In vivo cytogenetic and genotoxic effects of curcumin on mouse bone marrow

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الخلاصة

تهدف هذه الدراسة الى معرفة التاثيرات الوراثية الخلوية المتمثلة بمعامل الانقسام الخيطي , والانحرافات الكروموسومية , وتكوين النوى الصغيرة لخلايا نقي العظم للفئران (داخل الجسم) للمادة المطفرة (Mitomycin - C) والمستخدم بتركيز 2mg/kg بعد حقن يوم واحد, والتاثيرات الوراثية الخلوية للمستخلص الايثانولي الخام بواسطة حقن الحيوانات للمستخلص الخام لنبات الكركم *C. longa* لتراكيز مختلفة (400, 200, 100, 50 mg/kg) لسبعة ايام بواسطة الحقن تحت الغشاء البريتون, ودراسة قابلية المستخلص الكحولي الخام لنبات الكركم في تخفيض التأثير الوراثي السمي الذي تم تكوينه في خلايا نقي العظم في الفئران. لقد أظهرت النتائج أن العقار Mitomycin - C له تأثيرات سلبية تمثلت بانخفاض معامل الانقسام الخيطي, زيادة التغيرات الكروموسومية و زيادة نسبة تكوين النوى الصغيرة في خلايا نقي العظم في الفئران, هذه التأثيرات أشارت إلى امتلاك هذا العقار تأثيرات وراثية خلوية والذي اعطى 1.81% بالنسبة لمعامل الانقسام (Mitotic Index) , 4.12% بالنسبة للانحرافات الكروموسومية (Chromosome aberations), 8.13% بالنسبة لتكون النوى الصغيرة(Micro Nucleous) بعد حقن يوم واحد. وأظهرت النتائج بان التركيز 50mg/kg كان افضل تركيز والذي اعطى زيادة في MI والتي بلغت 5.40% وانخفاض نسبة CAs, MN والتي بلغت 1.801% , 0.774 على التوالي والذي كان قريب من السيطرة السالبة . وان المستخلص الخام للكركم كان له تاثيرات سمية وراثية في التراكيز الاعلى من 50mg/kg , بينما في التركيز 50 ملغم/ كغم فقد اظهر نسبة حماية ضد التأثيرات السامة للعقار MMC في خلايا نقي العظم للفئران, والتي اعطت نسبة حماية ضد التناثيرات المطفرة للمايتومايسين C .

Abstract

Objectives: Studying the cytogenetic effects of Mytomycin-C by using 2mg/kg by study of (mitotic index, chromosomal aberrations and micronucleus assays) on mouse bone marrow cells (*in vivo*) and Studying the cytogenetic effects of crude extract of *Curcuma longa* on mouse bone marrow cells and Studying the ability of crude extract of *C. longa* in reducing the genotoxic effects induced by Mytomycin-C by combination treatment on mouse bone marrow cells.

Material and methodology:

Administrative Arrangement: analytical study to period (1 / 5 / 2011 to 1 / 1 / 2012)conducted in Al-Nahrain research Centre for biotechnology, Baghdad, Iraq .

Methodology: distributed of mice on equal group each group contain five animals, negative control and injected Phosphate Buffer Solution only and positive control injecte Mitomycin - C 2mg/kg and four groups injected by ethanolic crude extract by uses concentration (400, 200, 100 and 50 mg/kg) depend on LD50 of curcumin extract and study interaction between crude extract and MMC after and befor treatment

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and determination of active the extract in preventiveor inhibition of side effect Mitomycin - C in vivo. **Results**: The results indicated that MMC has clear effects in reducing mitotic activity, increased spontaneous chromosomal aberration and increased micronucleus in mouse bone marrow cells (*in vivo*), these effects suggested that the drug has a genotoxic effect, the cytogenetic effects represented by mitotic index, chromosomal aberration and micronucleus (Mitotic Index, Chromosome abberations, Micro Nucleous) on mouse bone marrow cells (*in vivo*) of positive control Mitomycin-C use 2mg/kg for one day and gave 1.81% for MI and 4.12% for CAs and 8.13% for MN, and the cytogenetic effects of ethanolic crude extract of *Curcuma long extract* by administration the animals crude ethanolic extract at different concentration (50, 100, 200 and 400 mg/kg) for 7 days i.p., 50mg/kg was choice to as best dose which there ratio considered increase Mitotic Index (5.40%) and reduce Micro Nucleous (1.801%) and Chromosome abberations (0.774%) relatively was like negative control, the Interaction effect between extract administration pre and post treatment with drug (MMC) was estimated in cytogenetic parameters on mouse bone marrow cells, which gave a protective efficient against the genotoxic effect of Mitomycin - C in mouse bone marrow cells which gave 90% for M.I and 97.55% for CAs and 97.64% for MN, this effect was more efficient in post-treatment than in pre-treatment, ethanolic crude extract of *C. longa extract* had genotoxic effects at high doses exess of 50 mg/kg and showed Ethanolic crude extract *C. longa* was considered as fundamental biomutagene in the first degree and desmutagene in second degree as a result of its ability to increase mitotic activity, decrease micronucleus frequency and repair chromosomal aberration in mouse bone marrow cells.

Key word: Curcuma longa, invivo, cytogenetic effect.

Introduction

Curcumin, a naturally occurring yellow pigment derived from the rhizome of Curcuma longa, exhibits a variety of pharmacological actions including anti-inflammatory, anti-infectious, and anticancer actions (Aggarwal and Sung, 2009).

Curcumin (1,7-bis (4-hydroxy-3-methoxyphenol)-1,6-heptadiene- 3,5-dione) is a major polyphenolic compound isolated from the rhizomes of turmeric (Curcuma longa), Since the time of the old Asian medicine

curcumin has been used in the practice for the treatment of common cold, skin diseases, wound healing, inflammation.

Both in vivo and in vitro studies, including those concerned with the induction of genetic mutations, chromosome damage and single strand breaks in DNA, have confirmed mitomycin – C genotoxicity (Sebastia et al., 2012).

The supplements containing curcumin are commercially supplied for persons heavily consuming alcoholic beverages. Ethanol is suggested to decrease cellular energy state that appears to be an important factor in regulating apoptosis in some cell types, Ethanol increases apoptotic cell death of mouse thymocytes under in vitro condition. (Samuhasaneeto et al., 2009).

Curcumin is the flavoring agent of turmeric powder (Curcuma longa) with various therapeutic properties especially anti-tumor activity without any side effects on normal cells, Its anti-tumor activities embrace cancer growth inhibition and apoptosis induction by modulating different signal transduction pathways in vitro and in vivo, in spite of these tempting attributes of curcumin, insolubility of the compound in aqueous solutions, actually the main reason for its poor bioavailability in vivo, has limited its exploitation as therapeutic agent. To overcome this problem and increase the solubility of curcumin, numerous approaches have been taken by using adjuvants, liposomes and more recently nanoparticles, However, there has not been found any perfect formulation yet (Ravindran and Prasad, 2009).

Curcumin exerts both pro- and antimutagenic effects. At 100 and 200 mg/kg body wt doses, curcumin has been shown to reduce the number of aberrant cells in cyclophosphamide- induced chromosomal aberration in Wistar rats, Turmeric also prevents mutation in urethane (a powerful mutagen) models108. Contradictory reports also exist. Curcumin and turmeric enhance *g*-radiation-induced chromosome aberration in Chinese hamster ovary, Curcumin has also been shown to be non-protective against hexavalent chromium-induced DNA strand break. In fact, the total effect of chromium and curcumin is additive in causing DNA breaks in human lymphocytes and gastric mucosal cells (Shukla et al, 2011).

Alterations in peripheral blood lymphocytes, such as chromosomal aberrations (CAs) and sister chromatid exchanges (SCE) have long been applied in surveillance of human genotoxic exposure and early effects of genotoxic carcinogens. The use of these biomarker assays is based on the

fact that most established human carcinogens are genotoxic in short-term tests and capable of inducing chromosomal damage. From an other hand, chromosomal aberrations have been used as important cytogenetic biomarkers to study the mutagenic effects of different chemicals in vivo and in vitro, The relevance of CAs as a biomarker has been further emphasized by epidemiological studies suggesting that a high frequency of chromosomal aberrations is predictive of an increased risk of cancer (Sutiakova et al., 2012). On the other hand, SCE is a highly sensitive parameter for evaluating human occupational and environmental exposure to mutagenic and carcinogenic agents, Moreover, parameters such as Mitotic Index (MI) and Cell Proliferation kinetics (CPK) have been proposed as useful biomarkers for the pre-screening of the potential cytostatic activity of new drugs. The use of the cell proliferation kinetics and the mitotic index are recognized biomarkers in biological monitoring to evaluate lymphocyte proliferation in population as well as to evaluate normal or tumour cells (Norppa et al., 2006).

Material and methodology

- **Solutions of cytogenetics studies**
- **Phosphate buffered saline (PBS)**
- **Colchicin solution**
- **Mitomycin-C solution**
- **Fixative**
- **Sorenson's Buffer**
- **Giemsa Stain Solution**

Laboratory animals

forty adult of albino male mice which obtained from Biotechnology Research Center Al-Nahrain, were used. Their age ranged at (8-12) weeks and weighting (23-27) g. They were housed in plastic cages containing hard wood chips for bedding in an air-conditioned room at 25±2˚C, with 14/10 hour's light/dark cycle.

The animals were given water and fed with a suitable quantity of water and complete dietThe animal divided as following:

Experiment no. I:-

The animals were divided into three groups

Group I: Negative control (5 mice)

Treated with (0.1 ml) PBS.

Group 2: the animals injected intraperitoneally with mitomycin –C (2 mg/Kg) represented positive control (5 mice) (once dose) (Shubber *et al*., 1985).

Group 3: The animals injected intrapertoneally with different concentrations of *C. longa* rhizomes extract (400, 100, 150, and 50 mg / kg of body weight) 7 does/week we choice the concentrations depend on LD50 (200mg/kg of body weight) (Jagetia *et al.,* 2003) .

Group 4: the animals represented negative control when injected intrapertoneally with PBS for 7 days/week.

Animals sacrifice was carried out after 8 days of injection with plant extract expect MMC group which sacrificed after one day of injection with MMC.

Using twenty animals were used for each group, five animals were used for estimating mitotic index and five animals were used for chromosomal aberration in bone marrow and five animals were used for micronucleus test.

Pre-drug treatment with mitomycin-C.

Interaction treatment between ethanolic extract concentrations of *C. longa* and mitomycin-C.

The mice were divided into two groups:

Group 1: administrated with ethanolic extract at concentration of 50 mg/Kg (50% from LD50) by injected intraperitoneally (for 7 days) before injection with MMC at eighth day.

The mice in these groups were sacrificed, and bone marrow samples were taken for cytogenetic analysis (MI, CA, and MN)

Post-drug treatment with mitomycin – C

Group 1: administrated MMC at concentration (2mg/kg) by injected intraperitoneally once (1 day) then, administrated animals ethanolic crude extract at concentration 50mg/Kg for 7 days (from 2 days-7days) 1days MMC/1-7 days extract.

Animals sacrifices were carried out after 8 days and bone marrow samples were taken for cytogenetic analysis (MI, CA, and MN).

Using twenty animals for each group, five animals were used for estimating mitotic index and five animals were used for chromosomal aberration in bone marrow the other five animals were used for micronucleus test.

Cytogenetic analysis test

Mitotic index (MI) assay:

The slides were examined under light microscope with (40x) power, and 1000 of the divided and non divided cells were counted and the percentage rate was calculated for only the divided ones according to this equation (Stick and San, 1981).

Chromosomal aberration (CA) assay:

The prepared slides were examined under the oil immersion lens for 100 divided of mitotic index cells (metaphase stage) per/slide for each animal and the percentage of these aberrations was estimated.

Micronucleus test (MN) assay:

The number of MN in 1000 cells of polychromatic erythrocytes (PCE) was scored under the oil immersion lens, and percentage of MN was calculated, according to this equation :

MN= numbers of MN / total numbers of PCE

(Schmid, 1976)

The protective value of ethanolic extract of *Zingeber officianele* rhizome ethanol extract:

It was calculated according to the (Rawat *et al*., 1997).

Statistical analysis

Data were analyzed by 2-way analysis of variance with ANOVA- test. Data are presented as means ± SD. The level of significance was $P < 0.05$ was used for analysis of variance test (ANOVA) (Al-Mohammed *et al*., 1986).

Results

Cytogenetic Analysis

Cytogenetic effects of MMC on mouse bone marrow cells

MMC effects on mitotic index (MI):

Normal white mice had a mitotic index of (6.2%) in their bone marrow cells (table 1) and this is considered as a negative control. While the mice treated with MMC caused a significant reduction ($p<0.05$) in MI (1.8%) in comparison with negative control.

3-4-1-2: MMC effect on chromosomal aberrations (CAs):

The spontaneouse frequancy of the chromosomal aberrations in control untreated mouse bone marrow cells was (1.42%) as shown in the table (1) and this precentage was increased in comparison with the result

of CAs of the mice treated with MMC which was (4.12%).Dicentric and chromatid gap were significantly reduced ($p< 0.05$) to (0.12 - 0.3%) when compared with the negative control (0.26%). While the other types of aberrations : chromatid break, chromosome gap, ring and deletion were significantly increased ($p<0.05$) to (0.81, 0.32, 0.83, 0.64 and 1.4%) respectively when compared with negative contol which gives (0.19, 0.03, 0.13, 0.32 and 0.43%) respectivelly (table 1). This result agreed with (Abdul-Hasan *et al*., 2006) in which they noticed that CAs have been increased in bone marrow cells of treated mice with MMC.

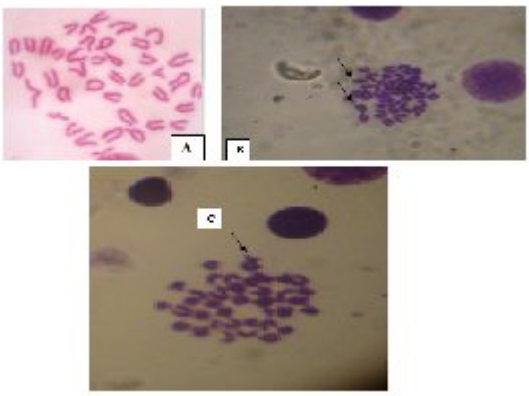
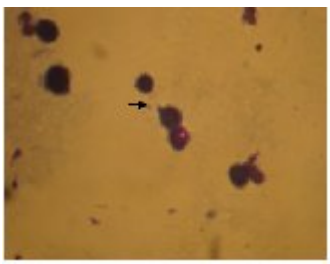


Figure (1): Cells in metaphase stage taken from mice treated with mitomycin-C by using Giemsa stain, showing: normal chromosomes(A) dicentric (B), deletion and chromatid gap (C) (1000 x).

MMC effect on micronucleus indection (MN):

Micronucleus frequencies of poly chromatic erythrocytes from negative control mice was (1.75%) as in table (1). This precentage was increased when are treated the animals with MMC and became (8.13%). This increased was significant ($p<0.05$) from the negative control. These results are in agreement with those reported by (Kumpati *et al*., 2003) that mentioned MMC induced the MN in bone marrow cells.



Abnormal micronucleated bone marrow cell from mouse treated with MMC.

The effect of Curcuma extract on mitotic index (MI):

Under normal experimental conditions, white mice have a mitotic index of (6.2%) and this considered as a negative control.

Table (1) pointed that the mitotic index of mouce bone marrow cells treated with five different doses of curcumin has significant differences ($p<0.05$) which gave different precentages of cell division The highest MI (5.4%) was seen in the bone marrow cells treated with 50 mg/kg. the ratio increase of decrease concentration compare with negative control.

The effect of curcuma extract on chromosomal aberrations (CAs):

The CAs of normal mice was (1.32%) this considered as negative control.The result in table (1) showed that the plant ethanolic extract cause and increase in CAs with increasing of doses concentration, this indicated that plant extract is genotoxic at high doses on mouse bone marrow cells. At dose (50 mg/Kg) gave CA equal to gives CA equall to (0.774%). This reduction in spontaneous frequency of CA was significant ($p<0.05$) in comparision with those of the control.

The CAs that were noticed are: chromatid break, chromosome gap, dicentric, ring and deletion which appeared when using different concentrations of curcuma. These types of aberration were appeared in the negative control(0.142, 0.533, 0.421, 0.327, 0.10 and 0.131%) respectively, and this percentages were decreased when mice treated with all doses of *C.longa* as shown in table (1) except increasing in percentage of chromatid break.

The effect of Curcuma extract on micronucleus induction (MN):

The significant decrease ($p<0.05$) in MN frequency was observed at 50 mg/kg which gave 2.5% when compared with negative control .

Table (1) shows that there are decreasing in MN percentage which were (4.2 , 2.8 , 2.5 , 1.80)% with decreasing the concentration of curcuma extract used (400 , 200 , 100 , 50 mg/kg) respectively. This results agreed with (Natividad et al 2012).

suggested about this results is may be due to antioxidant contain of curcumin extracted acting as scaving agent (Topic *et al*., 2002).

Table (1): Cytogenetic effects of different concentration of *C. longa* in comparison with negative (PBS) and positive (MMC) controls on mouse bone marrow cells (*in vivo*)

Groups	MI (mean±SD)	MN (mean±SD)	Chromosomal aberration (mean±SD)						Total%
			Chromatid break	Chromatid gap	Deletion	Dicentric	Ring	Chromosom gap	
Control –ve (PBS) only	A 6.20±0.200	A 1.750±0.173	A 0.112±0.012 5	A 0.533±0.009 8	A 0.424±0.004 9	A 0.101±0.01 7	A 0.131±0.03 6	A 0.100±0.030	1.32
Control +ve (MMC)mg/kgB.W	B 1.81±0.25	B 9.13±0.404	B 0.250±0.20 0	B 0.42 ±0.251	B 0.675±0.05 0	B 0.810±0.10 1	B 0.74±0.085	B 1.201±0.351	4.12
Concentration 400mg/kgB.W	C 5.01±0.25	C 4.20±0.264	C 0.560±0.15 0	AC 0.274±0.02 6	C 0.280±0.02 6	A 0.249±0.01 7	A 0.142±0.05 0	A 0.103±0.035	1.680
Concentration 200mg/kgB.W	C 4.52±0.35	A 2.810±0.401	C 0.421±0.100	AC 0.280±0.04 0	CD 0.241±0.03 6	A 0.240±0.02 6	A 0.232±0.03 2	A 0.110±0.040	1.56
Concentration 100mg/kgB.W	C 5.60±0.34	A 2.500±0.057	C 0.371±0.10	C 0.195±0.02	D 0.142±0.02	A 0.51±0.036	A 0.121±0.02	A 0.10±0.020	1.239

Differences a,b,c are significant (P<0.05) to compression column
Differences A, B, C are significant (P<0.05) to compression rows

Interaction between MMC and Curcuma ethanolic extract:

This experiment was designed to know the effect of plant extract on mutagenic effect of MMC which shows a high precentage of CAs, increase in MN and decrease in MI of bone marrow of mice. Therefore; we would like to know if curcumin extract has the ability to reduce the effect of MMC by injecting the animal before and after the time of MMC treatment to detect its inhibitory effect of curcuma ethanolic extract 50mg/kg was choice to as best dose which there ratio considered increase MI and reduce MN and CAs relatively was like negative control.

Treatment with Curcuma extract before MMC:

The results of this experiment were represented clearly in table (2), which shows the ability of curcuma extract to reduce the effect of the drug in the mouse bone marrow on MI.

the MI of mice treated with different concentration revealed of curcumin extract before dose of the drug was 2.11%. This result has non significant different (p>0.05) comparing with mitotic index in posative control 1.8% that treated with the drug only.

So the plant extract provided 17.04% the plant extract have the ability to reduce the effect of MMC. figure (1)

The percentage of CA was reduced significantly (p<0.05) when curcuma extract used before MMC treatment, which reached 2.041 % in comparison with the positive control 4.12% while Before-treatment with curcumin plant extract provided 77.66% protection against the genotoxic effect of MMC. Figure (1)

The percentage of MN cells was also reduced 2.24% when the extract give to mice before MMC treatment; this result was significant ($p<0.05$) from the positive control 8.13%, and The pretreatment with the plant extract provided a protection on MN (92.31%) against the effect of MMC. figure (1)

Treatment with Curcuma extract after MMC:

The results of this experiment were displayed in table (2). Post treatment with curcuma extract caused significant increase ($p<0.05$) in MI 5.8% comparing with MI in positive control 1.8%, so that the post treatment with the plant extract provided protection on MI 90.90% from the effect of drug on MI . Figure (1)

The percentage of CAs after treatment with the extract was 1.486%, which was significant ($p<0.05$) compared with positive control (4.12%) all of the CAs were decreased significant when compared with positive control post drug treatment with the extract provided protection on CAs (97.55%) from the effect of the drug MMC on CAs . figure (1)

The percentage of micronucleus was also reduce to reach 1.9% post treatment with the extract. Which significantly different ($p<0.05$) compared from the positive control 8.13% the extract provided protection 97.64% from the effect of MMC on MN induction .Figure (1)

Table (2): Interaction between *C. longa* and MMC (before and after treatment)

Groups	MI (mean±SD)	MN (mean±SD)	Chromosomal aberration (mean±SD)						
			Chromatid break	Chromatid gap	Deletion	Dicentric	Ring	Chromosome gap	Total
Control –ve (PBS) only	A 6.20±0.200	A 1.750±0.173	A 0.176±0.025	A 0.433±0.098	A 0.43±0.049	A 0.263±0.047	A 0.16±0.036	A 0.18±0.03	1.65
Control +ve (MMC)mg/kgB.W	B 1.81±0.25	B 8.13±0.404	B 0.81±0.01	AB 0.300±0.05	B 0.83±0.043	B 0.15±0.05	B 0.62±0.026	B 1.466±0.35	4.12
Before	B 3.110±0.283	A 2.241±0.293	C 0.273±0.02	B 0.29±0.005	C 0.19±0.01	C 0.49±0.02	C 0.49±0.051	A 0.37±0.02	2.04
After	A 5.800±0.300	A 1.901±0.360	C 0.194±0.005	D 0.280±0.015	C 0.167±0.005	A 0.271±0.03	D 0.281±0.042	A 0.292±0.024	1.486

* Differences A, B, C are significant ($P<0.05$) to compression row

Discussion

MMC effects on mitotic index (MI):

This reduction is may be due to the proteins required for mitosis were not produced at the same quantities, or may be the code was not reach to the cell to induce it to proliferate, or may be the drug cause the death of bone marrow cells, These will lead to decrease (or not produce) the quantities of proteins required for mitosis, or the code did not reach the cells to induced it for proliferation (Vijayalaxmi and Venu, 1999).

My suggestion the mitotic activity of the cell which affected with MMC could not repaired, or due to defect occurred in the mitotic spindle composition during cell division

These results were agreed with the results of (Al-Khait, 1999 and Abdul- Hasan *et al*., 2006) who found that MMC had cause reduction in MI of bone marrow cells.

While disagreed with the result of (Al-Duliemy, 2005) who faild to demonstrate apotential for genetic damage *in vitro* which may due to tissue and /or species differences.

The effect of Curcuma extract on mitotic index (MI):

Our work reflected that MI was significantly ($p < 0.05$) increased in several concentrations whereas the Protective value was not significantly modified in any case. This behavior indicates that curcumin could stimulate the cell division process, acting as a mitogen such as phytohemaglutinin, but it would not modify the kinetics of this process .

factors viz., ‘‘culture shock’’ and ‘‘mitotic clock’’ especially considering that increases in these parameters were not observed in the negative controls.The results agreed with results by the Azmi et al. (2006).

The effect of C.longa extract on chromosomal aberrations (CAs):

For CAs, Cao et al. (2006) observed that curcumin, at high doses, imposed oxidative stress and damaged the DNA of HepG2 cells. Moreover, the same group observed that curcumin significantly increased the frequency of chromosomal aberrations at doses higher than 8 lg/ml, which evaluated the genotoxicity of curcumin in PC12 cells obtained from rat pheochromocytoma. They observed an induction of micronuclei in binucleated cells at curcumin concentrations of 2.5 and 10 lg/ml, offering for the last one a difference statistically significant ($p < 0.05$) from the control group. Both studies and our work indicated that at low concentrations curcumin is not genotoxic;

however, at high concentrations, curcumin presented genotoxic potential in vitro models. Studies conducted at the national institute of nutrition (NIN), that some of the spices and vegetable stimulate specifically the levels of glutathione –s- transferase (G S T), group of enzyme which are known as cellular detoxification enzyme and inhibition of carcinogenesis (Mendonca et al. 2010).

Interaction between MMC and Curcuma ethanolic extract:

The anti-oxidant and pro-oxidant properties according to its concentration may be the main reason responsible for its biphasic effects , Alaikov et al. (2007) showed that curcumin has protective and anticlastogenic activity by enhancing the scavenging of free radicals against cisplatin. The DNA damage by the ability called ‘‘Topoisomerase II poisoning’’ was also observed in an ethanolic extract of propolis which caused a concentration-dependent increase in chromosome- type breaks such as acentric fragments (Montoro et al., 2012). Other polyphenols have shown their pro-oxidant, and therefore DNA-breaking, properties. In these study of Our work reflected that MI was significantly ($p < 0.05$) increased in several concentrations whereas the PI was not significantly modified in any case, This behavior indicates that curcumin could stimulate the cell division process, acting as a mitogen such as phytohemaglutinin, but it would not modify the kinetics of this process.

It could be seen the curcumin gave striking result as protective agent against the genotoxic effect of MMC if it was given after MMC treatment. This might related to the chemical constituent of the extract which may be by the following action:-

- The plant extract linked with the drug.
- or linked with its metabolites to form non-absorbable complexes.
- or act to prevent activation of the drug by inhibition cytochrome p450 enzyme and other ODC and AHH enzymes. or any act as antioxidant or scavengers for the free radicals in the cells, The protective effect of curcumin has been well documented and has been observed against several stressing agents, such as hydrocortisone, nicotine, ethanol and irradiation (Ragunathan and Panneerselvam, 2010). Antioxidant protection against oxidant challengemay decrease the rate of mutation. Oxidant challenge can induce potentially mutagenic DNA damage by direct action of ROS on DNA, or indirectly, via aldehydic lipid peroxidation degradation products. Several intracellular antioxidant DNA protection mechanisms exist, including ROS scavengers,

enzymatic inactivation of ROS and iron binding. In cultured cells curcumin exhibited properties of an iron chelator. Activation and altered transcription of antioxidant and DNA repair enzymes are also important to maintain low-baseline levels of DNA damage under normal intracellular conditions of continuous oxidant challenge of varying intensity (Szeto et al, 2011).

Contain a combination of different chemical constituent in which might act together to reduce genotoxic effect of MMC of this compounds are flavonoids, saponin, phenols.

Those active compound may play on important role in reducing the genotoxicity of the drug, for example flavonoid are shown anti oxidant that prevent DNA damage at low concentration and have ability to scavenging the hydroxyl radical , superoxide anions and lipid peroxy radicals eg. Green tea, Onion, Apples, Grapes, Ginkgo and Tomato are just a few of the many of plants that contain flavonoid antioxidant, Treatment with C.longa extract after the drug provided protection ratios for MI, MN and CAs more than this ratios when it given before drug treatment. So, curcuma extract could be classified as "bio antimutagene" in the first order, and "desmutagene" in the secand order.

The reason behind this is that the extract or the plant extract contain several vitamins and poly phenolic compounds and this play vital role in the inhibition mutagenic effect of MMC, these agreed with the result up (Al-Hassan, 2006).

This results were disagreed with (Al- Sudany, 2005), curcuma extract could be classified as “desmutagen” in the first order, and “bioantimutagen” in the second order.

Curcumin exerts both pro- and antimutagenic effects. At 100 and 200 mg/kg body wt doses, curcumin has been shown to reduce the number of aberrant cells in cyclophosphamide- induced chromosomal aberration in Wistar rats Turmeric also prevents mutation in urethane (a powerful mutagen) models . (Shukla, 2011)

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