution 2: 2.5 g of Potassium iodide was dissolved in 10 ml distilled water.

Few drops of the freshly prepared reagent were added to 5 ml of sample, a white precipitate will appear If alkaloids are present (Souse*k et al.*, 1999)

Detection of glycosides:

Benedict test

137 g of sodium citrate and 100 g of $Na_2CO_3.H_2O$ were dissolved in 800 ml of distilled water, the mixture were filtrate and added 17.3 g cupric sulfate was dissolved in 100 ml of distilled water and added to the previous filtrate then volume completed to 1000 ml with distilled water.2ml of the prepared indicator was added to a test tube containing 1 ml of plant extract and placed in water bath for 5 minute at 50 C^0 and left to cool, Red precipitate will appear if glycosides are present (Sheikhly and Al-azawy, 1993).

Detection of flavonoids:

Few drops of 0.5 N potassium hydroxide were added to 5 ml of extract sample. The presence of flavonoids was indicated by the formation of dark color (AL-Shahaat, 1986).

Detection of tannins:

Few drops of the freshly prepared ferric chloride (1%) were added to 5 ml of extract sample. if tannins were present, the solution should turn blue-green (Cannell, 1998)

Detection of saponins:

A sample of plant extract was shacked in a test tube, formation of foam indicating to presence of saponins (Harborn, 1984).

Detection of phenol:

Mixing equal volumes of 1%aqueous ferric chloride solution, reagent and alcoholic plant extract. The appearance of blue-green colour indicates to the presence of phenols (Harborne, 1984).

Detection of essential oils:

Ten ml of plant extract was saturated with filter paper then exposed to Ultra Violet (UV) light source for 10 min. The presence of pinkish color indicates the presence of essential oil (Harborne, 1984).

Detection ofterpene and steroid:

Two ml of chloroform was add to one drop of glacial acetic acid and one drop of concentrated sulfuric acid (H_2SO_4), mixed and added to one ml of crud extract, the appearance of pink colour indicates the presence of terpen while formation of blue color after leaving the sample for one minute indicates the presence of steroid (Cannell, 1998).

Detection of resins:

Ten ml of plant extract were added to 50 ml ethanol 95% the mixture were left in waterbathat temperature 50 C^0 for two minute then filtered and added to 100 ml acidic distilled water with hydrochloric acid, appearance of turbidity indicates positive test (Harborne, 1984).

Detection of coumarins:

Five grams of the dried water plant extract was dissolved in some drops of alcohol in a test-tube, covered with filter paper, sprayed with NaOH, then placed in a water bath until boiling, and then the filter paper was placed under UV light spectrum. The appearance of greenish-yellow color indicated the presence of coumarins (Harborn, 1984).

Cell line:

Hep-2 cell line:

The origin and description of this cell line was mentioned by (Moore *et al*., 1955). The passage number was 224 were used in this study. It was a human laryngeal carcinoma excised from 57 years old man, then transplanted in immune suppressed rat by cortisone after growth of the tumor in the rat, it was then excised and transplanted as an *in vitro* tissue culture by using culture media RPMI-1640 provided with 10%fetal calf serum(FCS).

Murine Mammary Adenocarcinoma (AMN-3) cell line

The origin and description of this cell line was first mentioned by Al-Shamery, (2003) from balb/c species female mouse carrier of cell line AMN-3 in vivo (murine mammary adenocarcinoma). The passage number was 186were used in this study. Maintained in RPMI- 1640 containing 10% bovine calf serum.

Rat Embryo Fibroblast cell line (REF)

The transformed culture of the rat embryo is the most important source for the undifferentiated fibroblastic culture. This cell line was supplied by tissue culture unit / ICCMGR, Baghdad, Iraq. The passage number was (92) were used in this study. The specimen was taken from rat embryo then killed and analysed by Trypsin then it was maintained in RPMI-1640 medium with 10% FCS.

RESULT:

The results of in vitro studies revealed the cytotoxic, of the incidence when ethanolic extracts of Curcuma longa were tested.

Extraction of Curcuma longa

Extraction was accomplished on 50 gm for the extraction, ethanolic crude extract (ECE).

Table (1): Detection of active compounds in *C. longa*.

Active compound	Reagent	indicator	Result of detection
Glycosides	Benedict reagent	Red precipitate	_
Alkaloids	Myer's reagent Dragendorf test	White precipitate orange Precipitate	_
Saponins	A- extract mixing B- $HgCl_2$ 1%	Thick foaming White precipitate	+
Resins	Ethanol, boiling, D.W.	Turbid	+
Flavonoids	Petroleum ether + Ammonia	Dark color	+
Phenols	Ferric Chloride 1%	Blue-Green color	+
coumarins	Filter paper+ NaOH+ U.V.	Blue-Green color	_
Essential Oils	Filter paper +Extract+ UV	Rosy or pink	+
Terpine	Chlorophorm+non $CH_3COOH+H_2SO_4$	pink color	+
Steroids	Similar for terpine reagent	Blue color	+

The result of this table shows that the chemical detection for the active compounds in the ethanolic crude extract of *C. longa*. which contain flavenoid, saponin, phenols, resins, Volatile Oils, tannins, terpins and steroids are positive results while alkaloids, glycosids, and cumarines gave negative results, pH of the extract were 4.2, this value refers to acidic properties of the extract because of the absence of alkaloid.

Cytotoxicity effects of ethanolic crude extract of Curcuma rhizome on REF transformed cell line in vitro:

The extract was found to reduce the viability of cell culture. The inhibition percentage was 93% for concentration 100µl at 48hr.

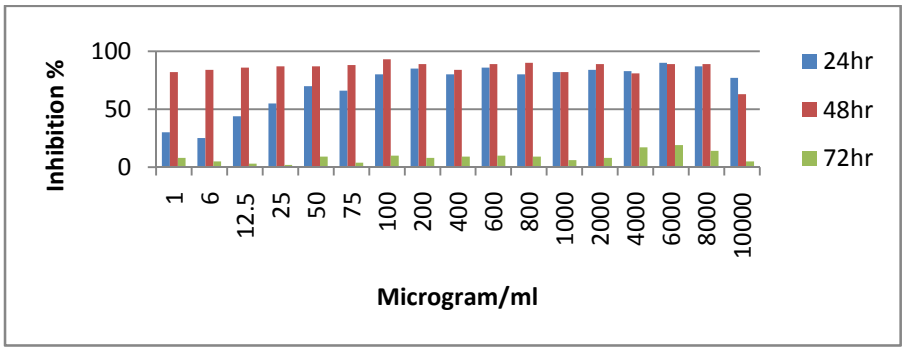


Figure (2): Cytotoxicity effects of ethanolic crude extract *C.longa* rhizome on REF transformed cell line in vitro during different exposure time

The results revealed that all the concentrations of the extract after 24 and 48 hrs exposures decreased the cell number and their optical density. While continuous exposure to various concentration of ethanolic extract of curcuma in time and dose dependent caused an increase in the number of REF cell line highly significantly ($p<0.05$) at all concentrations. The present study indicated that the extract had more effect on REF cell after 24 and 48 hrs of exposure time.

Table (2): Summary Statistics that comparing between different exposure times in different concentrations of Ref cell line

Statistics	EXPOSURE TIME		
	24hr	**48hr**	**72hr**
Mean	72.294	88.000	8.824
Std. Deviation	**± 21.268**	± 1.837	**± 4.531**
Minimum	25.000	82.000	**2.000**
Maximum	**93.000**	90.000	19.000
t- Test	**t=14.005 *(HS)**	**t=197.389 (HS)**	**t= 7.984 (HS)**

*HS= P<0.000

In table (2) observed the comparison between different exposure time, highly significant (HS= P<0.000), and observed mean after 24hr is 88.000 (HS= P<0.000).

Cytotoxic effect of ethanolic crude extract of curcuma on AMN-3 cell line in vitro:

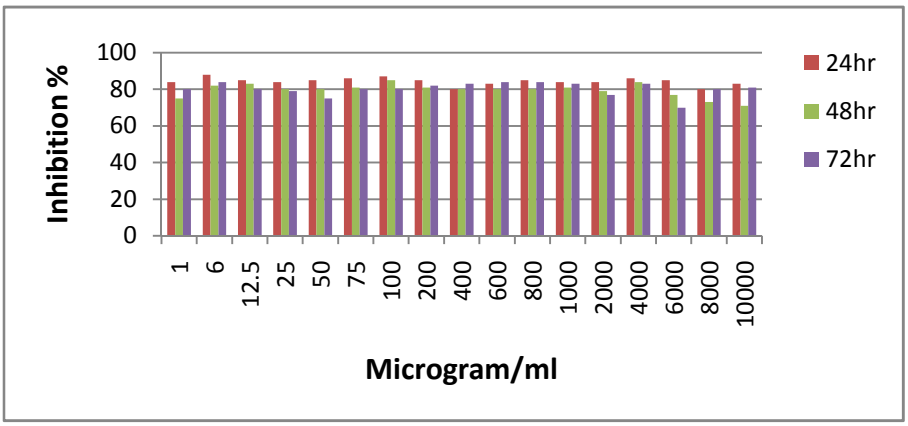


Figure (3): Shows the cytotoxicity activity of the ethanolic of *C.longa* rhizome on AMN-3 cell lines *in vitro* during different exposure time

Figure (3) shows the cytotoxicity assay on AMN-3 cell lineduring 24, 48 and 72 hrs. The extract was found to reduce the viability of cell culture at three exposure time for all concentrations used. This inhibitory effect was reduced after contentious exposure until reach 72 hr of exposure, that there was highly significant differences at level ($p<0.05$) for all concentrations(1,6,12.5,25,50,75,100,200,400,600,800,1000,2000,4000,6000,8000,10000 µg/ml) compared with control. The highest inhibition was 88.3% at concentration 6 µg/mloptical density (0.051) after 24hr. from exposure time.

Table (3): Summary Statistics that comparing between different exposure time in different concentrations of AMN-3 cell line

Statistics	EXPOSURE TIME		
	24hr.	**48hr.**	**72hr.**
Mean	84.765	**80.000**	82.882
Std. Deviation	**±1.786**	± 3.841	± 2.147
Minimum	83.796	77.975	**81.728**
Maximum	**85.633**	81.925	83.936
t- Test	**t=195.528 *(HS)**	**t=85.832 (HS)**	**t= 159.059 (HS)**

*HS= P<0.000

In table (3) show comparesion between different exposure time and observed highly significant(HS= P<0.000), and mean after 24hr is 80.000 (HS= P<0.000).

Cytotoxic effect of ethanolic crude extract of Curcumin on HEP-2 cell line in vitro:

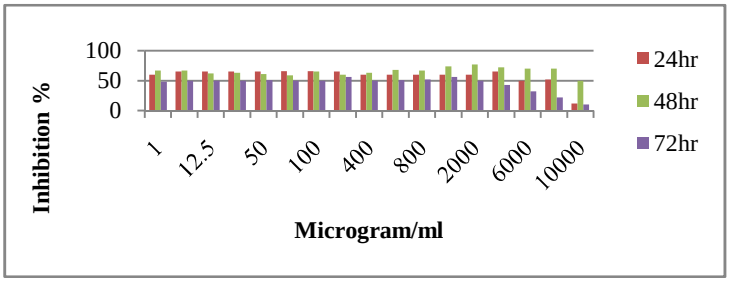


Figure (4): Cytotoxicity activity of the ethanolic extract of *C. longa* rhizome on HEP-2 cell lines *in vitro* during different exposure time

The result of this figure shows of cytotoxicity assay of crude extract of *C. longa* on HEP-2 cell line during 24 , 48 and 72 hrs and how the extract were reduced the viability of HEP-2 cell line show in Figure (3). This effects were begun after 24 hr of exposure time of the cells to the extract, the extract shows low cytotoxicity on the cells in 24 hr. The highest inhibition were seen at 48hr of exposure time at the concentration 1000, 2000, 4000 µg/ml and then decreased with decreasing the concentrations.

The cell inhibition were decreased at 72hr of exposure period, and this may be due to the toxic effect of the extract on the cells exhausted of the media leads to decreased cell viability. the significant differences at level ($p<0.05$) between the concentrations starting from the concentration 1000, 400, 200, 75, 25 and 1 µg/ml compared with control treatment after 24 hr of exposure period.

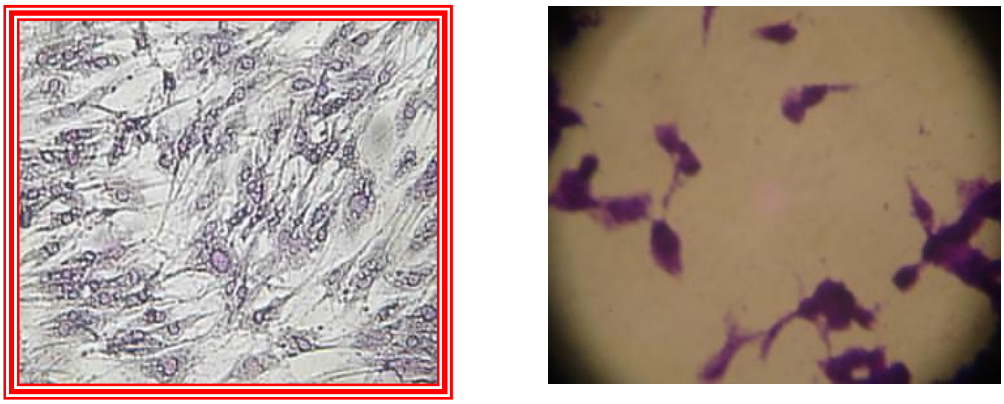
The concentration 2000 µg/ml optical density (0.057) gave the highest inhibition effect on cell growth at 77.1% after exposure time 48hr.

Table (4): Summary Statistics that comparing between different exposure time in different concentrations of Hep-2 cell line

Statistics	EXPOSURE TIME		
	24hr.	**48hr.**	**72hr.**
Mean	58.538	**65.538**	45.302
Std. Deviation	± 12.884	**± 6.414**	**± 12.429**
Minimum	51.914	62.241	**38.912**
Maximum	**65.163**	68.835	51.694
t- Test	**t=18.733 *(HS)**	**t=42.133 (HS)**	**t=15.028 (HS)**

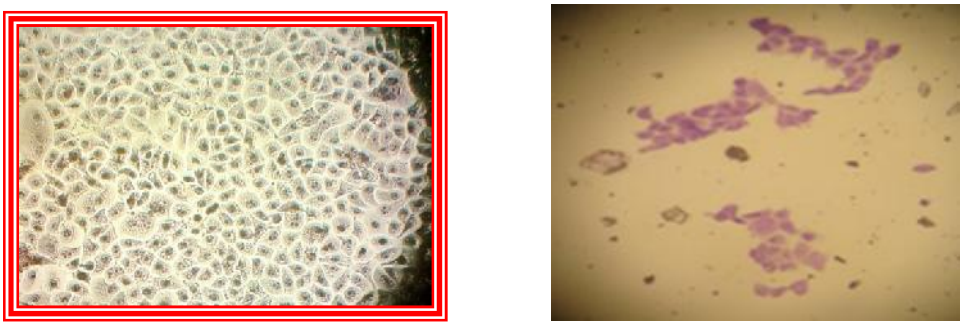
*HS= P<0.000

In table (4) show comparesion between different exposure time, highly significant (HS= P<0.000), and mean after 24hr is 65.538 (HS= P<0.000)



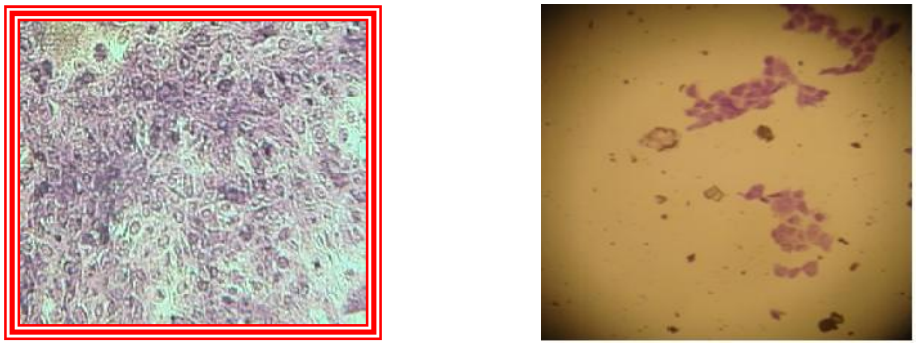
A B

Figure (5): A- Show negative control REF cell line shows confluent monolayer (200 X), B- REF cell line after their exposure to 100 µg/ml for 24 hr of curcuma extract (200 X).



A B

Figure (6): A- Show negative control of AMN-3 cell line shows confluent monolayer (200 X), B- AMN-3 cell line after exposure to 6µg/ml of curcuma extract after 24hr. (200X).



A B

Figure (7): A- Hep-2 cell line shows negative control confluent monolayer (200 X), B- HEP-2 cell line after their exposure to 2000 µg/ml for 48hr. of curcuma extract (200X).

DISCUSSION:

Cancer chemotherapy with strategies using foods and medicinal herbs has been regarded as one of the most visible fields for cancer control (Jo *et al*.,2004).These natural products include, flavenoid, saponin, phenols, resins, Volatile Oils, tannins, terpins and steroids while alkaloids, glycosids, and cumarines gave negative results, this result agree with Kathi and Kamper, (1999) andSobaihat (2008).*C. longa*is characterized by pungent taste and aromatic smell due to the presence of Terpenes and volatile oil (Awang, 1982).

Extraction

The amount of crude product resulted from the extraction process was consistent to that resulted in the study of Al-Khazraji (1991).The relative proportion between the amount of plant used for extraction and crude product was variable depending on several factors, such as the method of extraction and solvent used in extraction process as well as the type of study plant (Henning *et al*.,2003).

Cell growth assay (*In vitro* study):

The results showed that growth inhibition was significantly progressed as the concentrations of extract increased as well as the time of exposure. While the effect of extract type was variable according to type of cell line and the time of exposure. The obvious result obtained in the growth inhibitory assay was the dose- and time- dependant cytotoxic effect of the two extracts on both study cell lines.The present results were consistent with that reported in the study of Campbell *et al.* (2002) in which dose-response curves were obtained for several of the most potent crude extracts. Flow cytometric analysis of breast cancer cells suggest that the herb arrests cells in the G2/M phase of the cell cycle. They are also agree with the results of Al-Qadoori (2004), Sa'eed (2008) and Aggarwal*et al* (2009)They were mentioned that the plant extracts of their studies have cytotoxic effects on different cell lines such as Hep-2, AMN-3 and RD in a dose- and time-dependant manner.

Cytotoxicity effects of ethanolic crude extract of Curcuma rhizome on REF transformed cell line:

The cell viability reached its lowest limit extended exposure time for 72 hr. for all concentration figure (2). The extract show more activity against REF cells. This selectively effect may be due to many factors including PH and osmolality. Despite most cell-lines grow well in PH 7.4, the optimum PH for cell growth varies for different cell strains, and some epidermal cells may be maintained at PH 5.5 (Freshney, 1994). Since curcumin extracts are relatively acid (Sobaihat, 2008), therefore, it may alter the PH of the solution in which the cells were suspended particularly at its highest concentration, while this effect was diminished as concentration dropped, or this selective effect of extractions may appears on the cell adhesion.The other factor may be osmolality effect, in which extracts of curcuma is rich in proteins, carbohydrates, minerals and other constituents which make it hypertonic solution and may cause dose-dependent osmotic shock to cell-lines(Combest, 2007).

Cytotoxicity effects of ethanolic crude extract of Curcma rhizome on AMN-3 cell line:

The ability to bind with plasma membrane receptor of AMN-3 cell differs according to the origin and type of cells. A variety of highly specific receptors molecules are integral component of the cell surface. These constitute re cognition sites that receive chemical signal are often transferred to the cell. Such signals may initiate a variety of chemical activities and may to turn of ultimately signal specific genes (Sugden and Davidson, 2004). Both the nutritive and the non-nutritive components of the diet play a significant role in the inhibition of carcinogenic process, The non-nutritive constituents

exert their anti-carcinogenic effect by various mechanisms via:1. By virtue of their anti-oxidant property.

2. Deactivating the carcinogens

3. Or enhancing the tissue levels of protective enzymes in the body(Kim *et al* 2005). Toxic metabolites of harmful drugs and chemicals are detoxified by the bodiesdefense system. Phytochemicals in species like turmeric, mustard and allium vegetables may active more than one way to confer their beneficial effect (Krishnaswamy and Polasa, 2001).

Cytotoxicity effects of ethanolic crude extract of Curcuma rhizome on HEP-2 cell line:

This result agree with Padma*et al*., (2007) mention that 80% of inhibition could obtain when treating HEP-2 cancer cell lines with curcuma crud extract and using concentrations from 1000-100 µg /ml and gave inhibition rate 80% for HEP-2 and agree with Growth inhibitory effect of Cabbage extracts on Hep-2 cell line refers to have an antitumour effect (Hussien*et al*., 2008).The inhibition activity of extract against REF and AMN-3 cell growth observed during long period at all concentrations while their inhibitions against the growth of HEP-2 cell line were observed during short period at low concentrations. This can be attributed to sensitivity of REF and AMN-3 cell and or may be due to the cytotoxic activity of active compounds in the extract during exposure periods. The differences in Hep-2 response toward different treatments might indicate a presence or absence of specific cellular receptors in each type of cell lines; making the cells interact at same concentration in different manners. Moreover the metabolic pathways in response to each treatment differed from one line to another. This fact was mentioned in different studies which investigated at different plant extracts in treating several types of cell lines (Li *et al*., 2003).*C. longa* contains bioactive phenolic substances with potent antioxidative and chemopreventiveproperties. The antioxidant activity of phenolic compounds is mainly due to their properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and treble oxygen or decomposing peroxides, The phenolic compounds in many plant and vegetables, including curcumin may contribute directly to antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily is ingested from a diet rich in fruits and vegetables (Gulcin*et al*., 2002). Apoptosis is a major form of cell death and essential for normal development and for maintenance of homeostasis. In addition, current anti-neoplastic therapy, chemo-therapy and radiation therapy are likely to be effected by the apoptosis tendencies of cells, thus, this process has obvious therapeutic implication, The ability in inhibition or in enhancing apoptosis by plant extracts depends on several factors such as; extract concentration, concerted action of multiple micronutrients, cell type (Palozza *et al*., 2004). Recently, curcumin root and its main poly-phenolic constituents (turmeric , zerumbone) has been shown inhibit of the transcription factor (Nuclear

Transcription Factor Kappa-),NF-κB plays an important role in tumorigenesis, given its ability to control the expression and function of numerous genes involved in cell proliferation, sustained angiogenesis, and evasion of apoptosis. Different tumor types, including ovarian cancer, have been shown to express high constitutive nuclear factor-kappa-B(NF-κB) activity (Pacificoand Leonardi, 2006). Curcumin has also been shown to alter the expression and function of COX2 and 5-LOX at the transcriptional and post-translational levels. Thus, it is possible that many of the cellular and molecular effects observed in curcumin treated cells might be due to downstream effects rather

than direct interactions with curcumin**(**Seungand Sigrid**,** 2012).Curcumininduces apoptosis and inhibits cell-cycle progression, both of which are instrumental in preventing cancerous cell growth in rat aortic smooth muscle cells, The antiproliferative effect is mediated partly through inhibition of protein tyrosine kinase and c-myc mRNA expression and the apoptotic effect may partly be mediated through inhibition of protein tyrosine kinase, protein kinase C, c-myc mRNA expression and bcl-2 mRNA expression, Curcumin induces apoptotic cell death by DNA-damage in human cancer cell lines, TK-10, MCF-7 and UACC-62 by acting as topoisomerase II poison. Recently, curcumin has been shown to cause apoptosis in mouse neuro 2a cells by impairing the ubiquitin–proteasome system through the mitochondrial pathway (Martin-Cordero *et al* 2003).

Expression of glutathione S-transferase (protein 1) P1 (GSTP1) is correlated to carcinogenesis, and curcumin has been shown to induce apoptosis in K562 leukaemia cells by inhibiting the expression of (GSTP1) at transcription level. The mechanism of curcumin-induced apoptosis has also been studied in Caki cells, where curcumin causes apoptosis through down regulation of Bcl-XL and protein A1 (IAP), release of cytochrome *c*, which are markedly blocked by *N*acetylcysteine, indicating a role of ROS in curcumin induced cell death. In LNCaP prostate cancer cells, curcumin induces apoptosis by enhancing tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). The combined treatment of the cell with curcumin and TRAIL induces DNA fragmentation, cleavage of procaspase 3, 8 and 9, truncation of Bid and release of cytochrome *c* from mitochondria, indicating involvement of both external receptor- mediated and internal chemical-induced apoptosis in these cells (Duvoix, 2003). In colorectal carcinoma cell line, curcumin delays apoptosis along with the arrest of cell cycle at G1 phase, Curcumin also reduces *P53* gene expression, which is accompanied with the induction of HSP-70 gene through initial depletion98 of intracellular Ca2+. Curcumin also produces nonselective inhibition of proliferation in several leukaemia, nontransformed haematopoietic progenitor cells and fibroblast cell lines. That curcumin induces apoptosis and large-scale DNA fragmentation has also been observed in V*g*9V*d*2+ T cells through inhibition of isopentenyl pyrophosphate-induced NF*k*B activation, proliferation and chemokine production. However, curcumin affects different cell lines differently. Whereas leukaemia, breast, colon, hepatocellular and ovarian carcinoma cells undergo apoptosis in the presence of curcumin, lung, prostate, kidney, cervix and CNS malignancies and melanoma cells show resistance to cytotoxic effect of curcumin (Choudhuri *et al* 2002).

CONCLUSION

From the results of the present study, the following conclusions could be considered:-

The ethanolic crude extract of Curcuma longa Which contain flavenoid, saponin, phenols, resins, Volatile Oils, tannins, terpins and steroids while alkaloids, glycosids, and cumarines gave negative results, In the *in vitro* study,The ethanolic crude extract of *C. longa* has a potent cytotoxic activity against REF transformed cell line, AMN-3 and Hep-2 cell lines in a concentration and time dependent manners (in vitro), The cell lines REF and AMN-3 were the most sensitive to the inhibitory effects of ethanolic extract of *C. longa* rhizomes, The cell line Hep-2 showed less response at low concentration while the extract had cytotoxic effect at highest concentration after exposure 48 hr.

RECOMMENDATIONS:

1. Using further different cancer cell lines as well as normal cell lines to study the cytotoxicity of *Curcuma longa* extract

such as oral , stomach and colon cancer cell lines.
2. Using the comet test technique to detect the effects of plants extracts on DNA.
3. Use different solvent to extract*Curcuma*longa for example methanol.

REFERENCES

1. **Balachandran, S.; Kentish, S.E. and Mawson, R. (2006).** The effect of both preparation method and season on the super critical extraction of curcumin and ginger,.J.Purif. Technol., 48: 94-105.
2. **Seung, J. L.** and **Sigrid, A. L. (2012).**Anaphase-promoting complex/cyclosome protein Cdc27 is a target for curcumin-induced cell cycle arrest and apoptosis.*BMC Cancer*, 12**:**44 doi: 10.1186/1471-2407-12-44.IVSL.
3. **Lee SJ, Krauthauser C, Maduskuie V, Fawcett PT, Olson JM, Rajasekaran SA (2011)**: Curcumin-induced HDAC inhibition and attenuation of medulloblastoma growth in vitro and in vivo. *BMC Cancer*, **11:**144. PMCID: 3090367.IVSL.
4. **Karunagaran D, Joseph J, Kumar TR**: **Cell growth regulation (2007).** *AdvExp Med Biol*, **595:**245-268.
5. **Ishita C., Kaushik B., Uday B. And Ranajit K. Banerjee (2010).** Turmeric and curcumin: Biological actions and medicinal applications. *Cancer Lett*., 2010, 94, 79–83.
6. **AL-Taee,F.A.; Barakat, N.T.; Jaafer, T.H.; AL-Saady,K.R.. (2008).** Effect of Curcumin crude extract on cancer cell line. Iraqi Journal of Cancer. 1: 10-12.
7. **Hussien, S.M.; AL-Taee, F.S.; AL-Saady, K.R.; Barakat, N.T. and Huseen, R.A. (2008).** Growth Inhibitory Effect of Cabbage Extracts on Hep-2 Cell line. 1: 53-56
8. **Zhang L, Fujita T, Wu G, Xiao X, Wan Y (2011):** Phosphorylation of the anaphase-promoting complex/Cdc27 is involved in TGF-beta signaling. *J BiolChem*,286(12)**:**10041-10050.IVSL.
9. **Song, E. K.,(2001):** Diarylheptanoids with free radical scavenging and hepato protective activity *in vitro* from *Curcuma longa*. *Planta Med*., 67, 876–877.
10. **Harborne, J. B. (1984)**. Phytochemical Methods. 2nd ed. Chapman and Hall, London. p. 5.
11. **Cannell, P. (1998).** How to Approach the Isolation of NaturalProduct. 1st ed. Human. Press. Inc.
12. **Al-Jeboory, A. (1994).** Natural pharmacology, the future of medical plant in drug and medicine industry. 1st ed. Baghdad. Dar Al Huria press. p.75.
13. **Sousek J, Guedon D, Adam T, Bochorakova H, Taborska E, Valka I and Simanek V. (1999).** Alkaloids and organic acid content of eight *Fumaria* species. J. Phytochemical Analysis, 10: 6-11.
14. الشيخلي، محمد عبد الستار; العزاوي، فريال حسن فياض (1993). المركبات الطبيعية في النبات. دار الكتب للطباعة والنشر، الموصل ص 221-224 .
15. **Al-Shahaat, N. A. Z. (1986).** Plants and Medicinal Herbs. Dar AI-Behaar, Beirut. pp. 140-146. Cited in: Sa'eed, O. F. (2004). The Effect of Green and Black Tea Extracts on Different Cell Lines *in Vitro*. M. Sc. Thesis, College of Pharmacy, University of Mosul, Iraq.
16. **Moore, A.E.; Sabachewesky, L. and Toolen, H.W. (1955).** Culture characteris of four permanent lines of human cancer cells. J. Cancer Res., 15: 598-605.
17. **Al-Shamery, A. M. H. (2003).** The study of Newcastle disease virus effect in the treatment of transplanted tumor in mice. M.Sc. Thesis. College of veterinary medicine, University of Baghdad, Iraq.
18. **Kathi, J.; Kamper, MD, MPH. (1999).**Curcumin (*Curcuma longa*) The Longwood Herbal task force and the

center for Holistic pediatric Education & Research Pp: 1-18.

19. **Jo,E.H.;Hong,H.D.;Ahn,N.C.;Jung,J.W.;Yang,S.R.;Park,J.S.;Kim,S.H.andKang,K.S.(2004):**Modulations of the Bcl-2/Bax family were involved in the chemopreventive effects of licorice root in MCF-7 human breast cancer cell line.J. Agric. Food Chem.,52:1715-1719.
20. صبيحات، مروة حسين خضير(2008). تقييم الفعالية البيولوجية لمستخلصات نبات الزنجبيل *Zingeberofficinale*Rosc . رسالة ماجستير. كلية العلوم، جامعة بغداد.
21. **Awang, D. (1982).**Curcumin Canadian. J. pharmaceutical. 4:125-126.
22. **Al-Khazraji,S.M.(1991):**Biopharmacological study of *Artemisia herbaAlba*,M.Sc.Thesis, College of Pharmacy. University of Baghdad, Iraq.
23. **Henning, S.M.; FajardoLira, C.; Lee, H.W.; Youssefian, A.A.; Go, V.L.W. and Herber, D.(2003).**Catechine content of 18 teas and a green tea extract supplement correlates with the oxidant capacity. J. Nutrition and Cancer, 45:226-235.
24. **Campbell,M.J.;Hamilton,B.;Shoemaker,M.;Tagliaferri,M. and Cohen, J.(2002):**Antiproliferative activity of Chinese medicinal herbs on the breast cancer cells *in vitro*. Anticancer Res.Nov-Dec.,22:3843-3852.
25. **Sa'eed,O.F.(2004):**The effect of green and black tea extracts on different cell lines *in vitro*. M.Sc. Thesis, College of Pharmacy, University of Mosul, Iraq.
26. **Aggarwal and Sung, B. (2009).** Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targetsTrends Pharmacol. Sci., 30 (2009), pp. 85–94
27. **Combest. W.L. (2007).** Herbal pharmacy: curcuma pharmacology. Campbell University school of pharmacy, Greek, NC.
28. **Sugden, D. and Davidson K. (2004).** "Melatonin, melatonin receptors and melanophores: a moving story". Pigment Cell Res.17: 454-460.
29. **Kim, E.; Jeong, K.; Tae-yoonkim , Shin-Jeong L.; Hyun-Ok Y.; Sanghwa, H.; Young-Myeong, K. and Young-Guen Kwon, (2005).** Biochemical and Biophysical Research Communication 335 (2) : 300-308
30. **Krishnaswamy, K and Polasa, K. (2001).** Non-nutrient and cancer prevention. J.ICMR Bull, 31:1-3.
31. **Padma, V.V.; Christie, S.A.D.; Ramkuma, K.M. (2007).**Induction of Apoptosis by curcuma in HEp-2 Cell Line Is Mediated by Reactive Oxygen Species. J. Basic and Clinical pharmacology and toxicology. Tamilnadu. India. 100: 302-307.
32. **Stacey L. F.**, **Misty D. B.**, **Jiayuh L.**, **Chenglong L.**, **Eric B.S.**, **Pui-Kai L.**, **James R. F.**, **Joelle F.**, **William C. K.** and **Cheryl A .L. (2011).**The novel curcuminanalog FLLL32 decreases STAT3 DNA binding activity and expression, and induces apoptosis in osteosarcoma cell lines. *BMC Cancer* 2011, 11:112 doi:10.1186/1471-2407-11-112.IVSL.
33. **Li, Y. M.; Ohmo, Y.; Minatoguchi, S.; Fukuda, K. and Fujiwora,H. (2003)**. Extracts from the roots of *Linderastrychifolia* induces apoptosis in lung cancer cells and prolongs survival of tumor. Wearing mice. Am. J. Clin. Med., 31: 857 – 869.
34. **Gulcin, I., M. Oktay, O.I. Kufrevioglu and A. Aslan, (2002).** Determination of antioxident activity of lichen *Cetraria islandica* (L.) Arch. J. Ethnopharmacol., 79: 325-329.
35. **Palozza, P., S. Serini, F. Di Nicuolo and G. Calviello, (2004).** Modulation of apoptosis signaling by carotenoids in cancer cells. J. Arch. Biochim. Biophys., 430: 104-109.
36. **Pacifico, F. and Leonardi A(2006).** NF-kappa B in solid tumors. J. BiochemPharmacol, 72:1142-1152.
37. **Duvoix, A.,** Induction of apoptosis by curcumin: mediation by glutathione S-

38. transferase P1-1 inhibition. *Biochem. Pharmacol*., 2003, **66**, 1475–1483.
39. **Martin-Cordero, C., Lopez-Lazaro, M., Galvez, M. and Ayuso, M. J.(2003):**Curcumin as a DNA topoisomerase II poison. *J. Enzyme Inhib.Med. Chem*., 18, 505–509.
40. **Choudhuri, T., Pal, S., Aggarwal, M. L., Das, T. and Sa, G.(2002):**Curcumin induces apoptosis in human breast cancer cells through p53- dependent Bax induction. *FEBSLett*.,512, 334–340.