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Effect of the *Spirulina* alga on chromosomal abnormalities in albino mice treated with Gasoline

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ABSTRACT

This study aims to investigate the effect of *Spirulina* algae at a concentration of 1.5%, 3% and 6% on chromosomal abnormalities of bone marrow cells in male albino mice towards the toxicity of benzene. The study included 48 mice divided into two groups: the first group (negative control group = 6 mice) and the group The second contained 42 rats divided into seven groups of 6 rats per group, where the positive control group was injected with benzene at a concentration of 0.2% twice a week diluted with corn oil in the peritoneal cavity, and three interference groups were dosed orally with *Spirulina* algae powder at a concentration of 1.5%, 3% and 6% once a day. They were injected with benzene at a concentration of 0.2% diluted with corn oil twice a week in the peritoneal cavity, and the last three groups were dosed orally with *Spirulina* powder only at a concentration of 1.5%, 3%, and 6% once daily. The results of the experiment indicated a significant improvement in chromosomal abnormalities in bone marrow cells in the overlap groups. The three groups (the third, fourth and fifth group) compared to the positive control group. The best results were recorded in the interference group of mice that took *Spirulina* powder at a concentration of 6% and injected with Gasoline at a concentration of 0.2% (the fifth group), where the rate of chromosomal abnormalities was (0.23 ± 1.98) compared to the positive control, which amounted to (4.03 ± 0.32), while the groups that were dosed orally with *Spirulina* powder at a concentration of 1.5% and 3% 6% only did not record any significant differences (P>0.05) compared to the negative control group, so the study recommends using *Spirulina* powder as a food supplement and an effective substance towards toxicity Gasoline for people exposed to it.

Keywords: *Spirulina powder, Gasoline, chromosomal abnormalities, bone marrow.*

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1 - INTRODUCTION

Man has used herbs and natural plants since ancient times in the treatment of diseases and recovery from them, and man's knowledge of the properties, benefits and uses of medicinal plants has increased over time [1]. And that one of the most important benefits of using plants in the treatment of diseases is the lack of side effects because the active substances are in small concentrations in plants, and therefore the human body receives these active substances in natural concentrations, while the use of manufactured chemical drugs is often accompanied by various side effects and because of these side effects many resort to Researchers have turned to plants and algae to explore materials that could be used as an alternative treatment [2]. *Spirulina*spp is one of the most common blue-green algae, as it is used in the medical, industrial and food fields, because it contains high levels of active compounds [3]. Cytogenetic biomarkers are used in many studies due to the sensitivity of genes to toxic agents, where these studies have a major role in early detection of the risk of cancerous diseases, and therefore these indicators play a role in helping to prevent toxic factors. Chromosomal aberatioms (CAs) are tests. Micronucleus (MN) is one of the most important of these tests [4]. Benzene is a highly volatile and flammable hydrocarbon aromatic liquid chemical substance. It dissolves slightly in water and quickly dissolves in oil, ether, acetone, chloroform and alcohol. It is colorless or tends to a yellowish-white color when present in gasoline [5,6]. Humans inhale the vapor of gasoline. The body quickly absorbs it, where it is metabolized in some organs of the body such as the liver and bone marrow, and the metabolites are excreted with urine after being associated with many reactions [7]. Blood, where it is absorbed by the membranes of red blood cells and accumulates within tissues that contain a high percentage of fat, and that about 50% of inhaled benzene is exhaled, and the rest is metabolized mainly in the liver [8]. Benzene metabolism results in compounds that are Catechol and Hydroquinone, which are transported to the target tissues via the hepatic portal vein, where these substances cause damage directly or indirectly [9]. These

compounds pass into the bone marrow, where they are transformed into benzoquinone, which is toxic to the bone marrow [10]. Where benzoquinone causes genotoxic effects, including mitotic recombination, an increase or decrease in the number of chromosomes, and chromosome translocation[11].

2 - Material and Methods

2 – 1 : Laboratory animals

Forty-eight male white mice were used in the experiment, their weights ranged between 25-30 grams and their ages ranged between 2-3 months. They were obtained from the University of Kufa, College of Science, Animal House. It was distributed into eight groups, each group consisted of 6 rats.

2 – 2 *: Spirulina platensis* powder

Spirulinaplatensis powder obtained of Chinese origin in a bag weighing 450 grams. The required concentrations were prepared according to the method of Reeves(1993) by adding 1.5, 3, 6 grams of *Spirulinaplatensis* powder on 100 ml of normal saline, where special containers of 100 ml were used, and they were kept in the refrigerator until use.

2 – 3 :Gasoline

Gasoline was supplied by the Al-Karrar fuel station affiliated to the Middle Euphrates branch for the distribution of oil derivatives in Al-Najaf Al-Ashraf Governorate, the Iraqi Ministry of Oil.

2 – 4 : Examination of chromosomal abnormalities

Using a 100x oil lens optical microscope, the slides were examined considering the condition that the cells are in the equatorial phase, in which the chromosomes are clear, and the percentage of aberrations was estimated [12].

2 – 5 : Chemical solutions

Chemical solutions for cytogenetic analyzes were prepared in this experiment by the method of Allen et al. [12]

2 – 6 :Statistical analysis

The results were analyzed statistically for the purpose of identifying the significant differences for the treatments included in the study, where the standard deviation rate was calculated for the variables, as well as one-way ANOVA was tested, and the LSD calculation was the least significant difference intended to identify the significant differences that were identified at the level of probability 5% [13].

Figure 1: Chromosomes at metaphase of a bone marrow cell in a mouse treated with benzene 0.2% and *Spirulina* 3% (Group IV) .

Figure 2 : Chromosomes in metaphase of a bone marrow cell in a mouse treated with 0.2% Gasoline (positive control group) .

3 - RESULTS AND DISCUSSION:

The results of the statistical analysis showed that there was a significant increase ($P<0.05$) in the rate of chromosomal abnormalities in the positive control, which reached (4.03) compared to the negative control group, which amounted to (1.91 ± 0.5), while the use of *Spirulina* algae contributed with concentrations of 1.5%, 3% and 6 % in the groups of interaction with benzene (the third, fourth and fifth group) reduced the rates of chromosomal abnormalities, as it recorded a significant decrease ($P<0.05$), which reached, respectively (1.98 ± 0.23, 2.52 ± 0.24, 3.08 ± 0.3) compared to the positive control, which amounted to (4.03 ± 0.32), and the results showed that there were no significant differences ($P>0.05$) in the rate of chromosomal abnormalities in the groups (sixth, seventh, and eighth groups) in which only *Spirulina* algae was used at concentrations of 1.5%, 3%, and 6%, as it reached, respectively (1.66±0.38, 1.79 ± 0.41, 1.84 ± 0.26) compared to the negative control, which was (1.91 ± 0.5).

Structural changes to chromosomes occur because of exposure of living organisms to many chemicals that interfere with the genetic material (DNA), leading to major chromosomal changes, including chromosomal breakage, chromatid segregation, mutations and deletions [14]. The results of the current study, as shown in Table 1 , showed a significant increase in the rate of chromosomal abnormalities in bone marrow cells, which included chromosomal breakage, chromatid breakage, formation of chromosomal breaks, and deletions in the positive control group, which was injected with benzene at a concentration of 0.2%. These results agreed With Fujie *et al*. [15], where they indicated in their study that benzene caused an increase in chromosomal abnormalities, which include the occurrence of break and gap separation in the chromosomes of bone marrow cells, and they indicated that this could be attributed to the compounds that are formed as a result of benzene metabolism In the liver, where it is metabolized to several compounds, including phenol, hydroquinone, and benzoquinone. Kalf [16] indicated that benzene is metabolized to phenol by the cytochrome p-450 pathway, whereby phenol goes to the bone marrow and in turn is metabolized to catechol and hydroquinone, which work to form free radicals that attack DNA. Others believe that these substances can interfere with the two strands of DNA, causing a group of chromosomal abnormalities that include chromosomal breakage and chromosomal segregation [14]. Or it may be attributed to the fact that one of the benzene metabolites (1-4 benzoquinone) can inhibit the enzyme opoisomerase II, which works to anneal the breaks between the DNA strands and thus the occurrence of chromosomal changes [17]. Table 1 also showed that the use of *Spirulina* algae powder helped mitigate chromosomal abnormalities caused by benzene to bone marrow cells. The best results were recorded in the fifth group, where *Spirulina* algae was used at a concentration of 6%, and this is consistent with the findings of Aly *et al*. [18] where they indicated that *Spirulina* algae has an antidote effect towards the destruction of the genetic material DNA, due to the fact that these algae possess polysaccharides that have the ability to enhance the work of DNA repair enzymes.*Spirulina* algae has the potential to enhance antioxidant activities and DNA repair processes [19]. This improvement in the rate of chromosomal abnormalities may be attributed to the ability of *Spirulina* algae to restrict the activity of ROS, which would reduce chromosomal abnormalities [18]. The health benefits of *Spirulina* are due to the chemical compounds it contains, as there are three biologically active compounds: phycocyanin protein, γ-linolenic acid, and sulfated polysaccharides [20].

4 –Conclusions:

The use of spirulina powder contributed significantly to reducing or preventing the cytogenetic toxicity caused by Gasoline.

Table 1: The effect of using *Spirulina* algae against the toxicity of Gasoline, on the percentage of chromosomal abnormalities in bone marrow cells of male albino mice.

Parameters / Group	Deletion	Chromosome Gap	Chromatid break	Chromosome break	Total
Negative control (1st group)	0.235±0.16B	0.131±0.11C	0.263±0.15B	0.129±0.17B	1.91±0.5D
Positive control Benzene 0.2% 2nd group	0.481±0.18A	0.555±0.15A	0.424±0.12A	0.505±0.12A	4.03±0.32A
Benzin + *Spirulina* 1.5% (3rd group)	0.466±0.13A	0.300±0.12B	0.245±0.13B	0.274±0.17B	3.08±0.3B
Benzin + *Spirulina* 3% (4th group)	0.356±0.15AB	0.330±0.14B	0.217±0.16B	0.194±0.12B	2.52±0.24C
Benzin + *Spirulina* 6% (5th group)	0.268±0.14B	0.260±0.11BC	0.134±0.12B	0.181±0.11B	1.98±0.23D
***Spirulina* only 1.5% (6th group)**	0.222±0.13B	0.166±0.1C	0.253±0.14B	0.141±0.12B	1.84±0.26D
***Spirulina* only 3% (7th group)**	0.209±0.12B	0.213±0.14BC	0.208±0.13B	0.204±0.12B	1.79±0.41D
***Spirulina* only 6% (8th group)**	0.192±0.13B	0.274±0.09BC	0.242±0.08B	0.167±0.15B	1.66±0.38D
LSD (0.05%)	**0.172**	**0.150**	**0.160**	**0.165**	**0.403**

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