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Evaluation of antioxidant and anti-mutagenic activity of naturally fortified honey with *Curcuma longa* extract

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Abstract

Honey considered as natural product, also it has a great influence on health and can be used as antibacterial, anti-inflammation and antioxidant as wellndue to exiciting of some organic materials like amino acids, enzymes, peroxide and much more. The current research planned to explore the activity to anti-mutagenic and anti-oxidant to naturally fortified honey with *Curcuma longa* extract. The fortified honey was evaluated by GC MASS method. The activity of the anti-oxidant elevated due to the activity of the free radicals in 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH), nitric and super oxide. Scavenge is achieved by alkaline DMSO and lipid per oxidation method, While anti mutagenic activity were assessed by mitotic index (MI), chromosomal aberration (CA) and Micronuclei (MN,) tests. The outcomes data from the current work shows fortified honey had a highest antioxidant activity in compare with *Curcuma longa* extract of genotoxic activity showed that furfural increased formations of MNs, CA and MI in blood culture associated with control, opposing, fortified honey by itself didn't display any effect in geno-toxic while the effect in anti-mutagenic of honey could be related the capability of scavenge power to antioxidant for free radical. Fortified honey reduced the mitotic index in all actions.

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Introduction

Honey composition depends on its flower sources, ecological influences (Alvarez-Suarez, *et al.,* 2013). Many Studies have shown that honey with dark color have the highest concentration of antioxidant than the light color (Bogdanov *et al.,* 2008). Honey usually consider as a product come formed in nature and because of its high nutrition value in addition to the properties that influence the health of human being as antioxidant, antibacterial and anti-inflammation, all these properties and more rise its consumption (Alvarez-Suarez *et al.,* 2010).

Antioxidants existing in honey are so special because it contains both ascorbic acid which is not an enzyme compound with peroxidase and catalase which are enzymes (Orhan *et al.,* 2003), moreover honey contain tocopherol (Schramm *et al.,* 2003), organic acids like amino acids, carotenoids in addition to proteins, Maillard reaction (Keen 2003) *Curcuma longa* belong to the family: Zingiberaceae it´s extract have antioxidant properties (Srivastava *et al.,* 1995).

Curcumin sulphate and glucuronide is the major constituents of the extracts. It causes apoptosis for cancer cell including colon, fore-stomach, duodenum and etc. (Zhang *et al.,* 2004). The aim of this study is to detect the antioxidant activity of fortified honey with *Curcuma longa* active component, and studying the active component of fortified honey and its ability to prevention Cell mitosis.

Material and methods

Spectrophotometrical method used for determination total polyphenols content, we used by using Folin-Ciocalteau reagent. One gm of honey fortified with *Curcuma longa* mixed with 5 ml of meth-alcohol to determine it, after mixing the sample and make it homogeneous, 0.5 ml of it was taken using pipette and placed it in volumetric flask (10 mL) and 0.5 mL of D.W. to dilute it, then 0.5 ml of Folin-Ciocalteau was placed in the solution then a solution of sodium carbonate (1.2 ml) was also add to the solution. Then a 20 minutes was gave to the mixture complete the reaction, after that the spectroscopy measurement was achieved using 765 nm. The expression of TP is (mg GAE 100/g) which represent the weight of Gallic acid in milligrams (mg) per weight of honey (normally 100 g).

Detection active component

Active component of control honey and *Curcuma longa* extract and fortified honey with date palm fruit by:

GC-MS

GC/MS analyses were begun with injected 1 µL of the sample in split mode 1/10; The Injection carried at 250 Celsius. Using DB-5MS column (30 m × 0.25 mm × 0.25 µm) was applied in a continuous current of the carrier gas (1 mL/ minute).

The start degree of the temperature was 5 °C, and kept for 3 minutes then rise 10 °C every minute until it reached 290 °C. Mass spectroscopy was achieved and the data base and the peaks was collected at 230 °C, the mode of ionization was 70 eV and the (m/z) was 35–450.

The activity of antioxidants (In-vitro)

Standard solutions were prepared using meth-alcohol (methanol) as a solvent to dissolve ascorbic acid to prepare five different concentrations (starting from 10 to 50 µg/ml) rising 10 µg/m to test the free radicals activity of DPPH.

Preparation of DPPH solution

Dissolved about 8.6 mg of DPPH in 6.6 ml meth-alcohol (methanol); test tubes incubate in dark to protect from light.

Estimating the scavenge activities to DPPH (Protocol)

Solution of DPPH around (150 µL) was added to 3 ml meth-alcohol (methanol), the absorptivity was immediately taken using 516 nm, the read was used as control for comparison. Changing the volume in the levels of test sample (from 100 to 200) 20 µl every time using methanol as solvent to increase the volume.

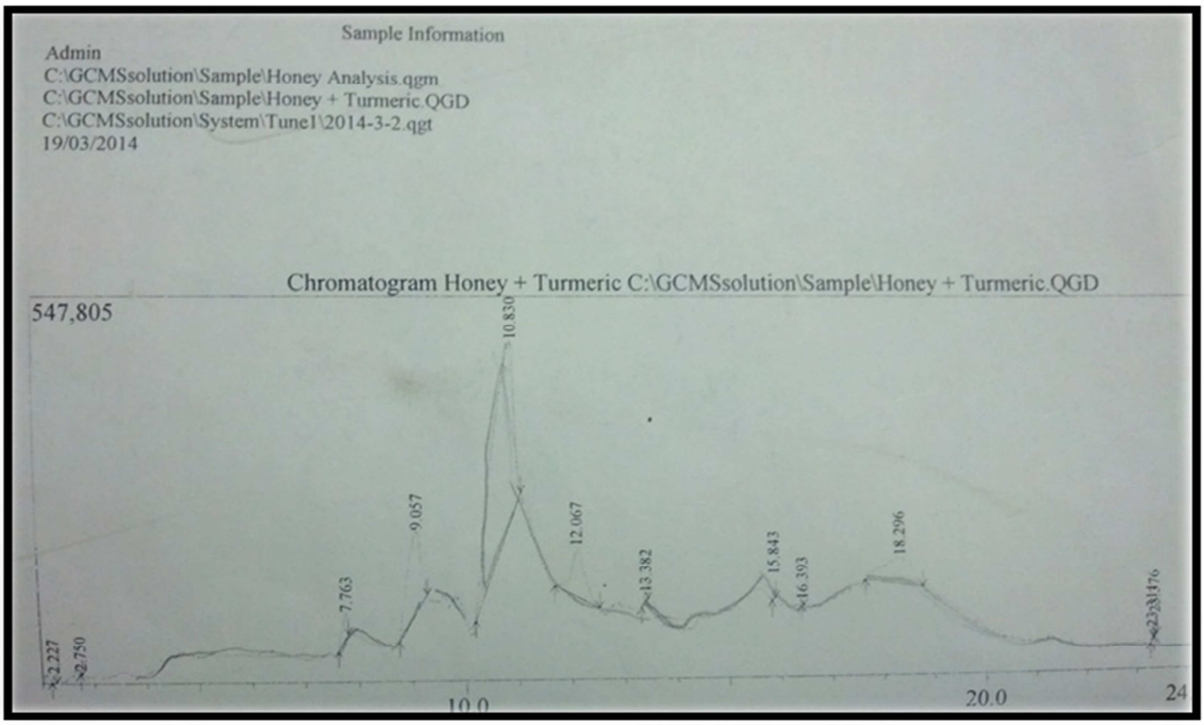

Fig. 1. Active component in fortified honey by GC-MS.

The absorptivity was taken to each solution using (Shimadzu, UV-1700 spectrophotometry) at the same wavelength.

The calculations of IC_{50} and the decreasing percent were done as depicted in the below equation:

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\%FRSA = \frac{\text{Absorbitivity of control} - \text{Absorbitivity of sample}}{\text{Absorbitivity of Control}} X\ 100$$

(Where FRSA represent the scavenging activities of the free radicals)

Each testing was repeated three times and outcomes actually are the average of them (mean % ± SD10).

Reagents

DMSO (dimethyl sulphoxide), Nitro-blue tetrazolium.

Preparing stock solutions

Dissolve 1 gram of curcumin in 1 mL of distilled water (DW) then dilute this solution using DW to achieve different concentrations (10 to 50 µg / Ml). Using the equal quantities of methanoic extract powder and dissolve it same volume of DMSO to achieve a (1 mg/ml solution), dilute the stock solution by using DMSO to prepare different concentration (from 100 to 600 µg / mL).

To prepare Alkaline DMSO: take 2 ml alkaline DMSO containing, 10 mMol of sodium hydroxide in 0.2 ml of DW and 1.8 mL of DMSO. Nitro-blue tetrazolium (NBT): Dissolve 50 mg of NBT was in 50 mL of DMSO to achieve (conc. 1 mg/mL).

Estimating the scavenge activities to superoxide

Take 0.2 mL from nitro-blue tetrazolium (2 mg / mL stock prepared above) and 0.6 mL of the standard extracted in DMSO, 2 mL from alkaline DMSO (10 m M of sodium hydroxide dissolved in 0.2 mL DW) the two stock solutions were added to each other's to get 2.8 mL volume, the absorptivity was taken at the same wave number (560 nm), (1.2 mL, 80 mM) of hydrogen peroxide was added to the above stock solutions (100-600 µg / mL), 600 µL of DMSO, 0.2 ml NBT solution and 2 ml alkaline DMSO were mixed and the absorptivity of this solution was used as control reading.

The percent of was determined as depicted below:

$$\%\ Super\ oxide\ scavening\ activity = \frac{Test\ absorbance - control\ absorbance}{Test\ absorbance} X\ 100$$

Culture of Lymphocyte

Lymphocytes culture from human peripheral was used as the test. This research was achieved due to donation of blood from three not smokers and healthy donators and there ages were (between 20 to 30 years ages) with no medication for at least 4 weeks. For the sister chromosomal exchange (SCE), chromosomal abnormalities (CA) researchers, add 5.0 from chromosome to 0.4 of blood (contains heparin, phytohemagglutinin, fetal bovine serum and antibiotics) enhanced with 20 lg/mL bromo-deoxyuridine. All these cultures incubated at 37 Celsius for three days (72 hr).with fortified honey, no changes in acidity of medium were noticed.

Anthracene $40x10^{-5}$ were used to treat the human lymphocytes for one to two days, the positive and negative controls (mitomycin-C = MMC, 0.40 lg/ mL) were also preserved in all experimentations. The preparation of CA was achieved according to methods (Al-Mamary *et al.,* 2002) in and SCE tests with minor modifications (9). 200 from each donor of well-spread metaphases (in total 400/dose) chromosomal deviations score. Mitotic Index (MI) were specified by counting 2000 cells from every giver. For the SCE assess, the samples were marked with Giemsa, according to Evans methods (Evans and O'Riordan 1975; Kilbey *et al.,* 1984). The number of SCEs studied according (Yavuz-Kocaman *et al.,* 2008).

Micronuclei

Preparation of micronuclei was achieved as the protocols in references (Speit and Houpter1985; Schneider *et al.,* 1981). Propagations of cells were assessed using CBPI (Cytokinesis Block Proliferation Index) which showed the middling quantity of cell series, specified cell had undertaken (Fenech 2000; Surrales *et al.,* 19965; Wang *et al.,* 2005).

The main damage in DNA was due to the presence of Anthracene which was specified by means of comet evaluation (aldecott *et al.,* 1994), with few changes. The details of method that follows the comet assess in human lymphocytes was specified in the work (Hagmar *et al.,* 1994).

To distinguish the impracticality of cells, we used exclusion assessment of trypan blue which were (>98%). Lymphocytes were isolated and incubated using stimulated honey with (1 mg / ml for 1 hour at room temperature). Positive and negative controls (Anthracene $20x10^{-5}$) involved as well.

The percent of tail moment were around (100/slide) which represent 200 comets in total for each concentration which were resolute through certain image. The investigation method was (Comet Assess IV, Observant Instruments Shimadzou).

The data of the outcomes was studies carefully and the z-test was realistic to specify the percent of unusual cells with MI, CBPI, MN, CA, CA/cell, Dose–response, RI, SCEs. Relations and correlations were resolute from the abnormal cells percentage, MN, SCE, CA/cell.

Result

Table one show the differences between total phenols in (mg of GAE/g) and the total flavonoids for the *Curcuma* water extract, Honey non fortified with *Curcuma* active component and Fortified with *Curcuma*, while table 2 shows the active component in fortified honey by GC-MS for the three components Fortified Honey, Curcuma and the Control Honey.

In table 3 the values of IC_{50} to different antioxidant activity like nitric oxides DPPH in control honey, fortified honey and *Phoenix dactylifer*a. Table 4 shows the chromosomal aberration for different types of honey.

Table 4 shows the distribution of meningioma (MN) on the cell in different types of honey as well, last but to least is Figure one which represent the FT-IR Spectrum that shows the active components in fortified honey. Figure (1)and Table(2) showed the active component of all samples، we found there is

many new anticancer component and anti clinical diseases were found in fortified honey as:2propanone1,3dihydroxychromelin, Ethyl Ester, Dl-Glyceraldehyde dimer, Dl-Glyceraldehyde dimer, phosphoric triamide, n-Hexanal, α -phellanderene, Propranolol, 1-propanol,2- aminoalaninol , Octane, p-cymene, phosphoric triamide, Heptanoic acid, cineol, Methylene asparagines, Coumarin, 2-Phenylethanol, Benzylalcohol, Botanic acid, L- propline, Succinic acid, 1,3 Propandiol, 3-Deoxymannoic acid, (+)Ascorpic acid, 8,11-Octadecaoic acid, Tetradecane, Inositol. Table (3) Illustrates increases the dose of DPPH free radicals scavenging according to the high capability to scavenge to fortified honey extract.

Table 1. Total Phenol and total flavonoid of *Curcuma* water extract, Honey none fortified with *Curcuma* active component and Fortified with *Curcuma*.

Material	Total Phenols (mg of GAE/g)	Total Flavonoids
Curcuma water extract	17.13± .0.024	17.03± .0.08
Honey non fortified with *Curcuma* active component	22.65± .0.032	18.98± .0.022
Fortified with *Curcuma*	35.14± .0.017	25.47± .0.024

Discussion

Table one and table 2 only the components of the types of honey and from results that were gathered in this research it was found that fortified honey decreased chromosomal aberrations in all treatments as shown in table (3) and the Anthravene cause increases in the frequencies of MN depending on doses.

Table 2. Active component in fortified honey by GC-MS.

No	Chemical Component	Fortified Honey	Curcuma	Control Honey
1	2propanone1,3dihydroxychromelin	+		
2	Ethyl Estar			
3	Dl-Glyceraldehyde dimer	+	+	
4	phosphoric triamide	+		+
5	n-Hexanal	+		+
6	α –phellanderene		+	
8	α –zingiberene		+	
9	Propranolol	+		
10	1-propanol,2- amino alaninol	+		
11	Octane	+		+
12	p-cymene		+	
13	phosphoric triamide	+		
14	Heptanoic acid	+	+	
15	Cineol	+	+	
16	Methylene asparagines	+		+
17	Terpinolene		+	
18	n-Nonane			+
19	Coumarin	+		+
20	2-Phenylethanol			+
21	Benzylalcohol	+		+

22	2-Furaldehyde			+
23	Botanic acid	+		+
24	β-Caryophyllene		+	
25	L- propline	+		+
26	Succinic acid	+		
27	1,3 Propandiol	+		+
28	3-Deoxymannoic acid	+	+	
29	(+)Ascorpic acid	+		+
30	Curcumin		+	
31	α –turmerone		+	
32	8,11-Octadecaoic acid	+	+	
33	Heptadecanoic acid	+		+
34	9,15-Octadeca-dien-1-ol	+		
35	9,12,15-Octadecatrienoic acid	+		+
36	β –bisabolene		+	
37	Sesquiphellanderene		+	
38	r-turmerone		+	
39	Tetradecane	+		
40	Inositol	+	+	

In addition to its reflection to genomic variability, MN analyzes both chromosomes clast (breakage of chromosomes) and chromosomes lag because of dysfunction (Inoue *et al., 1997*) as was depicyed clearly in table 4.

The experimental data that was gathered in Table 4 indicate also the breaks in chromosome as the main deviation which lead to rearrangement in the structure that increase the irregularities in chromosome isolation during mitosis (Liou SH *et al.,* 2002). The increases in the risks of cancers were related to increases in CA level (Gisselsson 2001).

Table 3.IC_{50} value of different antioxidant activity.

Test	Fortified honey	*Phoenix dactylifer*a	Control honey
DPPH scavenging	8.02±0.012	25.7±0.011	18.68±0.022
Nitric Oxide Scavenging	22.14±0.015	137.03±0.005	120.94±0.033
Super oxide Scavenging	53.04±0.024	108.31±0.022	155.13±0.044

Our experiments also concluded that fortified honey reduced the mitotic index in all actions. Due to the reaction between (alkaline DMSO) and the (NBT) the free radicals were formed to give the painted diformazen.

The radical oxides in fortified honey cause the formation and the inhibitions of formazan as shown in Table 4. (The concentration of ascorbic acid used was 53.04µg/Ml).

The data gathered from all tests shows toxicity ofadditives in all their concentration, while the treating time reduced the mitotic index. The value of IC_{50} IN table 4 was 8.02µg/ml in compare with other samples. The scavenge activity to the active oxygen of

nitric oxides were affect the pathology action. Nitrogen species reactivity are in the following sequence NO_2, N_2O_4, N_3O_4 respectively, while the nitrate NO_3^- and nitrite NO_2^- shows high activity. We found that fortified honey had the lowest value IC_{50}value 22.14 µg / ml, the scavenging activity of radicals liberate from superoxide's using alkaline DMSO process are well-known in its harmfully to cells components.

Table 4. Chromosomal aberration by Furfural.

Treatment	MI	Type of chromosomal aberration						Total CA
		G'	G"	B'	B"	Deletion	SF	
Negative Control	9.88							
Fortified honey negative control	9.39	0.10	0.02					0.12
Positive control furfural ($10x10^{-5}$)	15.08	0.68	0.57	0.28	0.27	0.05	0.05	1.9
Fortified honey after furfural	11.38	0.31	0.18	0.12	0.10	-	-	0.99
Fortified with furfural	12.26	0.47	0.33	0.20	0.0.12	-	-	1.12
Furfural after fortified honey	12.57	0.51	0.38	0.23	0.13	0.01	0.01	1.27

In vitro genotoxicity exams discovered the complexes that cause the harms in genes, in both direct and indirect ways, in diverse routes. They were counted as a primary biologic signs things of exposure to carcinogenic chemicals (Mamur *et al.,* 2010).

Furthermore the frequencies of CA / Cell were increases in every treated group comparing to negative control. Six categories of chromosome deviation where caused by furfural showing its clastic effect.

Table 5. Number of Distribution of MN on the cell.

Treatment	No of the testing cell	Distribution of MN on the cell				No of the cells contain MN	No of MN	No MN/ Total cells No
		0	1	2	3	3	3	0.003
Negative Control	1000	998	3	-	-	5	5	0.005
Fortified honey negative control	1000	997	5	-	-	5	5	0.005
Positive control furfural (20x10-5	1000	972	13	3	2	18	25	0.025
Fortified honey after furfural	1000	995	7	-	-	7	7	0.007
Fortified with furfural	1000	988	10	2	-	8	14	0.010
Furfural after fortified honey	100	988	9	2	1	12	16	0.016

MI in table 5 was lessening may be because of G2 block which prevent cells to enter mitosis otherwise it could be due to lessening in the levels of ATP pressure resulted from the production of energy centers. Inhibition of certain cell cycle-specific enzymes, like polymerases of DNA, and its necessarily in generation of DNA, and other enzymes as well were involved directly with their orientations or the assemblies, this could clarify the effect of anti-mitosis and the frequency changes of diverse cell steps as well (Albertini *et al.,* 2000). Nuclear divisions and duplications indicate the of chemical additives effect, Table (5) Chromosomal aberration by Anthracene. (Arslan *et al.,* 2008). (B'= Chromatid break, B"=Chromatid break, G' Chromatid gapG' Chromosomal gap, while SF=Simple fragment)

Reference

Alvarez-Suarez JM, Giampieri F, Battino M. 2013. Honey as a source of dietary antioxidants: Structures, bioavailability and evidence of protective

effects against human chronic diseases. Current journal in Medicinal Chemistry, **20**, 621–638.

Bogdanov S, Jurendic, T, Sieber R, Gallmann, P. 2008. Honey for nutrition and health: A review. American Journal of Collision and Nutrition, **27**, 677–689.

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M. 2010.Contribution of honey in nutrition and human health: Mediterranean Journal of Nutrition and Metabolism**3,** 15–23.

Orhan F, Sekerel BE, Kocabas CN, Sackesen C, Adaliog˘lu G, Tuncer A. 2003. Complementary and alternative medicine in children with asthma. Annals of Allergy, Asthma & Immunology,**90(6),** 611-615.

Schramm DD, KarimM, SchraderHR,Holt RR,Kirkpatrick NJ, Polagruto JA,Ensunsa JL, SchmitzHH, Keen CL. 2003.Food effects on the absorption and pharmacokinetics of cocoa flavanols. Life Science , **73**, 857–869.

Keen CL. 2003. Honey with high levels of antioxidants can provide protection to healthy human subjects. Journal of Agricultural Food Chemistry, **51**, 1732–173.

Srivastava KC, Bordia A, Verma SK. 1995.Curcumin, a major component of food spice tumeric (Curcuma longa) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. prostaglandins leukot essent fatty acids**,52,** 223-227.

Zhang J, Qi H, Wu C. 2004.Research of anti-proliferation of curcumin on A549 human lung cancer cells and its mechanism**.** Zhongyaocai = Journal of Chinese medicinal materials**, 27(12),** 923-927.

Al-Mamary M, Al-Meeri A, Al-Habori M. 2002.Antioxidant activities and totalphenolics of different types of honey. Nutr Res, **22**, 1041–1047.

Evans HJ, O'Riordan ML.1975. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat Res, **31(3),** 135-48.

Kilbey BJ, Legator M, Nichols W, Ramel C. 1984. Handbook of Mutagenicity Test Procedures. Elsevier Science Publishers, Amsterdam, 405–427 p.

Yavuz-Kocaman A, Rencuzogullari E, Ila HB, Topaktas M. 2008. The genotoxic effect of potassium metabisulfite using chromosome aberration, sister chromatid exchange, micronucleus tests in human lymphocytes and chromosome aberration test in bone marrow cells of rats. Environmental and Molecular Mutagenesis.**49**, 276–282.

Speit G, Houpter S. 1985.On the mechanisms of differential giemsa staining of bromodeoxyuridine-substituted chromosomes. II. Differences between the demonstrations of sister-chromatid differentiation and replication patterns. Human Genetics. **70**, 126–129.

Schneider EL, Nakanishi Y, Lewis J, Sternberg H. 1981. Simultaneous examination of sister-chromatid exchanges and cell replication kinetics in tumor and normal cells in vivo. Cancer Research.**41**, 4973–4975.

Fenech M. 2000.The in vitro micronucleus technique.Mutation Research. **455**, 81– 95.

Surrales J, Xamena N, Creus A, Catalan J, Norppa H, Marcos R. 1995.Induction of micronuclei by five pyrethroid insecticides in whole blood and isolated human lymphocytes cultures. Mutation Research.**341**, 169–184.

Wang H, Rosidi B, Perrault R, Wang M, Zhang L, Windhofer F, Iliakis G. 2005.DNA ligase III as a candidate component of backup pathways of nonhomologous end joining. Cancer Research. **65,** 4020 4030.

Aldecott KW, McKeown CK, Tucker JD, Ljungquist S, Thompson LH. 1994. An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. Molecular and Cellular Biology. **14**, 68–76.

Hagmar L, Brogger A, Hansteen IL, Heim S, Hogstedt B, Knudsen L, Lambert, B, Linnainmaa K, Mitelman F, Nordenson I, Reuterwall C, Salomaa SI, Skerfving S, Sorsa M. 1994. Cancer risk in human predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Research.**54,** 2919–2922.

Mamur S, Yuzbas_10gˇlu D, Unal F, Yılmaz S. 2010. Does potassium sorbate induce genotoxic or mutagenic effects in lymphocytes? Toxicology In Vitro 24, 790–794. Michaelsson, G., Juhlin, L., 1973. Urticaria induced by preservatives and dye additives in foods and drugs. British Journal Dermatology.**88,** 525–532.

Liou SH, Chen YH, Loh CH, Yang T, Wu TN, Chen CJ, Hsieh LL. 2002. The association between frequencies of mitomycin C-induced sister-chromatid exchange and cancer risk in arseniasis. Toxicology Letters.**129**, 237–243.

Gisselsson D. 2001. Chromosomal instability in cancer: causes and consequences. Atlas of Genetics and Cytogenetics in Oncology and Haematology.

Inoue A, Yokomori K, Tanabe H, Mizusawa H, Sofuni T, Hayashi Y, Tsuchida Y, Shimatake H.1997. Extensive genetic heterogeneity in the neuroblastoma cell line NB(TU)1. International Journal of Cancer **72**, 1070–1077.

Albertini RJ, Anderson D, Douglas GR, Hagmar L, Hemminki K, Merlo F, Natarajan AT, Norppa H, Shuker DEG Tice, Waters MD, Aitio A. 2000. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. Mutation Research.**463,** 111–172.