The Ability of Green Tea (*Camellia sinensis*) Extract in Modulating the Cytogenetic and Haematological Effects of Mitomycin C in Albino Male Mice

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Abstract

The present study aimed to investigate the toxic and mutagenic and anti – mutagenic effects of the aqueous extract (5, 10 and 15 mg/kg) of green tea (*Camellia sinensis*) in modulating the genotoxic effects of mitomycin C (MMC). Albino male mice (*Mus musculs*) were employed as a biological system and four parameters were performed *in vivo*; total leucocyte count, mitotic index, chromosomal aberrations and micronucleus formation.

The plant extract was evaluated through three types of treatments. In the first, the extract was given alone orally. While the second and third treatment included two types of interactions with MMC; pre – and post – MMC treatments. All treatments were paralleled by negative and positive controls. In the first treatment, the dose 15 mg/kg of green tea extract enhanced the parameters investigated and a significant increase was observed in total count of leucocyte (8070 cells/cu. mm. blood) as compared to either negative (6900 cells/cu. mm. blood) or positive (5060 cells/cu. mm. blood) controls, Such observation was positively correlated with the mitotic index. In contrast, the spontaneous formation of micronuceli and chromosomal aberrations were decreased in the three investigated doses of the extract. The results showed that the plant extract had no genotoxic or mutgenic effects.

In the second and third treatments, green tea extract showed a good performance in protecting the bone marrow cells in mice against genotoxic MMC effect by increasing the total leucocyte count and mitotic index and decreasing the chromosomal aberration and mironuclei when the treatment were before or after the MMC.

Introduction

Two hundred and fifty years ago, there were few or no synthetic medicines. The 250,000 – 300,000 species of higher plants were the main source of drugs for the world's population. Today, 75% of the world's population, still relies on those plants and other tools of traditional medicine (1). Green tea (*Camellia sinensis*) a member of the Theaceae family, is one the most widely consumed beverages in the world and its many pharmaceutical properties have been described (2). It has become clear that Green tea have demonstrated significant antioxidant, anticarcingenic, antimutagenic, anti-inflammatory, and antimicrobial properties (3,4). On the basis of a large body of evidence, green tea is an effective chempreventive agent for many types of cancer (5,6). The chemical composition of green tea revealed several compounds such as, polyphenolic compounds, which account for 30 - 40 percent of the extractable solids of dried green tea leaves, with most of the polyphenols being flavanols more commonly known as catechins (7,8). Other polyphenols include flavanols and their glycoside and depsides, such as cholorogenic acid, coumaroylguinic acid, and one unique to tea, theogallin (3 – galloylginic acid) (9). Also quinic acid, carotenoids, trigalloylglucose, lignin, protein, caffeine, theaflavicacid, volatile

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compound, and vitamins C and E (9,10,11). The present study was carried out to shed light on the effects of green tea aqueous extract in modulating the genotoxic effects of mitomycine C (MMC) in albino male mice.

Materials and methods

1- Laboratory Animals: Albino male mice (*Mus musculus*) were the tested animals, which were 9 – 10 week old at the beginning of experiments, and during the experiments, they had free access to water and food (*ad libitum*).

2- Extraction of green tea: The dried leaves of green tea were purchased from a local medicinal plant store in Baghdad. The leaves were powdered using a coffee grinder, and 50 grams of the dry plant were immersed in 100 ml distilled water for 30 minutes with gentle stirring. The mixture was centrifuged (3000 rpm for 15 minutes), and the supernatant was collected. The aqueous extract was evaporated using a rotary evaporator (45°C), and the residue was dissolved in distilled water to make a stock solution, from which three doses (5, 10 and 15 mg /kg) were prepared.

3- Experimental Design: Three doses (5, 10 and 15 mg /kg) of the green tea extract were evaluated to test their effect in the animals for the investigated parameters through three types of experiments. In the first, 0.25 of each dose was given orally to the animals (number = 5) for seven days and in day 8, they were investigated. Such treatment was paralleled by negative (dosed with distilled water) and positive (dosed with MMC; 5 mg/kg) controls. In the second experiments, an interaction between plant extract and MMC was carried out through two types of treatment. In the first, the extract was given for seven days, while in day 8, they were given MMC, and the investigation was carried out in day 9 (pre – treatment). In the second type, MMC was given in day 1, while in the next seven days, the plant extract was given (post–treatment), in both interaction, a control sample was investigated in similar sequence, in which the plant extract was replaced with distilled water for each experiment.

4- Laboratory Methods: Total leucocyte count (TLC) was performed on blood obtained from the tail of animals using the conventional method of blood cell counting (12). The mitotic index (MI) and chromosomal aberrations (CA) were assessed in the bone marrow cells after injecting the animals with colchicine (13). For micronucleus (MN) assessment, the femur cleaned from tissue and muscles, then gapped from the middle with forceps in a vertical position over the edge of a test tube by a sterile syringe (1 ml), and heat inactivated human AB serum was injected so as to wash and drop the bone marrow in the test tube, then the test tubes were centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded, and one drop from the pellet was smeared on a clean slide. The slides were fixed with absolute method for 5 minutes. Then stained with Giemsa stain for 15 minutes (14).

5- Statistical Analysis: The data were presented in terms of means ± standard errors, and significant differences between means were assessed by the least significant difference (LSD) using the computer program SPSS (version 10).

Results

The 15 mg/kg dose of green tea extract increased significantly the count of leucocytes (8070 cells/cu. mm. blood) as compared to either negative (6900 cells/cu. mm. blood) or positive (5060 cells/cu. mm. blood) controls (Table 1).

Similar increases in the mitotic index were observed in the dose 15 mg / kg (17.02%), as compared to negative positive controls (13.04 and 6.1%, respectively). The MMC induced the formation of micronucli in the bone marrow cell, and reached a mean of

12.38%, which was significantly higher than the spontaneous formation of such micronuclei. in the negative controls (0.52%), in contrast, a reduction in the spontaneous formation of micronucleus was observed in the three doses of green tea extract (0.33, 0.35 and 0.30%, respectively) (Table 1). In the chromosal aberration assay, animals treated with MMC showed a high frequency of chromosal aberration (7.06%), and a significant difference was revealed as compared to negative controls (1.4%).

Table 1: Total count of leucocytes, mitotic index, micronucleus formation and chromosomal aberration in albino male mice treated with green tea aqueous extract.

Groups		Mean ± Standard Error			
		Leucocyte Count (cells / cu. mm. blood)	Mitotic Index (%)	Micronucleus (%)	Chromosomal Aberration (%)
Negative Control		6900 ± 65.2 a	13.04 ± 0.09 a	0.52 ± 0.06 a	1.4 ± 0.23 a
Positive Control		5060 ± 120.8 b	6.1 ± 0.18 b	12.38 ± 0.24 b	7.06 ± 0.21 b
Green Tea Extract mg/kg	5	6878 ± 115.7 a	14.08 ± 0.12 a	0.33 ± 0.02 c	1.3 ± 0.34 a
	10	7040 ± 82.8 a	15.1 ± 0.16 a	0.35 ± 0.6 c	1.5 ± 0.42 a
	15	8070 ± 217.7 c	17.02 ± 0.23 c	0.30 ± 0.07 c	1.3 ± 0.91 a

Different letters in the same column: Significant difference ($p \leq 0.05$).

In table 2, the results of interaction between green tea extract and MMC was carried out through two types of treatments (pre–and post–treatment). The two interactions with the MMC showed that the extract was able to modulate the mutagenic effect of MMC, especially the dose 15 mg/kg, which enhanced the leucocyte count and the mitotic index. Also a significant reduction in the induced micronucleus formation and chromosomal aberrations was observed (Table 2).

Table 2: Total count of leucocytes, Mitotic index, micronucleus formation and chromosomal aberration in albino male mice after interaction between green tea aqueous extract and mitomycin C.

Groups		Mean ± Standard Error			
		Leucocyte Count (cells / cu. mm. blood)	Mitotic Index (%)	Micronucleus (%)	Chromosomal Aberration (%)
H_2O + MMC		5620 ± 846.6 a	8.12 ± 0.24 a	10.84 ± 0.15 a	7.1 ± 0.15 a
Extract + MMC	5 mg/kg	5710 ± 79.7 a	8.28 ± 0.25 a	4.98 ± 0.21 b	3.9 ± 0.07 b
	10 mg/kg	6130 ± 88.9 a	10.44 ± 0.18 b	4.06 ± 0.18 b	2.3 ± 0.11 c
	15 mg/kg	7800 ± 85.5 b	10.94 ± 0.23 b	4 ± 0.26 b	1.9 ± 0.05 c
MMC + H_2O		5380 ± 166.3 a	6.72 ± 0.26 a	12.06 ± 0.14 a	6.2 ± 0.11 a
MMC + Extract	5 mg/kg	6030 ± 495.9 a	7 ± 0.16 a	6.82 ± 0.22 d	4.1 ± 0.07 b
	10 mg/kg	6670 ± 128.1 c	8.94 ± 0.15 b	7.22 ± 0.16 d	3.3 ± 0.15 b
	15 mg/kg	6870 ± 104.4 c	10.08 ± 0.13 c	4.92 ± 0.06 b	2.8 ± 0.14 b

Different letters in the same column: Significant difference ($p \leq 0.05$).

Discussion

The results of the present study showed that the leucocyte count was increased in all groups of green tea treated mice, especially the dose 15 mg/kg increased the count significantly. The increase counts of leucocytes can be justified in the ground of green tea nature. According to Sun (15), some plant natural products are absorbed in the intestine and enter the blood circulation, where they act as hydrophilic anti – oxidants and increase tissue concentration of vitamin C and both actions have the potential to enhance the immune functions. Treatment with green tea extract caused an increase in mitotic index. In contrast, chromosomal aberrations and spontaneous formation of micronuclei were significantly decreased with no significant differences between the three doses. These results indicated that the planet extract has no cytotoxic or mutagenic effects.

These results agreed with the literature which revealed that the green tea is a safe, and his no genotoxic effects (16, 17). Both types of interaction with MMC,

as a carcinogen, rendered similar results and it could be seen there was no significant difference recorded when plant extract given before or after MMC, however, both interaction were effective in modulating the effect of MMC. Therefore, green tea extract could be classified as desmutagen and bioantimutagen, and such effects can be explained through two pathways. As a desmutagen, the chemical constituents of the extract which may be linked with the drug or with its metabolites to form non-obsorbable complexes (18), or may act as antioxidant or scavengers for the free radicals in the cell or act to prevent activation of the drug by inhibiting enzymes (19). As bioantimutagen, it was clear that the plant extract given after drug may increase the error-free rapair fidelity in the cell (20), or may activate the agent, or activate the promoters of DNA rapair (17, 21).

These results were in agreement with Hara (10) who indicated the possibility that oral administration of green tea extract can inhibit chromosomal aberrations after treatment with MMC, and similar inhibition was observed when green tea extract administered orally to rats prior to administration to aflatoxin B1 (22).

Much of research into the effects of green tea has been focused on its chemical constituents. The polyphenols are generally considered to be the most important elements of green tea, mainly epigallocatechin – 3 – gallate (EGCG), and current researchers are focusing on the antioxidant and antiproliferative effects of polyphenolic compouneds (23). Green tea polyphenols may inhibit carcinogenesis by blocking the endogenous formation of N-nitroso compounds, suppressing the activation of carcinogens and trapping genotoxic agents (24). Also, green tea polyphenols may inhibit biochemical marker of tumor initiation and promotion (25).

Green tea contains theaflavins as a important component having the ability of antioxidant and prevention of carcinogenesis (11). Theanine is an amino acid found in high concentrations in green tea extract and has been found effective in increasing the antitumor activity of cancer drugs (26). Numerous other active compounds in green tea have been and identified include chlorophylls, pheophytins, lutein and beta–carotene, all of them have antioxidant properties and also anti–carcinogenic (27, 28). Also, the plant extract contains vitamin C, which is antimutagen active to block the target sites in DNA and prevent the mutagen form reaction with DNA (29).

In conclusion, the profiles of plant's toxicity and/or genotoxicity can be excluded and that the extract has no mutagenic effects. It was also able to modulate the gentoxic effects of MMC. Further investigation should be considered in the green tea extract on other cytogenetic parameters and careful consideration should be given to the biological activity of plant compounds, because they may play a significant role in anti-carcinogenesis effects and to understand the mechanism by which these compounds acts.

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قابلية مستخلص الشاي الأخضر *Camellia sinensis* في تعديل التاثيرات الدمية والوراثية الخلوية للمايتومايسين س في ذكور الفئران البيض

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الخلاصة:

هدف هذا البحث الى دراسة التاثيرات السمية والتطفيرية والمضادة للتطفر للمستخلص المائي لنبات الشاي الاخضر (10.5 و 15 ملغم/كغم) في تعديل التاثيرات الوراثية لعقار مايتومايسين س (MMC) باستعمال ذكور الفئران البيض بوصفها نظاما اختباريا وبالاعتماد على بعض التحليلات الدمية والوراثية الخلوية (العد الكلي لخلايا الدم البيض، معامل الانقسام الخلوي، تكون النوى الصغيرة والزيغ الكروموسومي لخلايا نقي العظم). قيم المستخلص النباتي من خلال ثلاثة انواع من المعاملات، في المعاملة الأولى جرع المستخلص النباتي عن طريق الفم لوحده، في المعاملتين الثانية والثالثة، فقد قيم التداخل ما بين المستخلص والمطفر ومن خلال نوعين من التداخل (قبل وبعد المطفر). في المعاملة الاولى، اظهرت الجرعة 15 ملغم/كغم تعزيز معنوي واضح لقيم الفحوصات المدروسة، وان العد الكلي لخلايا الدم البيض اظهر زيادة معنوية (8070 خلية/ملم^3 دم) مقارنة بالسيطرة السالبة (6900 خلية/ملم^3 دم) او السيطرة الموجبة (5060 خلية/ملم^3 دم). كذلك اظهرت النتائج بان المستخلص كان فعالا في رفع قيم معامل الانقسام، بالمقابل فان الدراسة سجلت انخفاض في التردد التلقائي لمعدل تكون النوى الصغيرة ومعدل الزيغ الكروموسومي وللجرع الثلاث المستخدمة. اظهرت النتائج عدم امتلاك المستخلص النباتي تاثيرات سمية او تطفيرية. اما بالنسبة للمعاملتين الثانية والثالثة، فقد اظهر مستخلص الشاي الاخضر كفاءة عالية في حماية خلايا نقي العظم للفئران من تاثيرات عقار مايتومايسين س (MMC) السامة، من خلال رفع قيم العد الكلي لخلايا الدم البيض ومعامل الانقسام وخفض نسبة الزيغ الكروموسومي وتكون النوى الصغيرة عند المعاملة قبل وبعد العقار.