Effect of Polluted Water on Chromosomes of Rats

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Abstract

There are several materials which have negative effect on the genetic material. Scientific studies in many well-known universities all over the world confirm that water pollutants are one of the materials which have great impact on the DNA.

This study aimed to clarify the effect of environmental pollution on some genetic processes of Rat. The study include three groups, the first one is the control group which treated with tap water, the second group treated with cyclophosphomide in single acute dose (20 mg/kg) , and the last one treated with industrial waste water collected from Hilla fabric factory.

Chromosomal aberrations were used as an index to evaluate the relationship between the genetic processes and water pollution. The results clearly shows the significance increase in both structural and numerical chromosomes, there is a significance increase in Chromosome break, aneuploidy, Analytical chromosome, chromosome breakage, and Ring chromosome.

The industrial water pollutant show a high increase in the acceptable limits set by WHO. This increase has a great effect on chromosome aberrations both structural and numerical.

The predominant types of aberrations by exposure to industrial pollution were centromeric attenuation, break and ring, gap and end to end association.

Keywords: Environmental pollution, Chromosomal aberrations, Genetic processes

الخلاصة

اكدت الدراسات العلمية المنجزة في مراكز بحثية عديدة على التاثيرات الكبيرة لملوثات الماء في المادة الوراثية ان هذه الدراسة هو التحري عن تاثيرات الملوثات البيئية في المادة الوراثية للجرذان . تتضمن الدراسة تقسيم الحيوانات الى ثلاث مجاميع ، الاولى وهي مجموعة سيطرة والتي تم معاملتها بمياه الشرب ، والمجوعة الثانية عوملت بمادة السايكلوفوسفومايد بجرعة واحدة مقدراها ٢٠ ملغم/كغم كسيطرة موجبة اما المجموعة الثالثة فقد تم معاملتها بماء ملوث ناتج عن المخلفات الصناعية من معمل نسيج الحلة. استخدمت الانحرافات الكروموسومية كدليل لتقييم العلاقة بين الفعاليات الجينية وتلوث المياه. تم تحليل عينات الماء لتحديد المعادن المتواجدة مثل الحديد والرصاص والكادميوم والنحاس والزنك والفضة واخيرا النيكل(بيانات غير منشورة حاليا). اظهر الماء الصناعي الملوث زيادة كبيرة عن المستويات المقبولة والمصرح بها من قبل منظمة الصحة العالمية. اذ تودي الزيادة في الملوثات الى تاثيرات كبيرة في الانحرافات الكروموسومية وبكلا الاتجاهين التركيبي والعددي. ان الانحرافات المتكررة بسب التعرض للمياه الصناعية الملوثة هي ضعف القسيم المركزي والكسر الكروماتيدي والكرموسوم الحلقي والفجوات والارتباط من طرف النهايتين.

Introduction

Recent decades have brought increasing concerns for potential adverse human and ecological health effects resulting from the production, use and disposal of numerous chemicals that offer improvements in industry, agriculture, medical treatment, and common household conveniences(Daughton C.G.,1990). Over the years, there has been a continuous expansion of the fabric industry, and considerable advances made in the discovery of massive production lines and mega plants that produced a huge amount of pollutants (e.x. Carcinogen, Teratogen and mutagen).

Fabric plants generate a wide variety of wastes during manufacturing, maintenance and housekeeping operations. While maintenance and housekeeping activities are similar from one plant to the next, the actual processes used fabric manufacturing vary widely. With this diversity of processes comes a similar diverse set of waste streams. Typical waste streams include spent fermentation broths, process liquors, solvents, equipment wash waters, spilled materials and used processing aids (FEBA). The disposal of this type of wastes is of environmental concern. Little is known about the extent of environmental transport and ultimate fate of many synthetic organic chemicals after their intended use, particularly hormonally active chemicals (Staykova, *et al* 2005).

The deficiency of the DNA is the most important causes of mutating the genetic material, cancer, and the other diseases which might happen to mankind. (Kapiszewka, 2005) Therefore, the nutriments are used during what is called therapy preparations or Nutraceutical, which is a portmanteau of the words "nutrition" and "pharmaceutical", is a food or food product that reportedly provides health and medical benefits, including the prevention and treatment of disease (Hardy, G. 2000). Among the plants used to purify water, the members of the Cruciferae are mentioned. These plants protect the vital systems because they contain active chemical materials such as alkaloid, phenols and glycosides (Potter 1990, Steinmetz, 1991.

The genetic cellular tests

A- The factor of Cleavage: It is the percentage of the divided cells in the periods of the division divided by the whole number of a sample including 1000 cells. (Ghosh *et al*., 1991).

Mitotic Index = The number of the dividing cells X 100 / The total number

The mitotic index factor is used to discover the toxicity of genetic effect for several physical and chemical agents which mostly affect the average of division or cleavage (De-Bonis *et al*., 2004). It is also used to discover the effects of the agricultural germicides. For example, the treatment of a group of rats with gradual doses of the Nuracron, which is one an organic phosphoric germicide or organophosphorus, to a significant decrease of the MI in comparison with the negative control (Zahran et. al. 2005). It also used to observe the effects caused by materials used in the industry. The addition of kosibol recovered from the cotton seeds to the animal fodders resulted in the decrease of the percentage of the dividing cells of the bone marrow of the rats. This resulted from its effect on the tiny pipes of the spindle (Aire and Akinogbemi, 1994).

B- The chromosome malformations

The chromosome changes are used in different stages of division to evaluate the damages happening in the genome under the effect of different physical and chemical agents (Meteuca *et al*. 2006). The chromosome malformations differ according to the effective factors. These include:

- Chromatid fractures which occurs only in one branch of the chromosome.
- Chromosome fractures which occur in both of chromosome branches.
- Fractured chromosomes in which they appear in the form of widespread tiny pieces.
- The decomposed chromosomes in which we cannot distinguish between one chromosome and others.
- Circular chromosome in which it appears in a form of a circle as result of the concourse of its two ends.

Aim of the study: To define the effects of water pollutants on genetic material.

Materials And Methods

1- Experimental design

Female rat (*Rattus norvegicus*) weighing 300±50 g , 12 weak age , grouped by the following order:

A- Negative control: drinking tap water .

B- Positive control: Treated with cyclophosphomide in single acute dose (20 mg/kg).

C- Treatment: The animal treated with polluted water for 30 days .

2- Cytogenetic assay

At the end of the experiment, the animals were sacrificed for analysis the cytogenetic assays , which include:

A- chromosome aberration , according to Tolliver and Robbins (1991) .

B- Mitotic index , according to (Ghosh *et al*., 1991) .

C- DNA fragmentation test , DNA extract according to Promega . USA . leaflet . and the test according to Prifer (1984) .

Statistical analysis was done using SPSS ver.17 with the LSD test under 0.05 probability level.

Results and Discussion

The treatment of the animals with water polluted with the remains of the textiles factories for 30 days increases the percentage of the chromosome malformations of the bone marrow of the animals to 50.32% in comparison with the negative control which showed 3.29%. The difference is significant in all types of malformation except chromatid fractures on the level of $P< 0.05$ in comparison with the positive control as seen in table no.1.

Table 1: Types of chromosome malformations

Treatment	Ring chromatid	Breaking chromatid	Analytical chromatid	Aneuploidy	Chromatid break	Total
Negative control	٠.٦٦	٠.١٣	٠	٠.٨٠	١.٧٠	٣.٢٩
Positive control	*٥.٠٦	*٧.٠٦	*٧.٨٣	*٤٢.٥٣	١٤.٦٦	٧٧.١٣
Treatment by pollution water	*٧.٠٠	*٣.٣٣	*٧.٣٣	*١٧.٦٦	*١٥.٠٠	٥٠.٣٢

* significant at 0.05

Table no. 2 showed the value of the factor of the division of the bone cells for the animals treated with polluted water which has been increased to 10.2, which is highly significant in comparison with the negative control.

Table 2: The differences of mitotic index between treatment.

Treatment	Mitotic Index
Negative control	٥.١٦
Positive control	*١.٢٦
Treatment by pollution water	*١٠.٢٠

* significant at 0.05

The reason of the high percentage of the chromosome malformations Table (1) and the value of the factor of division Table (2) is due polluting water with different kinds

of polluting chemical and biological materials such as different chemical compounds which are produced as factory residues. These residues are not correctly treated, therefore, they lead to stimulate the chromosome malformations as a result of the exposure to those agents (Fig. 1).
Moreover, those agents cause fractures, replacement of the pieces of the chromosome, or numerical changes some of which may cause the death of the cell and others may cause genetic effects both in the somatic cells and germ cells (Swierengo *et al*. 1991).
These changes lead to lose in the genome during the process of division, or imprecise isolation of the chromosomes from one another, therefore, the cell is liable to death (Tueker and Preston, 1996).

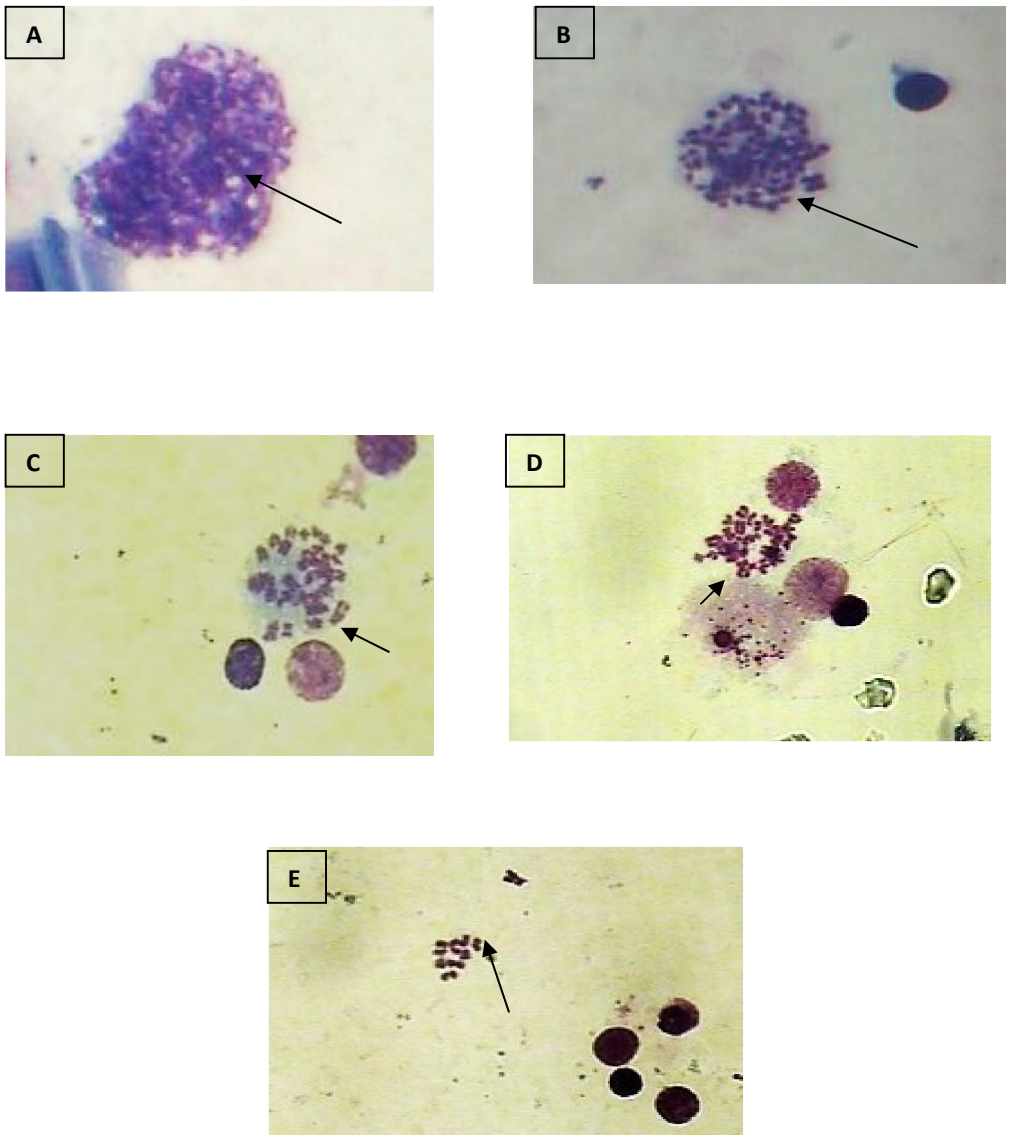

Figure 1: Chromosome malformations in bone marrow of animal that treated by industrial polluted water. A: Analytical chromosome, B: Chromosomes breakage, C: Chromatid break, D: Ring chromosome, and E: Aneuploidy

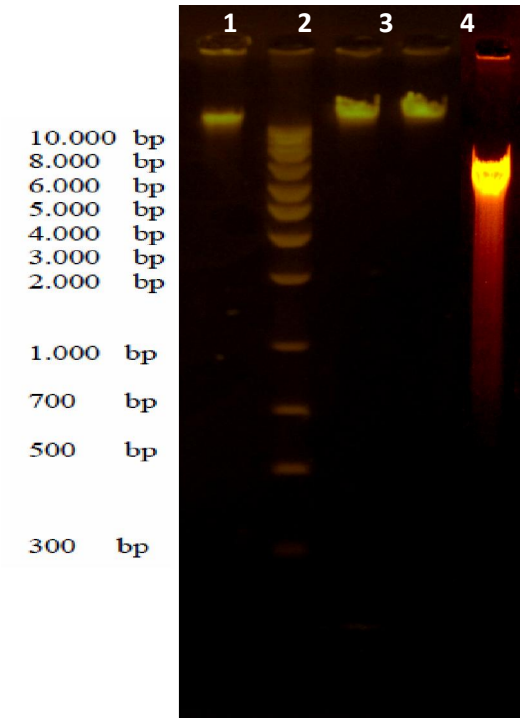

Figure 2: Electrophoresis pattern of DNA extracted from animal blood, the DNA was electrophoresed on 2% agarose gel , 70 V, 20mA for 2 hours. Direct visualization with Ethidium Bromide under UV light.

Lane 1: DNA extracted from animals in negative control group.

Lane 2: DNA marker (10000 bp). Lane 3,4: DNA extracted from animals treated with polluted water.

Lane 5: DNA extracted from animals in positive control group.

The changes in the chromosomes during different stages of division are also used to evaluate the damages which happen in the genome because of different physical and chemical factors (Meteuca *et al*., 2010). The chromosome malformations are either quantitve which include decrease or increase of the number of the chromosomes, or qualitative such as the exchange of the chromatid (Anderecscu *et al*., 2010).

The increase of the division factor of the animal treated with polluted water is considered important evidence that the polluting chemicals, the factory residues and the biological remains are existent. The study of the river polluted water manifests that there are significant differences of the factors of cells division of fish and the onion roots when they are treated with the polluted river water (Maschio, 2009).

The result of DNA fragmentation test (Fig. 2) show the DNA extract from treated animal blood don't affect by treatment , the molecular size of bands more than 10000 bp thus no hydrolysis in DNA comparison with DNA extract from animal treated by cyclophosphomide, the molecular size of bands approximately 8000 bp and the hydrolysis level is 700 bp . The reason in which DNA didn't affected by treatment because the blood pass through liver and kidney thus it cleaned and removal all toxic material happen in this route.

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