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The Role of *Curcuma Longa* Rhizomes Ethanolic Extract on Human Lymphocytes Treated by Bickel by Using G Banding Technique and Karyotyping

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ABSTRACT

Curcuma longa L., which belongs to the Zingiberaceae family, is a perennial herb distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries. Curcuminoids are inherent compounds of the species *C. longa*, there are three main compounds of this pigmented curcuminoid complex, namely, curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the major component of this plant, being responsible for the activities. Crude extract of *Curcuma longa* rhizomes was prepared by using ethanol and Chemical detection was performed in order to investigate the secondary metabolites compounds of ethanolic crude extract. Reversed high performance liquid chromatography carried out for the detection and estimation of curcumin in the crude extract. Otherwise cytogenetic study of the effect of *C. longa* crude extract and standard curcumin on the normal human lymphocyte blood cells done after exposure to nickel chloride (NiCl₂), furthermore, karyotyping was performed by using Metasystemkaros. Chemical detection of crude extract showed positive result for alkaloids, tannins, glycosides, phenols, Terpens, flavonoids, cumarines and resins while showed negative result for Saponins and Steroids. While the results of the cytogenetic study revealed that the effect of *C. longa* crude extract and standard curcumin reduced the genotoxic effect of nickel chloride (NiCl₂) on the normal human lymphocyte blood cells. Karyotyping showed that genotoxic effect of nickel chloride appeared as aberration in chromosome number 18. While lymphocytes treated with *C. longa* crude extract or standard curcumin showed no chromosomal aberration, at the same time no chromosomal aberration were observed after treatment with combination of NiCl₂ and crude extract or combination of NiCl₂ and standard curcumin which indicated the ability of standard curcumin and *C. longa* crude extract to prevent the effect of nickel chloride. Further medical cytogenetic and molecular testing to get better understanding the protective role of *Curcuma longa* rhizomes, crude extract against heavy metal induced chromosomal aberrations in human blood cultures.

| *Curcuma longa* | Nickel chloride | Karyotyping | Curcumin | Secondary metabolites |

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1. INTRODUCTION

Curcuma longa is a tropical plant native to southern and southeastern tropical Asia, A perennial herb belonging to Zingiberaceae family. The parts used are the rhizomes which are called turmeric. Turmeric is widely consumed in the countries of its origin for a variety of uses, including as a dietary spice, a dietary pigment, pharmaceutical industries and a folk medicine for the treatment of various illnesses such as biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis [1].

The most active component in turmeric is curcumin, which may make up 2 to 5% of the total spice in turmeric. Numerous reports suggest that curcumin has chemopreventive and chemotherapeutic effects. Curcumin has been shown to inhibit the proliferation of a wide variety of tumor cells [2].

Increased risks for cancer was found to be associated with exposures to nickel which is used in a wide variety of metallurgical processes such as electroplating and stainless steel production. The International Agency for the Research on Cancer (IARC) concluded that nickel compounds are human carcinogens and The Department of Health and Human Services (DHHS) has determined that nickel metal

Human Services (DHHS) has determined that nickel metal may reasonably be anticipated to be a carcinogen and that nickel compounds are known human carcinogens [3]. Nickel compounds that are soluble in water including nickel chloride being the most genotoxic than insoluble nickel [4], moreover its induced DNA damage and gene mutation in human lymphocytes [5].

The aimed of this study to detect secondary metabolites compounds and to evaluate the protective role of *Curcuma longa* rhizomes, crude extract on heavy metal induced chromosomal aberrations in human blood cultures.

2. EXPERIMENTAL

2.1 Materials, method and instruments

Ethanolic crude extract of *C. Longa* rhizomes were obtained by Soxhlet while used ethanol as solvent in extraction process. Curcumin contents in ethanolic crude extract of *C. Longa* rhizomes were detected and determined by Agilent 1100 HPLC apparatus and carried out by using Reversed C18 column (4.6 x 250 mm, 2µm) as stationary phase with THF aliquot (Tetrahydrofuran : distilled water)

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as mobile phase. Ikarosmeta system were used for capture, processing and karyotyping of chromosomes.

2.2 Preparation of *Curcuma longa* rhizomes Extracts

Turmeric rhizomes were purchased from a local market in Baghdad. Dry turmeric rhizomes were powdered by electrical grinder, 20 grams of the powder were soaked in 100 ml of petroleum-ether for 24 hr. then evaporated and the residue were extracted in 100 ml of the solvent (95% ethanol) using the Soxhlet apparatus at 50 0 C. The crude extract then evaporated at 45°C using oven and the resultant crude extract was collected and stored at 4oC until use to prepare the required doses and concentrations [6][7].

2.3 Chemical analysis of ethanolic crude extracts

The chemical analyses of plant ethanolic extracts were carried out to detect the following compounds as in Table 1.

Table 1. Detection of phytochemical compounds in *C. longa*ethanolic extract.

Chemical compounds	Reagents	Indication
Tannins	lead acetate 1% [8]	white-precipitate
Glycosides	ked's reagent [8]	violet ring
Flavonoids	95% ethanol + water bath + KOH [8].	yellow -precipitate
Saponins	2-mercuric chloride [9]	white-precipitate
Alkaloids	1-myers reagent [10]	brown-precipitate
	2-wagner's test [9]	
Terpens	concentrated sulfuric acid [8]	pink color
Steroids	similar reagent for terpens/ leaving sample for 1 Min.	blue color
Phenols	ferric chloride 1% [11]	blue-green color
Resins	ethanol, boiling, D.W [8]	turbid
Coumarines	Filter paper + NaOH + U.V. [11]	blue-green color

2.4 High performance liquid chromatography method

2.4.1 Standard preparation

The stock solution was prepared by dissolving 1mg of curcumin in 1 ml of tetrahydrofuran aliquot 40 : 60

(THF : distilled water) then diluted to get final concentrations (0.5µg/ml, 1 µg/ml, and 5µg/ml) [12] .

2.4.2 Sample preparation

The stock solution was prepared by dissolving 1mg of crude extract in 1 ml of THF aliquot 40 : 60 (THF : distilled water) then diluted to get final concentration 10µg/ml [12] .

2.4.3 Mobile phase preparation

The mobile phase consisting of 40: 60 (THF: distilled water) and 1% citrate buffer was passed through a 0.22µm membrane filter then degassed by ultrasonication before use [12].

2.4.4 Application

An aliquot 40 µl of each concentration of the standard and the crude was injected into injector twice; the system was run at a flow rate of 0.7 ml/min, curcumin was detected at a wavelength of 420 nm. [12]Peak height and peak area were recorded and measured. Then the correlation coefficient in linear regression equation was used to record the quantity of curcumin ($Y = a + bx$) [13] .

2.5 Chromosomal aberration assay using G banding technique and karyotyping by Ikarosmetasystem

The experiments were designed to evaluate in vitro the cytogenetic effects of ethanolic *C. longa* crude extracts and curcumin standard in human blood lymphocytes cells, as well as, its role in protect lymphocytes from effect of nickel chloride. Therefore, such evaluations were carried out through six groups with two flasks for each group: Group I: human lymphocytes without treatment as negative control. Group II: human lymphocytes were treated with 10 µl of Nickel chloride which (8 µg/ ml) as positive control. Group III: human lymphocytes were treated with 10 µl of pure curcumin (10 µg/ ml). Group IV: human lymphocytes were treated with 10 µl of *C.longa*ethanolic extract (10 µg/ml). Group V: human lymphocytes were treated with combination of 10 µl of Nickel chloride and 10 µl of pure curcumin. Group VI: human lymphocytes were treated with combination of 10 µl of Nickel chloride and 10 µl of ethanolic extract of *C.longa*.

Chromosomal aberration assay were set-up for each individual according to the standard protocol of [14] [15] , whole blood 0.5ml were added to 7ml RPMI 1640 culture medium containing Phytohemagglutinin and incubated at 37°C in 5% CO₂. After 72 h. colcimide added and cells were harvested and treated with warmed hypotonic solution.

(0.075 M KCl), after that suspended by fixative solution, washed at least three times to remove any amount of fixative solution.

Slides were prepared from the cell suspension then warmed by slide warmer at 37°C for three days. After that slides were treated with Trypsin and rinsed with phosphate buffer saline. Cells were stained with Giemsa stain, and then washed with phosphate buffer saline. Microscopic examination under 100X and the chromosomes were karyotyping by Ikaros Meta system karyotyping.

3. RESULTS & DISCUSSION

3.1 Chemical Analysis of Turmeric (*Curcuma longa*):

The results of chemical analysis of ethanolic extracts of Turmeric indicated the existence of alkaloids, tannins, glycosides, phenols, Terpens, flavonoids, cumarines and resins while Saponins and Steroids gave negative results. The strong fragrances of extract suggest the presence of a large quantity of phenolic compounds and volatile oils in these extracts as suggested by [16].

An explanation for these findings may be referred to the differences in environmental conditions like place of planting and period of harvesting. Highly contents of active compounds found in samples of turmeric rhizome were collected during winter–summer season which is the harvesting period and collected from the regions whereas have cool and dry weather during winter comparison with regions have not warm or cool humid weather during all seasons [17].

3.2 Detection and Determination of Curcumin by Reversed High Liquid Performance Chromotochraphy

Whether curcuminoids consists of different chemical entities as detected by HPLC at wave length 420nm. Three components were found in the standard compound Viz Cur, DMC and BDMC, in the following order

Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin. Curcumin was eluted first with retention time at 24.154 min. followed by Demethoxycurcumin and Bisdemethoxycurcumin at 28.762 and 34.025 min., respectively. The three components were found in *C. longa* crude extract with similar order to the components of standard compound and relative retention time which were for Curcumin at 24.271 min. followed by Demethoxycurcumin and Bisdemethoxycurcumin at 28.915 and 34.230 min., respectively as showed in figure (1- A and B).

The identity of standard curcumin and ethanolic crude extract chromatogram in the maximum wave length absorption and in the retention time explain the selectivity and accuracy of the applied method. The procedure followed in this study was refered to [12] who found that Curcumin was eluted first followed by Demethoxycurcumin and Bisdemethoxycurcumin and their retention time were 7.08, 8.13 and 9.28 min, respectively by using HPLC YMC ODS AQ-302/ Japan and Reversed C18 column 4.6 x 50 mm, 5 µm.

Estimation of curcumin in *C. longa* ethanolic crude extract calculated by standard curve. Three concentration of standard curcumin (0.5, 1, and 5 µg/ml) were injected twice for each concentration, followed by one concentration of *C. longa* ethanolic crude extract (10µg/ml). Table (2- A) showed peak area while table (2- B) showed peak height of first and second injection. Concentration of curcumin which calculated by peak area was 2.32627µg/ml. To confirm this result, concentration of curcumin from peak height was calculated and it appeared to be 2.531377µg/ml.

These differences in retention time and concentration of curcumin in ethanolic crude extract may be due to the different in the conditions of extraction method, kind of solvent, and type of RHPLC system used such as instrument and column dimensions.

This raw material estimation may be useful for small industry that does not own analytical method for curcumin quantitative analysis [13].

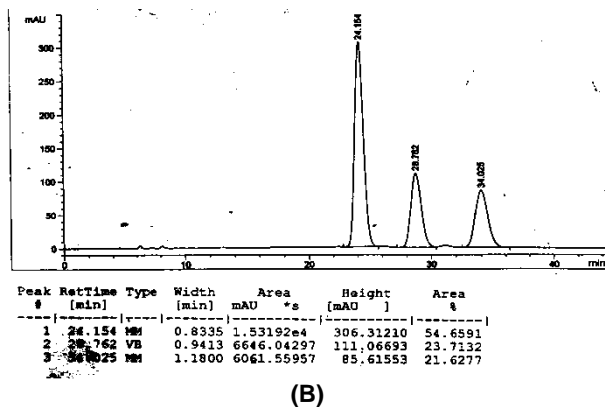
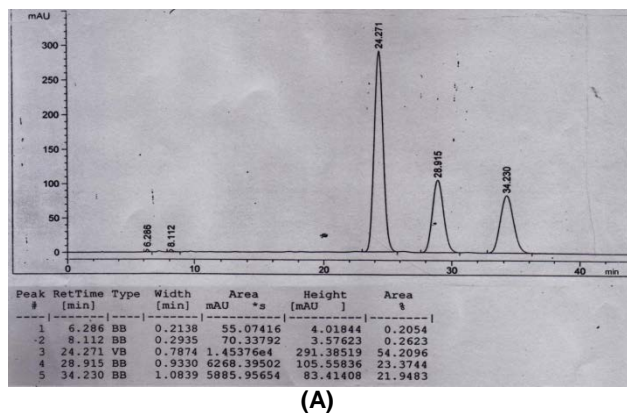


Fig. 1. RHPLC Chromatograms of *C. longa* ethanolic crude extract (A) and curcuminoids standard (B). The highest peak is curcumin followed by Demethoxycurcumin then Bisdemethoxycurcumin at wavelength 420 nm.



Fig.2 : Chromosomes at metaphase analyzed by Ikarosmetasystem images of isolated from negative control (human lymphocytes without treatment) (a), chromosomes treated with Nickel Chloride (NiCl₂) which caused chromosomal aberration in Ch. no. 18 (b) chromosomes treated with ethanolic crude extract of *C. longa* which showed no chromosomal aberration (c) chromosomes treated with pure curcumin which showed no chromosomal aberration (d) Chromosomes treated with a combination of Nickel Chloride and ethanolic crude extract of *C. longa* showed no chromosomal aberration (e) chromosomes treated with combination of Nickel Chloride and pure curcumin (f) showed no chromosomal aberration

Table (2-A). Peak area of first and second injection

Concentration of curcumin (st)	Peak area of 1st injection	Peak area of 2nd injection	Average
0.5 µg/ml	273.728	280.052	184.76
1 µg/ml	354.872	310.694	222.1887
5 µg/ml	864.309	862.743	577.3507
(Sample/crude extract) 10µg/ml	364.749	377.523	371.136

Table (2-B). Peak height of first and second injection

Concentration of curcumin (st)	Peak height of 1st injection	Peak height of 2nd injection	Average
0.5 µg/ml	4.764	4.762	4.763
1 µg/ml	5	5.617	5.3085
5 µg/ml	8.223	8.248	8.2355
(Sample/crude extract) 10µg/ml	6.343	6.479	6.411

3.3 Chromosomal aberration assay using G banding technique and karyotyping by Ikarosmetasystem

Nickel chloride have been reported to cause sister chromatid exchange and gene mutations by accelerates the formation of the free radicals which cause lipid peroxidation as well as DNA damage. These free radicals also inhibit the DNA repair enzymes. Curcumin supplementation brought about protective effect on nickel induced genotoxicity. Hence this herbal product scavenges and neutralizes free radical generated during toxic reactions in response to the chemical insult, by breaking their subsequent oxidative chain reactions [15]. Fig. (2-b) showed Karyotyping of chromosomes isolated from human lymphocytes treated with Nickel Chloride and showed chromosomal aberration in chromosome number 18 which compared with Fig. (2-a) showed karyotyping of normal control chromosomes at metaphase the karyotyping in figures(2-c) and (2-d) showed no chromosomal aberration in human lymphocytes treated with *C.longa* ethanolic crude extract and human lymphocytes treated with standard curcumin, respectively which indicate that curcumin has no toxicity, For Human clinical trials [18] demonstrated that no toxicity symptoms in administration of Curcumin at doses of 1–8 g/day and 10 g/day. Karyotyping of human lymphocytes chromosomes after exposure to combination of Nickel Chloride and *C.longa* crude extract and karyotyping of human lymphocytes chromosomes after exposure to combination of Nickel Chloride and standard curcumin showed no-chromosomal aberration as presented in figures (2-e) and (2-f), this obtained results was confirmed by the results of [15] who reported that curcumin supplementation is a very strong protective agent against Nickel Chloride induced genotoxicity, active compounds (curcuminoids) act as free radicals scavenger

which induced by Nickel Chloride by possible mechanism of protection against chemical carcinogenesis and could be mediated via-antioxidant-dependent. Hence this study presents the protective role of this herbal product on heavy metal induced chromosomal aberrations in human blood cultures.

4. CONCLUSION

Curcuma longa rhizomes were rich with poly phenols curcuminoids and can be a good source for curcumin health benefit. The results of RHPLC proved to be an effective procedure for detecting and estimating the concentration of plant active compound. Otherwise our result demonstrated that *C. longa* rhizomes ethanolic crude extract and pure curcumin had the ability to preventing genotoxicity effect of Nickel Chloride in human lymphocytes chromosomes.

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