



INHIBITION OF GENOTOXICITY EFFECT FOR ANTHRACENE IN LYMPHOCYTE IN VITRO USING FILTRATE LACTOBACILLUS ACIDOPHILLUS

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ABSTRACT

This study was conducted to determine the ability filtrate (*Lactobacillus acidophilus*) in the inhibition of the genotoxicity for anthracene on lymphocyte in vitro using cytogenetic analyses, which include tests (chromosomal aberration, mitotic index, micronuclei) The results showed that the filtrate *Lactobacillus acidophilus* have no genotoxicity in lymphocyte compared to the negative control, While concentrations of anthracene showed that there are a significant differences at ($P < 0.01$) with increasingly higher concentrations of anthracene. The Results showed that the treatment between filtrate *Lactobacillus acidophilus* and anthracene reduce the proportion of chromosomal aberrations and reduce a significant decrease in micronucleus formation. Was the best treatment are influential Add filtrate with anthracene or before and least influential is to add a filtrate after anthracene.

KEYWORD: genotoxicity, *Lactobacillus acidophilus*, anthracene.

INTRODUCTION

Anthracene is a chemical compound belongs to a cyclic group (polycyclic aromatic hydrocarbons) PAHs, molecular formula ($C_{14}H_{10}$) consists of three ring, source of anthracene could be a natural found in (coal coking, burning wood, cooking oil) and industrial (motor vehicle traffic on asphalt, diesel fuelled vehicles, aluminum and iron production), Anthracene is used in the production of the red dye alizarin and (azo dyes) (Enters in the industry naphthalene) It also is colorless but exhibits a blue (400-500 nm peak) fluorescence under ultraviolet light. (Algharbi, 2013; IPCS, 1998).

Mutations induced by PHAs

Continuous exposure of PHAs and the failure of repair mechanisms lead to induce mutagenesis in cells, Include These mutations are present in multiple genes as well that participate in cell survival, The danger of carcinogenesis by PAHs exposed individuals are associated with *p53* mutations due to the *p53* protein is a transcription factor that regulates cell proliferation, differentiation, apoptosis, and DNA repair, mutations induced in this important protein could lead to severe damage in cells and genes, (Mordukhovich, et al., 2010; Yoon, et al., 2003).

The ability antimutagenic for *Lactobacillus acidophilus*

One of the mechanisms by which working bacteria lactic acid to inhibit carcinogenesis is the metabolism of the probiotic products and perhaps the most important metabolic materials are (SCFA) Short Chain Fatty Acid including Butyrate that have a role in promoting apoptosis of cancer cells (Wong *et al.*, 2005). mention Mai, (2004) Metabolic activities for probiotic Produces that (SCFA) play a role in the prevention of Colorectal cancer (CRC) representing the most common malignancy of the gastrointestinal.

Cytogenetics Tests

Cytogenetic tests in lymphocytes culture are the most frequently applied in genetic toxicology and used in vitro to find out its ability to induce genetic mutations that may lead to the emergence of cancers and the chromosomal changes that can get Spontaneous or through the factors that influence either the nature of synthetic chromosomes or the mechanical movement (Ibrulj *et al.*, 2007).

METHODS

1-Bacterial identification

one capsule of *L.acidophilus* (natrol company) were dissolve in 1 ml of normal saline and then inoculated on

the MRS broth and incubated at 37 °C for 24 h. After observation turbidity of growth compared with McFarland tube, loopful by activated bacteria was streaking on MRS agar and incubation at 37°C for 24 h under anaerobic condition, cultural characteristics as described by Bergies manual (1984).

2-Microscopic examination

Isolated colony from MRS agar was examined under light microscope and the response to gram stain was also tested. (Hammes & Vogal, 1995) Colonies taken from

MRS agar medium appears white to pale in color round shape, soft, mucoid, convex and having smooth edges. figure (1).

3- Separation of the components of the bacteria L.B.

Inoculated (100) ml of MRS broth with bacteria *Lb. acidophilus* and at 37°C for 48 hours under anaerobic conditions, centrifuged for 15 minute at 13000 rpm, supernatant passed through filters accurate sterile (Milipore filter 0.22µ), put the filtrate in a sterile tube and kept at -4°C until needed.



Figure (1) colony of lactobacillus

4-Cytogenetic test

Lymphoid cell cultures Blood samples were collected from the peripheral (5) healthy people were non-smokers do not drink alcoholic beverages, Models cultured according to method of (Benn and Perle, 1992) Blood was cultured two groups For each group of five replicates.

A- The first group Lymphocyte culture for Mitotic Index, Chromosomal Abberations,

B-the second group: Lymphocyte culture for micronucleus assay Test tubes used for the second group in the preparation of micronuclei in according to the method of Fenech, and Morley, (1985).

Mitotic Index (MI) Assay

The slides were examined under high power (40 X) of compound light microscope and (1000) of divided and non divided cells were counted and the percentage rate was calculated for only the divided ones according to the following equation: (Ghosh *et al.*,1991).

$$\text{Mitotic Index} = \frac{\text{no. of the divided cells}}{\text{total no. of the cells}} \times 100$$

Chromosomal Aberration (CAs) Assay

The prepared slides were examined under the oil immersion lens for 100 divided cells for each animal or blood lymphocytes culture, and the cells should be at the first metaphase stage of the mitotic division where the chromosomal aberrations are clear and the percentage of these aberrations was estimated (Bauchinger *et al.*, 1983).

5 Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

RESULTS AND DISCUSSION

Biochemical identification of Lactobacillus

Selected *lactobacillus acidophilus* was depending on the biochemical characteristics, isolates were negative for oxidase tests, catalase tests, gelatin hydrolysis tests, *L.acidophilus* can grow at 45°C but not at 15 °C, These results were in agreement with those observed by Stukus *et al.*, (1997) and Collee *et al.*, (1996) and Isolation can not grow in nutrient agar medum, (Forbes *et al.*, 2002). table (1).

Biochemical tests	Result for lactobacillus acidophilus
Gelatinase Test	-
Oxidase Test	-
Catalase Test	-
Growth at 15 °C	-
Growth at 45 °C	+
Growth on nutrient agar	-

Experimental Design

Three stage were carried out to evaluation the cytogenetic effects of cell free extract of *Lactobacillus acidophilus*, and their Reducing the impact of the mutagen anthracene on lymphocyte.

1- Study the effect of genotoxicity probiotic on Lymphocytes in humans (in vitro)

Used in the evaluation of the genotoxicity to probiotic Cytogenetic test include Chromosomal Aberration and mitotic index and micronuclei The results shown in Table (2) and (3) there are no genotoxicity for probiotic when compared with the negative control Figuer (5) and (6).

A-Chromosomal Aberrationand Mitotic index

Table (2): Genotoxicity for probiotic in Lymphocytes (ML,CA)

Treatment	Mitotic index		Chromosomal Aberration							Statistical comparison
	MI%	SC	G'	G''	B'	B''	D	SF	Total CA	
Control (-)	9.70	A	0.034	0.018	0.0	0.0	0.0	0.00	0.052	A
CONTROL(+) P (P.S.B)	9.75	A	0.036	0.016	0.0	0.00	0.00	0.00	0.052	A
CONTROL(+) P (P.S.Ag)	9.70	A	0.03	0.014	0.00	0.00	0.00	0.00	0.044	A
P-value	--	--	--	--	--	--	--	--	--	NS

Similar Capital letters refer non significant between different groups (P.S.B) P= probiotic, S= standard *lactobacillus acidophilus*, B= filtrate from MRS broth/Ag = filtrate from MRS agar.

B-Micronuclei

Table (3) genotoxicity for probiotic in Lymphocytes (MN)

Treatment	Distribution micronuclei on cell				NO. of the cells contain MN	NO. of MN	NO. of MN\Total cells NO	Statistical comparison
	0	1	2	3				
Control (-)	997.6	2.4	0	0	2.4	2.4	0.0024	A
CONTROL(+) P (P.S.B)	998.2	1.8	0	0	1.8	1.8	0.0018	A
CONTROL(+) P (P.S.Ag)	998.2	1.8	0	0	1.8	1.8	0.0018	A
P-value	--	--	--	--	--	--	--	NS

Similar Capital letters refer non significant between different groups

The results showed absence of abnormalities chromosomal clear and distinct and not to increase the micronuclei in large numbers Compared with the negative control. Results obtained are compatible with the findings Yaseen., (1990).

The significant reduction in micronucleus formation associated with dose of treatment by filtrate of *L. acidophilus* particularly if our opinion that most cancers are preceded by mutations induced by different agents.

2-Study the effect of genotoxicity for Different concentrations of anthracene on Lymphocytes in humans (in vitro)

Cytogenetics is considered one of the important tests that have been adopted in many of the studies that are interested detects of effect carcinogenic materials or mutagen (Physical and chemical) environmental contaminants. Influence genotoxic of cell Which have the ability to be a mutagen materials (McGregor and Venitt, 1999) Results in table (4) which were conducted to detect the genotoxicity for anthracene on lymphoid cells showed that there are significant differences with (P<0.01) with increasing concentrations of anthracene. Concerning to variations chromosomal was The highest

percentage malformations recorded in G' (chromatid gap) a significant difference (P<0.01). Then it came later G'' (chromosomal gap), B' (chromatid break), While the show B'' (chromosomal break), SF (simple fragment), D (deletion) was the lowest percentage of distortions Figuer (7). In addition to increasing mitotic index With the increase in the concentration of anthracene. Also Results showed in table (5) The influence anthracene in the formation of micronuclei that there are significant differences at (P<0.01). With increase the percentage composition of micronuclei with increasing concentrations of anthracene as compared to control group Figuer (8).

Table (4) genotoxicity for anthracene in lymphocytes (MI, CA)

Treatment		Mitotic index		Chromosomal Aberration						Statistical comparison	
		MI%	SC	G'	G''	B'	B''	D	SF		Total CA
Control(-)		9.70	A	0.034	0.018	0.0	0.0	0.0	0.0	0.052	A
CONTROL(+) Anthracene	Con.A.1 2.5×10 ⁻⁵	9.85	B	0.096	0.044	0.028	0.012	0.0	0.0	0.180	B
	Con.A.2 5×10 ⁻⁵	10.03	C	0.2	0.106	0.09	0.05	0.014	0.016	0.476	C
	Con.A.3 10×10 ⁻⁵	10.92	D	0.328	0.120	0.104	0.078	0.040	0.040	0.710	D

The different capital letters refer significant between different groups at (P<0.01).

G': chromatid gap, G'': chromosomal gap, B': chromatid break, B'': chromosomal break, SF: simple fragment D: deletion

Acquired results are consistent with the findings of the Beck and lioyd , (1978) that The appearance of chromosomal variations in cells exposed to different concentrations of anthracene and increase these variations increase the concentrations of anthracene The effect on the DNA or the proteins present in the chromosome, Add to that the decline in the proportion of variations in the concentrations of low anthracene due to the inability of low concentrations of anthracene to reach

and influence the target molecules, whether DNA or proteins, Or to repair the system efficient in the cell, which works to rebuild the molecule DNA Rapidly in the case of low concentrations Unlike high concentrations as it works to slow the repair system and Increase for variations.

table (5) genotoxicity for anthracene in lymphocytes (MN)

Treatment		Distribution micronuclei on cell				NO. of the cells contain MN	NO. of MN	NO. of MN/Total cells NO	Statistical comparison
		0	1	2	3				
Control(-)		997.6	2.4	0	0	2.4	2.4	0.0024	A
Control(+) Anthracene	Con.A.1 2.5×10 ⁻⁵	994.8	4.2	1	0	5.2	6.2	0.0062	B
	Con.A.2 5×10 ⁻⁵	992	5.4	2	0.8	8	11.8	0.0118	C
	Con.A.3 10×10 ⁻⁵	985.6	6.4	4.2	3.8	14.4	26.2	0.0262	D

The different capital letters refer significant between different groups at (P<0.01).

As for The influence anthracene in the formation of micronuclei lead to increase percentage mention. pastor *et al* , (2002) The normal rate of occurrence of micronuclei nearly (4.7%) may reach (8.4%). Also addition Xia and Ichickawa, (1997) this percentage increase when exposed to chemicals that affect DNA.

3- interactions (before and after and together treatments) between lactobacillus isolated filtrate and Different concentrations of anthracene

At this stage can be observed viability of bacteria lactobacillus in inhibiting or reducing The damage in chromosomes or chromatids That result from lymphocyte exposed to the compound mutagen as anthracene and measure this damage through lower proportion of malformations.

filtrate *L. acidophilus* showed in Figure (2) the ability to reduce the proportion of malformations and chromosomal damage within percentage reasonable. The highest and best effect of treatment (together treatment) Add the filtrate with the concentration of anthracene at the same time, was reducing the highest rate of chromosomal aberrations (total C.A) for The highest concentration anthracene (Con.A.3 10×10^{-5}) from (0.710 to 0.188). Also it showed treatment (before treatment) Add the filtrate and then add the concentration of anthracene the ability to reduce the proportion of malformations and chromosomal damage But less than the first treatment. was reducing the highest rate of chromosomal aberrations (total C.A) for The highest concentration anthracene (Con.A.3 10×10^{-5}) from (0.710 to 0.210), Either treatment (after treatment) add anthracene concentration Then Add filtrate *L. acidophilus* It was less treatment influence reduce total C.A from) 0.710 to 0.342)

Figure (3) showed Mitotic index for all treatment (after and before and together) Decrease mitotic index compared to the control group anthracene indicates the effectiveness of a filtrate *L. acidophilus*.

From the above results we conclude that the (*Lactobacillus acidophilus*) ability to the treatment of the damage in living cells resulting from the mutagenic and that this ability comes maybe from the effective (SCFA) Short Chain Fatty Acid This is consistent with the findings Wong *et al.*, (2005) One of the mechanisms by which working bacteria lactic acid to inhibit carcinogenesis is the metabolism of the probiotic products and perhaps the most important metabolic materials are (SCFA) Short Chain Fatty Acid including

Butyrate that have a role in promoting apoptosis of cancer cells. Also reported Al-Khafaji, (2008) of the properties possessed by (*L.A.B*) is the ability of anti-mutagenesis by disabling mutagens in the intestines Adsorption.

Figure (4) As for the micronuclei for treatment between the filtrate *L. acidophilus* and the concentrations of anthracene was observed in (Together treatment) Recorded a decrease in the total number of micronuclei for The highest concentration anthracene (Con.A.3 10×10^{-5}) from 0.0262 to 0.004.

Also it showed treatment (before treatment) the ability to reduce the total number of micronuclei But less than the first treatment for The highest concentration anthracene (Con.A.3 10×10^{-5}) from 0.0262 to 0.005. Either treatment (after treatment) It was less treatment Recorded a decrease the total number of micronuclei for The highest concentration anthracene (Con.A.3 10×10^{-5}) from (0.0262 to 0.0054).

These results show what Caused the different concentrations of the damage in the DNA molecule and chromosomes, Resulting in chromosomal small fragments or loss centromere of the chromosomes. These fragment are connected During anaphase with non-affected chromosomes component of micronuclei as mentioned report Huang *et al.*, (2011). The results also showed the loss of genotoxicity of *L. acidophilus* and possession of anti- mutagenic susceptibility by reducing the number of micronuclei. This is consistent with what he referred to a report Ruqaya *et al.*, (2009).

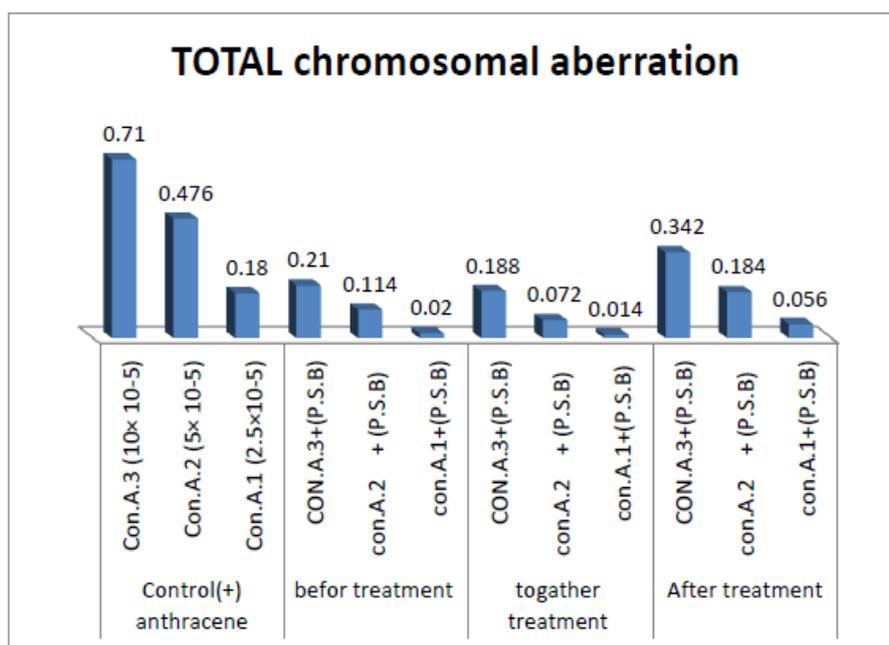
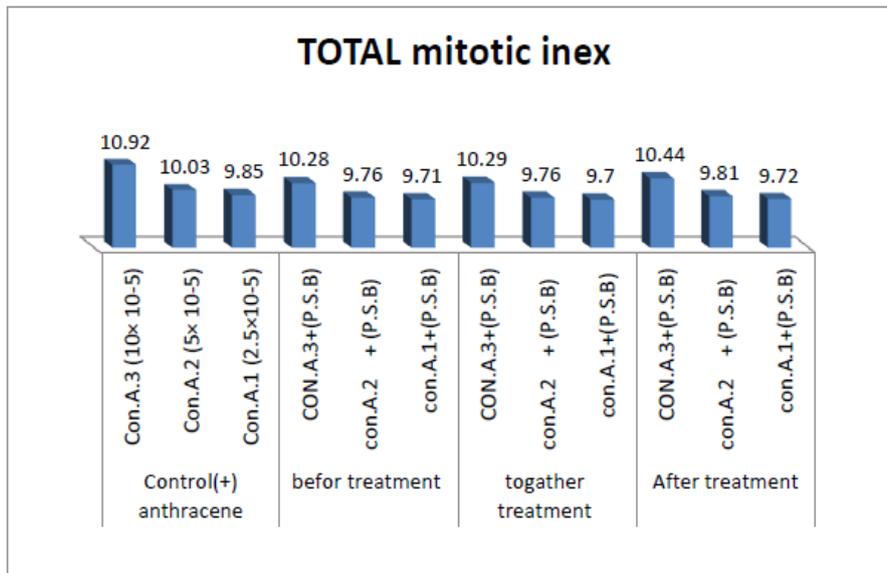
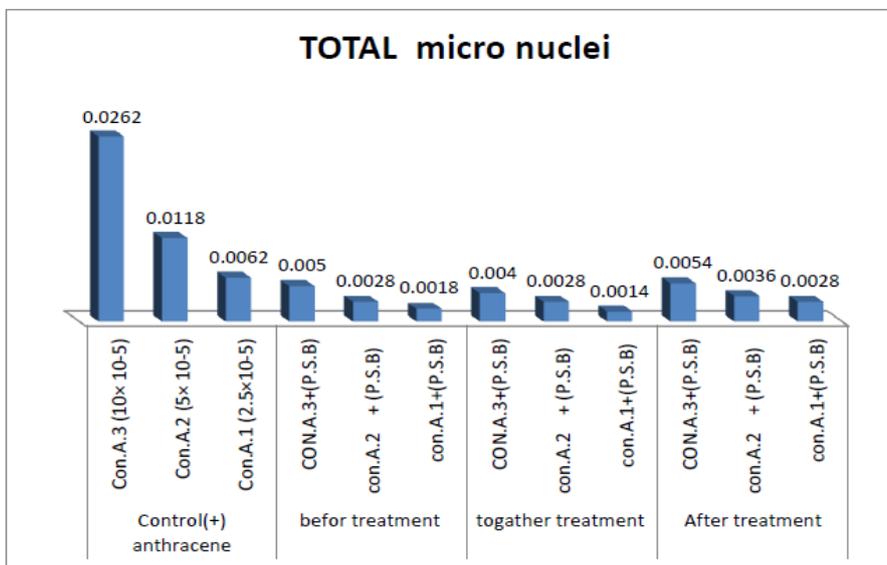


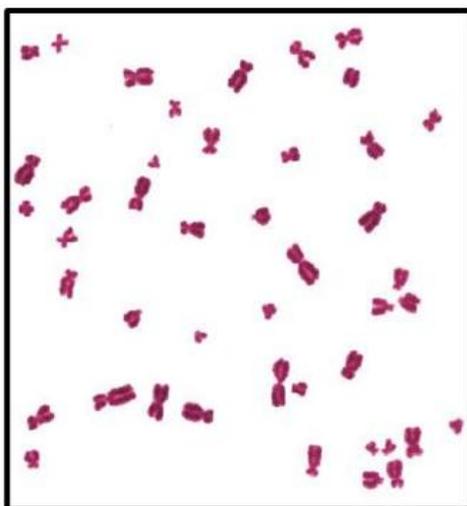
Figure (2) Decrease chromosomal aberration for treatment compared to the control group anthracene



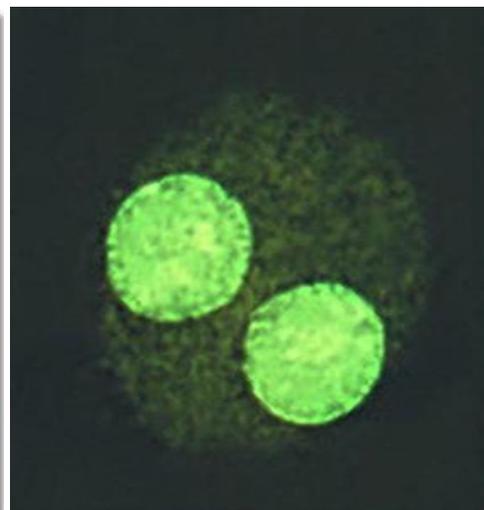
Figuer: (3) mitotic index compared to the control group anthracene



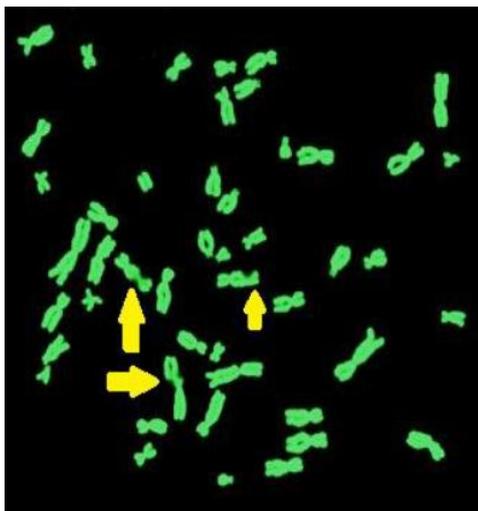
Figuer (4) Decrease micronuclei for treatment compared to the control group anthracene.



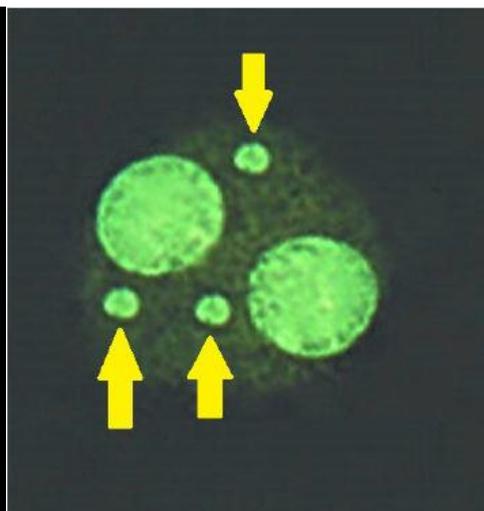
Figuer (5) Control (-)



Figuer (6) Binary cell nucleus without micronuclei



Figuer (7) A cell containing chromosomal changes of the type (gap chromatid, gap chromosome, break chromosome) Anthracene Con.A.3 10×10^{-5}



Figuer (8) Binary cell nucleus containing three micronuclei Anthracene Con.A.3 10×10^{-5}

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