



# Genetical Effect of Flavonoids Extracted from the Leaves of *Camellia sinensis* on Mice

Amina S. Jalal

Technical College of Health and Medical, Technical Education, Baghdad

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**Abstract:** This study was in AL-Nahrain Research Center/University of AL-Nahrain in 2009, examine the role of flavonoids extracted from *Camellia sinensis* leaves in the inhibition effects of genotoxicity of the chemical Methotrexate (MTX) in mice and depended on some genetic analyzes, which includes the Mitotic index, Chromosomal aberration assay and Micronucleus test in the bone marrow of the mice. This study showed that methotrexate (MTX) agent had toxic effect on cytogenetic by decreasing the mitotic index and increasing the chromosomal aberrations and micronucleus in the bone marrow while the flavonoids extract showed anti-mutagenic activity against methotrexate (MTX) by increasing the mitotic index and decreasing the chromosomal aberrations and micronucleus in the bone marrow.

**Key words:** Flavonoids, Methotrexate, Mitotic index, Chromosomal aberration assay, Micronucleus test.

## التأثير الوراثي للفلافونويدات المستخلصة من أوراق الشاي الأسود في الفئران البيضاء

آمنة صبيح جلال

كلية التقنيات الصحية والطبية، هيئة التعليم التقني، بغداد

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**الخلاصة:** تمت الدراسة في مركز أبحاث النهريين/جامعة النهريين سنة 2009 وتضمنت بحث دور الفلافونويدات المستخلصة من أوراق الشاي الأسود في تثبيط التأثير السمي للمادة الكيميائية الميثوتركسيت في الفئران البيضاء بالإعتماد على بعض التحليلات الوراثية والمتضمنة معامل الإنقسام الخيطي، فحص التشوهات الكروموسومية وفحص النوى الصغيرة في نخاع عظم الفئران البيضاء. وقد بينت هذه الدراسة إن مادة الميثوتركسيت لها تأثير سمي وراثي من خلال خفض معامل الإنقسام الخيطي وزيادة التشوهات الكروموسومية والنوى الصغيرة في حين إنملاك مستخلص الفلافونويدات تأثير مثبط لمادة الميثوتركسيت من خلال زيادة معامل الإنقسام الخيطي وإنخفاض التشوهات الكروموسومية والنوى الصغيرة في نخاع العظم.

## Introduction

The genetic mutation means that genetic changes occur to genes or chromosomes due to physical factors or chemical, can these genetic changes be passed on to subsequent generations. Chemical mutagens too many being used in important areas of life(1). Examples of chemicals that cause mutations Methotrexate drug (MTX) which is widely used to treat tumors, especially early disease leukemia and the treatment of psoriasis and chronic arthritis. As it produces its effects by facilitating the taking it by the cell causing rein in the metabolic processes that occur inside the cell, whether normal or infected (2).

The research showed the preventive action of materials and elements in the human food which have a characteristic anti-mutagenesis like the flavonoids present in black tea which works as materials of anti-carcinogenesis.

Black tea comes from a plant called "*Camellia sinensis*" and accounts for 74 percent of tea produced by the world. The polyphenols, especially those that are called "flavonoids", is a powerful anti-oxidants. The flavonoids include both "Alkatekinat", "Theflavinat" and "Therobeginat", which is mainly responsible for the health benefits and the beneficial effects of tea (3).

Flavonoids is a plant secondary metabolism products of multiple phenol materials typically found in glycoside materials and rarely in a free Aglycones. The natural compounds found widely in higher plants (4). Chemical formula of flavonoids is (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>). The actual number of these compounds at this time about (2000) composite differ among

themselves in terms of the number of groups hydroxyl and its molecule (5). Divided flavonoids into several types depending on their chemical composition and include Anthocyanides, Catechines, Flavonone, and Glycoside flavonones (3).

## Materials and Methods

Extract flavonoids: followed the method contained in the source (6) by the soaking (50) g of the powder plant dried for a period of 24 hours of methanol concentration (70%), and then filter the mixture through the papers whattman 1 evaporation alcohol after leaving in the incubator for a period of time, then suspended the size of a given distilled water, sterile filtration and preservation of the bottles in the dark (-20) until use. And attended the detection solution with adding 10 ml ethanol concentration %50 with 10ml solution of potassium hydroxide concentration %50, mixed equal sizes from each of this solution and extract, when see the yellow color returned a positive result to the presence of flavonoids.

## Study the ability of anti-mutagenesis

The mice were divided into five experimental groups. Each group consisted of three mice.

-A positive control group of mice that were intraperetonal injection of MTX concentration 30mg/kg has been anatomy after 24 hours (7).

- Mice that were injected by MTX and after 24 hours of injection they given orally by flavonoids that extract from the leaves of black tea with three doses are (4,40, 400) mg/ kg for six

days.

- A negative control group of mice.

### **Mitotic index**

Followed the method contained in the source (8), the mice was injected intraperitoneal with a single dose of 0.25 mL colchicine then killed after two hours then wash the bone with a solution PBS. Then centrifuge by 2000 rpm for 10 min, removed the filtrate and added 5 mL of a solution of (KCl). Then tubes placed in the incubator 37 degree for one hour. Then centrifuge by 2000 rpm for 10 min. Removed the filtrate and added 5 mL of a fixative solution gradually drops and on the wall of the tube with constant shaking. Then put the tubes in the refrigerator measured 4°C for half an hour, then centrifuge by 2000 rpm for 10 min. Thrown filtrate (repeated this process 2-3 times) and the last time suspended sediment about 1 ml of the fixative solution. Mixed the contents of the tube well then dropped a few drops of the contents of the tube on a clean glass slide and then left to dry and then colored dye giemsa slides and left to dry . The slides were examined under the microscope.

$$(\% \text{ MI}) = \frac{\text{number of dividing cells}}{\text{total number of cells}} \times 100$$

### **Chromosomal aberration assay (CA)**

Prepared slides were examined using 1600 times the power zoom (16X eyepiece and objective lens 100X) lens using oil as 100 cells were examined for each animal in the metaphase of mitosis and calculated percentages of them.

### **Micronucleus test (MN)**

Followed the method contained in reference (9) in this test as it was washing the content of the bone by syringe medical using a phase 2 ml of human serum, which underwent Heat Inactivation at a temperature of 56 °C for half an hour and then centrifuge the tubes containing bone marrow cells quickly 2000 rpm for 10 min and neglected the filtrate. Shake tubes well and put drops of it on the edge of a glass slide and the work of smear. Then left the slides to dry and then put drops of alcohol methanol on these slides for the purpose of fixative the cells and left to dry, then stained slides with Giemsa dye then washed with distilled water and left to dry and examined the slides using the lens of oil have been calculated the micronucleus in 500 cells.

### **Statistical analysis**

Results were analyzed statistically using the statistical packages for Social Sciences (SPSS). It was the moral test of differences between the averages of the application of a one-way analysis of variance (ANOVA) and Duncan test (10).

### **Results and Discussion**

Table(1) is clear that mutagen MTX which represents the positive control was the latest clear reduction in the coefficient of mitotic index for animals and this decline is significantly different at a level of (P> 0.05) compared with negative control.

When an overlap between MTX and flavonoids extracted from the leaves of black tea, showed significant increase in the overlap ratios of mitotic index and

legal teams at the level of ( $P > 0.05$ ) compared with positive control.

**Table (1):Overlap between the flavonoids and MTX in the MI values**

Concentrations mg / kg	control		% Inhibition	treatment	% Protection
	Negative	Positive			
	23.51 ± 0.18 a	10.13 ± 0.21 c	57		
4				15.26± 0.31b	22
40				18.44±0.15b	35
400				20.31 ± 0.29a	43

Table(2) shows that the treatment by the mutagen MTX (positive control) led to increase the percentage of chromosomal aberration which this is a moral difference at the level of ( $P > 0.05$ ) compared with negative control.

When an overlap between MTX and flavonoids extracted from the leaves of black tea, it was observed a decrease in the rates of chromosomal aberration when dosing animals (4,40,400) mg/kg and thus showed significantly different at a level of ( $P > 0.05$ ) compared with positive control.

**Table (2):Overlap between the flavonoids and MTX in the CA values**

Concentrations mg / kg	control		% Inhibition	treatment	% Protection
	Negative	Positive			
	1.21 ± 0.4 a	6.11 ± 0.22 c	80		
4				3.63 ± 0.21 b	40
40				2.39 ± 0.11 a	60
400				2.16 ± 0.26 a	65

Table (3) shows that treatment by MTX (positive control) led to increase in the micronucleus this is a moral difference at the level of ( $P > 0.05$ ) compared with negative control.

When an overlap between MTX and flavonoids, it was observed a decrease in

the rates of the micronucleus animals at the dosages (4,40,400) mg/kg and thus showed significantly different at a level of ( $P > 0.05$ ) compared with positive control.

Table (3):Overlap between the flavonoids and MTX in the MN values

Concentrations mg / kg	control		%Inhibition	treatment	% Protection
	Negative	Positive			
	1.44 ± 0.23 a	6.78 ± 0.24 c	78		
4				4.17 ± 0.92 b	38
40				2.63 ± 0.22 a	61
400				1.89 ± 0.19 a	72

It is clear from the results of the current study, the flavonoids extracted from the leaves of black tea at the dosages (4,40,400) mg/kg has led to the increase coefficient of mitotic index and decrease the rate of chromosomal aberration and micronucleus compared with the positive control drug for treatment of MTX as observed toxic effects of genetic in somatic cells of the mutagenic through its ability to reduce the coefficient of mitotic index and increase the chromosomal aberration and micronucleus and this is consistent with the findings of the studies(11) and the reason for toxic effects of the drug to its ability to overlap with the genetic material DNA from through the lack of an enzyme (DHFR) key in the process of growth and cell division(12) as it leads to deplete rules nitrogen deficient oxygen triple phosphate (dNTP) involved in building DNA, resulting in an objection reform process damage the automatic molecule DNA in addition to it leads to damage to this molecule, which leads to chromosomal changes (13).

The results show that flavonoids played a role in reducing the toxicity of the drug

MTX through raising the rate coefficient of mitotic index and low the chromosomal aberration and micronucleus. The influence of these compounds on the mechanism of cancer through the inhibition of the effectiveness of gene Bc1-2 and result from the imbalance of this gene into the stage of the cancer cell death (Apoptosis), as it works to remove these compounds (Scavenging) of free radicals generated in turning normal cells into cancerous cells, as these compounds contain a hydroxyl group that would increase their ability to remove free radicals generated in cancer cells that went to enter the cell in the process of programmed death (14,15).

It found also (16) that compound catachin, which is one of the phenolic compounds in tea leaf after treatment of hamster cells (V79) by mutagen U-Nitroquinoline (UNQO) leads to the reduction of the proportion of mutations occurring by this mutagen.

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