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Research Article

THE CYTOPROTECTIVE ACTIVITY OF AQUEOUS GREEN TEA EXTRACT AGAINST METRONIDAZOLE AND TINIDAZOLE GENOTOXIC EFFECT

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ABSTRACT

Objective: The study was designed to evaluate the genoprotective effect of green tea extract against genotoxicity induced by metronidazole and tinidazole.

Methods: A total of 36 mice were used, for each experiment. The animals were allocated into six groups: Group I - negative control administered distilled water; Group II - healthy mice treated with metronidazole alone, Group III - healthy mice treated with tinidazole alone, Group IV - healthy mice administered green tea extract alone, Group V - healthy mice administered of metronidazole anddd then green tea extract was administered, and Group VI - healthy mice administered of tinidazole and then green tea extract was administered.

Results: It has been found that there are significant differences in mitotic index and micronucleus (MN) appearance between the Groups II, IV, and V (7.76±0.8, 11.92±1.14, and 8.36±0.57 and 6.92±0.5, 10.18±1.19, and 7.52±1.05) in bone marrow cells and spleen cells for mitotic index, respectively (6.75±0.4, 5.5±0.41, and 5.92±0.68), in bone marrow for MN appearance. Furthermore, it has been found that there are significant differences in mitotic index and MN appearance between the Groups III, IV, and VI (4.36±0.88, 11.92±1.14, and 5.36±0.55 and 4.08±0.35, 10.18±1.19, and 5.08±0.35) in bone marrow cells and spleen cells for mitotic index, respectively (8.43±0.96, 5.5±0.41, and 6.84±0.66), in bone marrow for MN appearance.

Conclusion: The aqueous extract of green tea has a protective effect against the genotoxic effects induced by either metronidazole or tinidazole in mice.

Keywords: Green tea, Metronidazole, Micronucleus, Mitotic index, Tinidazole.

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INTRODUCTION

Human exposure to different types of chemical substances, some of these chemical substances are cytotoxic in nature like antineoplastic medications, cytotoxicity in these medications is a major pharmacological action against cancerous cells [1, 2]. Other medications have cytotoxicity as an adverse effect. Nowadays many approaches have been used to minimize this adverse effect without need to withdrawal these medications from the market [3] one of these approaches is the using of plants extracts. Some of these extracts have the ability to decrease the cytotoxicity to a significant level; these substances are considered as cytoprotective agents [4].

The *in vitro* and *in vivo* micronucleus (MN) assay is used for the detection of any chemical compound which may induce the formation MN which is a small membrane-bound DNA fragment. These micronuclei maybe originate either when chromosome fragments lacking a centromere or when whole chromosomes are unable to migrate with the rest of the chromosomes during the anaphase of cell division [5,6]. Metronidazole is antiprotozoal medication. It is used to treat many pathological conditions such as endocarditis, bacterial vaginosis, giardiasis, trichomoniasis, and amebiasis [7,8]. Metronidazole and its metabolites disrupted DNA integrity besides it interacts with cellular components, leading to cell death [9,10]. Tinidazole is an antiparasitic drug clinically use against amoebae, giardia, and trichomonas [11]. Authors have been utilized human cell lines to study the effect of tinidazole on cell division and they reported that the intended drug may possess genotoxic effect on the cell division [12]. *Camellia sinensis* (green tea) have many therapeutic benefit effects. Its leaves have been known to contain active ingredients such as polyphenols (catechins), caffeine (called theine), tannin (flavonols), theophylline, theobromine, saponins, essential oils, carotene, and many vitamins and trace elements and others [13]. The health benefits include the prevention of cancer and cardiovascular diseases besides it have the anti-inflammatory, anti-arthritic, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective, and cholesterol-lowering effects [14]. This study was designed to evaluate the protective effect of aqueous green tea extract against a genotoxic effect of two antiprotozoal medications (metronidazole and tinidazole) in bone marrow cells and spleen cells.

METHODS

Plants collection

The plant was brought from the Iraqi market and authenticated by the Department of Pharmacognosy and Medicinal plants, College of the Pharmacy, University of Baghdad. A voucher sample was kept at the Department of Pharmacognosy and Medicinal plants, College of the Pharmacy, University of Baghdad.

Extraction

Powdered plant material (200 g) was taken in a conical flask and extracted with 500 ml of distilled water (DW) with a mechanical shaker with temperature control (room temperature) at the constant stirring rate at 200 rpm. It was left for 24 h, and solids were filtered using Whitman No. 1 filter. The extraction was repeated 3 times until complete extraction [15].

Phytochemical investigation
A preliminary phytochemical investigation was carried out for all fractions using the following tests [16].

Test for alkaloids
About 20 mg of each fraction was mixed in 2 ml of 1% HCl, warmed, and filtered, and the filtrate was treated separately with both reagents (Mayer's and Dragendorff's). Detection whether the alkaloids were present or not is through the formation of turbidity or precipitate.

Test for saponins
Few milligrams from each fraction were dissolved in boiling water in a test tube, cooled, and shaken vigorously to form a stable, persistent froth.

Test for flavonoids
Few milligrams of each fraction were shaken with hexane, filtered, and dissolved in 3 ml of 80% ethanol and filtered. The filtrate was used for the following tests:

a. 1 ml of the filtrate was mixed with 1 ml of 1% aluminum chloride in methanol in a test tube. Formation of yellow color indicated the presence of flavonoids.
b. 1 ml of the filtrate was mixed with 1 ml of dilute ammonium hydroxide solution. A dark yellow color indicated the presence of flavonoids.

Test for phenols
1 ml from each fraction was treated separately with 1% ferric chloride solution in a test tube. The formation of deep green-blue color indicates the presence of phenolic compounds.

Test for methylxanthine
Caffeine reacts with the excess accurately known amount of iodine in an acidic environment, forming an insoluble precipitate.

Test for volatile oils
Dried leaves were collected and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus.

Animals
Animals were purchased from the Animal House of the College of Pharmacy, Baghdad University. 36 albino Swiss mice for each of mitotic index and MN assay, weighing 23–27 g, were used in this study in accordance to the guidelines of the Biochemical and Research Ethical Committee at College of Pharmacy, University of Baghdad. Animals housed for 2 days under standard conditions (temperature 22±2°C, relative humidity 50–60%, and 12 h day and night cycle). Food consisted of normal animal chow and water was provided *ad libitum*. All experimental procedures were performed from 8 to 10 a.m. For each experiment, the animals were allocated into six groups (6 mice each) and treated as follows: Group I - negative control (orally administered DW) for 7 consecutive days; Group II - healthy mice treated with metronidazole alone at dose 78 mg/kg orally once daily for 10 consecutive days; Group III - healthy mice treated with tinidazole alone at dose 52 mg/kg orally once daily for 10 consecutive days; Group IV - healthy mice orally administered aqueous green tea extract (1.25%) alone for 7 consecutive days; and Group V - healthy mice orally administered of metronidazole (78 mg/kg) for 10 days. At the day 11, 1.25% aqueous extract of green tea was orally administered once daily for 7 consecutive days; and Group VI - healthy mice orally administered of tinidazole (52 mg/kg) for 10 days. At the day 11, 1.25% aqueous extract of green tea was orally administered once daily for 7 consecutive days. The aqueous green tea extract at 1.25% concentration was administered to mice in Groups II, V, and VI as their sole source of drinking water.

Evaluation of genotoxicity in bone marrow cells and spleen cells
At the end of the experiment, each animal utilized in the present study was intraperitoneally injected with 1 mg/kg colchicine, and after 2 h, they were sacrificed by cervical dislocation. Bone marrow samples were aspirated from the femur bone and processed using an aseptic technique for evaluation of mitotic index as previously reported elsewhere [17].

MN test
Mice were sacrificed by spinal dislocation at the end of the experiment. Bone marrow smears were prepared as described by Agarwal and Chauhan [18], stained with Giemsa, and the calculating incidence of micronuclei appearance.

Statistical analysis
Data were expressed as mean±standard deviation. Statistical analyses were performed using unpaired Student *t*-test. If the overall F value was found statistically significant ($p<0.05$), further comparisons among groups were made according to *post hoc* Tukey's test. All statistical analyses were performed using SPSS 20.

RESULTS

Phytochemical results
In Table 1. phytochemical investigations that done on the green tea extract showed that there were different active constituents that present in this extract, these active constituents include saponins, polyphenols, volatile oil and flavonoids.

Table 2 shows that mitotic index in both bone marrow cells and spleen cells did not significantly differ in metronidazole-treated mice (78 mg/kg) (Group II) compared to negative control animals ($p>0.05$); however, in tinidazole (52 mg/kg)-treated animals (Group III), there was a significant reduction in mitotic index compared to negative control animals in both bone marrow cells and spleen cells ($p<0.05$). Besides, the aqueous extract of green tea at a concentration (1.25%) (Group IV) produced a significant elevation in mitotic index in both bone marrow cells and spleen cells when compared to negative control mice ($p<0.05$).

Furthermore, Table 2 shows that there are significant differences in mitotic index among three different group (metronidazole-treated group, aqueous green tea extract group, and metronidazole plus aqueous green tea extract group) in bone marrow cells and spleen cells ($p<0.05$). Furthermore, there are significant differences among the groups (tinidazole group, aqueous green tea extract, and tinidazole plus aqueous green tea extract) in mitotic index in bone marrow cells and spleen cells ($p<0.05$).

In Table 3, there was a non-significant difference in the MN appearance in metronidazole-treated group (Group II) (78 mg/kg) when compared to negative control in bone marrow cells ($p>0.05$), but there was a significant elevation in the appearance of MN in tinidazole-treated group of mice (Group III) (52 mg/kg) when compared to negative controls in bone marrow cells ($p<0.05$). Moreover, Table 2 shows that the aqueous extract of green tea at a concentration (1.25%) (Group IV) produced a significant reduction in the MN appearance in bone marrow cells compared to negative control mice ($p<0.05$).

In addition, in Table 3, there are significant differences ($p<0.05$) in the incidence of MN appearance among the groups (metronidazole group, aqueous green tea extract group, and metronidazole plus aqueous green tea extract group) in bone marrow cells (6.75±0.4, 5.5±0.41, and 5.92±0.68, respectively); furthermore, there are significant differences ($p<0.05$) in the incidence of MN appearance among the groups (tinidazole group, aqueous green tea extract, and tinidazole plus

Table 1: Phytochemical investigation of *Camellia sinensis* (green tea)

Type of test	Results
Test for alkaloids	Negative
Test for saponin	Positive
Test for flavonoids	Positive
Test for volatile oil	Positive
Test for methylxanthine	Positive
Test for phenol	Positive

Table 2: Effects of 1.25% concentration of aqueous green tea extract on metronidazole (78 mg/kg) and tinidazole (52 mg/kg) on a mitotic index in both bone marrow cells and spleen cells in mice

Number of groups	Names of groups	Bone marrow cells	Spleen cells
I	Water (negative control)	8.68±0.44	7.12±0.27
II	Metronidazole at dose 78 mg/kg	7.76±0.84^{A}	6.92±0.53^{A}
III	Tinidazole at dose 52 mg/kg	4.36±0.88*^{a}	4.08±0.35*^{a}
IV	Aqueous green tea extract 1.25%	11.92±1.14*^{Bb}	10.18±1.19*^{Bb}
V	Metronidazole (78 mg/kg) + green tea extract 1.25%	8.36±0.57^{C}	7.52±1.05^{C}
VI	Tinidazole (52 mg/kg)+green tea extract 1.25%	5.36±0.55^{c}	5.08±0.35^{c}

Data expressed as mean±SD; n=6 animals in each group. *Significant difference compared to the negative control group ($p<0.05$); values with non-identical small letters superscripts (a, b, and c) are considered significantly different ($p<0.05$) among Groups (III, IV, and VI). Values with non-identical capital letters superscripts (A, B, and C) are considered significantly different ($p<0.05$) among Groups (II, V and VI). SD: Standard deviation

Table 3: Effects of 1.25% concentration of aqueous green tea extract on metronidazole-treated (78 mg/kg) and tinidazole-treated (52 mg/kg) groups on MN appearance in bone marrow cells in mice

Groups	Names of groups	Bone marrow cells
I	Water (negative control)	6.67±0.42
II	Metronidazole at dose 78 mg/kg	6.75±0.4^{A}
III	Tinidazole at dose 52 mg/kg	8.34±0.96*^{a}
IV	Aqueous green tea extract 1.25%	5.5±0.41*^{Bb}
V	Metronidazole (78 mg/kg) + green tea extract 1.25%	5.92±0.68^{C}
VI	Tinidazole (52 mg/kg)+green tea extract 1.25%	6.84±0.66^{c}

Data are expressed as mean±SD; n=6 animals in each group; *significantly different compared to negative control ($p<0.05$); Values with non-identical small letter superscripts (a, b, and c) are considered significantly different among Groups (III, IV, and VI) ($p<0.05$). Values with non-identical capital letter superscripts (A, B, and C) are considered significantly different among Groups (II, IV, and V ($p<0.05$). MN: Micronucleus, SD: Standard deviation

aqueous green tea extract) in bone marrow cells (8.34±0.96, 5.5±0.41, and 6.84±0.66, respectively).

DISCUSSION

In the present study, it has been found that mitotic index of bone marrow cells and spleen cells significantly increases after the administration of green tea extract in groups that administered metronidazole or tinidazole previously when compared to the groups the administered metronidazole and tinidazole alone. MN appearance has been significantly decreased after administration of green tea extract in groups that administered metronidazole or tinidazole previously when compared to the groups the administered metronidazole and tinidazole alone.

Metronidazole and tinidazole considered as a first choice in the treatment of protozoa infections, and at the same time, these two medications have different adverse effects to make them unable to be used for a prolonged period. One of these unwanted effects is the cytotoxicity. Metronidazole has been reported to be cytotoxic in different studies [19]. Similarly, it has been reported that tinidazole possesses genotoxic which lead to either induce DNA repair system or induce apoptosis. The induction of DNA repair system leads to a decrease in cell division [12]. Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food. Phenolic compounds are considered secondary metabolites, and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified. Green tea may have a wide variety of pharmacological activity due to the presence of huge different types of active ingredients; thus, it is nowadays widely used for its health properties [20]. In the present study, the use of metronidazole and tinidazole for 10 successive days produced a decrease in cell proliferation and increase the incidence if MN appearance, but oral administration of 1.25% concentration of aqueous green tea extract for 7 successive days after the use of metronidazole or tinidazole group produced a significant increase in the cell proliferation with decrease in the MN appearance. Hydroxyl radical is one of the powerful reactive oxygen species in the biological system. Hydroxyl radical reacts with a polyunsaturated fatty acid of cell membrane phospholipids and causes harm to the cell. Moreover, the hydroxyl radical is considered a harmful species in pathophysiological processes and capable of damaging every molecule of the biological system and contributes to carcinogenesis, mutagenesis, and cytotoxicity [21]. The data of this study showed that there are a significant amount of flavonoids and alkaloids, as many previous studies have shown that flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [22]. The proposed mechanism of action may be scavenging through the hydroxyl radicals and prevent the degradation of 2-deoxyribose [23,24]. There are two major restrictions in the present manuscript, first one is the need of extensive phytochemical investigations to identify, purification and separation of active constituents, the second restriction is related to the dose and duration of administration of metronidazole and tinidazole, the use of more than one dose and extending in the duration of administration give us a more clear picture for the protective effect of green tea.

The recommendation for further work in manifested by using different dose and/or duration of metronidazole and tinidazole administration.

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AUTHORS' CONTRIBUTIONS

The first author performed the procedure and wrote manuscripts. The second author guided through the procedure, and third and fourth author discuss the final results. Fifth author help in performing the procedure. All authors are responsible for financial support to complete the research.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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