

Original Article

IMPACTS OF DIFFERENT CONCENTRATIONS OF AQUEOUS GREEN TEA EXTRACT ADMINISTERED DURING METHOTREXATE TREATMENT ON SOME SELECTED BLOOD INDICES IN RATS

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ABSTRACT

Objective: This study was designed to investigate the impacts of administering of different concentrations of Aqueous Green Tea Extract (AGTE) (0.625, 1.25 or 2.5%) during Methotrexate (MTX) treatment, and continued for 5 days, on blood indices in rats.

Methods: Six groups of white Albino rats were utilized: Group I- (Control, received water); Group II- [single i. p. injection (20 mg/kg) of MTX], Group III- [1.25% concentration of AGTE alone for 5 days], and Groups IV, V and VI- [rats were administered, respectively AGTE (0.625, 1.25 or 2.5%), during MTX treatment, and continued for 5 days].

Results: The results showed that MTX alone induced significant decreases in Hb, HCT, MCHC, MCH, RBCs, and total WBCs; while, it increased platelets count. Administration of different concentrations of AGTE during MTX produced significant decrease in total WBCs and platelets; while significant increase in Hb, HCT, MCHC, MCH; meanwhile, significant increase, or decrease, respectively in MCV (depending on the concentration of AGTE); and a significant increase in RBCs only was seen at 2.5% AGTE plus MTX), compared to MTX-treated animals.

Conclusion: Marked alterations in blood indices were obtained from this study when AGTE was administered during MTX treatment. So this study recommends taking into account such administration when MTX is therapeutically utilized.

Keywords: Methotrexate, Aqueous Green tea extract, Blood indices, Rats.

INTRODUCTION

Methotrexate (MTX), a dihydrofolate analog, inhibits dihydrofolate reductase enzyme, an enzyme that is important for conversion of dihydrofolate to tetrahydrofolate. Thus, MTX treatment causes depletion of folate stores [1, 2]. The drug is widely used for the treatment of various malignancies as well as in the treatment of rheumatoid arthritis and other chronic inflammatory disorders [3-5]. Along with its effective therapeutic power, MTX has adverse effects on several organs and tissues [6-9]. Green tea, made from the dried leaves of (*Camellia sinensis*, Theaceae) is one of the most popular beverages consumed around the world. Numerous experimental and epidemiological studies support the health benefits of green tea consumption, including chemo-preventive properties [10, 11], anti-inflammatory effects and antioxidants that scavenge free radicals to protect cells in normal and pathological states [12]. Most of the beneficial effects of green tea are attributed to its polyphenolic flavonoids, known as catechins, including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and the major flavonoid (-)-epigallocatechin-3-gallate (EGCG) (One cup (240 ml) of brewed green tea contains up to 200 mg EGCG) [13]. The haematological adverse effects that provoked by the chemotherapeutic drug MTX may be possibly influenced by the administration of green tea. There was no previously performed study to elucidate the impacts of administering different concentrations of AGTE during MTX on blood indices. Thus, the aim of this study was to investigate the impacts of administering of (0.625, 1.25 or 2.5%) concentration AGTE during MTX treatment, and continued for 5 days on some selected blood indices in rats.

MATERIALS AND METHODS

Preparation of Aqueous Green Tea Extract (AGTE)

Different concentrations of AGTE (0.625, 1.25, and 2.5%) were freshly prepared by soaking for 10 minutes 0.625 gm, 1.25 gm and 2.5 gm, respectively of green tea leaves in 100 ml of distilled water

at 90°C; then each solution of AGTE was filtered [14]. The aqueous extract was substituted water as the sole source of drinking fluid in animals administered only 1.25% AGTE, and in groups of animals administered 0.625, 1.25, or 2.5% AGTE during MTX, and continued for 5 days).

Experimental protocol

Thirty-six White Albino rats of both sexes, weighing 200-220g were used in this study; the animals were obtained from and maintained in the Animal House of the College of Pharmacy, University of Baghdad under conditions of controlled temperature.

The animals were fed commercial pellets. Groups of animals that selected and served as control or those treated with MTX alone were allowed access to tap water *ad libitum*; the remainder groups of animals that utilized in this study were allowed access to specific concentrations of AGTE as their sole source of drinking fluid. Ethical Committee in the College of Pharmacy, University of Baghdad was approving this study.

Rats were divided into six groups of six animals each:

I- Animals were administered water this group served as negative control.

II- Methotrexate was administered to rats in a dose of 20 mg / kg, i. p., for one day. Following this dose, saline was administered for 5 consecutive days [15].

III- Aqueous green tea extract (AGTE) alone, at a concentration of 1.25%, was given to group of animals as a sole source of drinking water for 5 consecutive days.

IV, V, and VI- Groups of animals was received respectively, different concentrations (0.625, 1.25 or 2.5%) of AGTE, as their sole source of drinking water, during MTX treatment, and continued for 5 days [14].

Analysis of Blood Indices

Animals were sacrificed by cervical dislocation 24 hours after the end of the treatment (at day 6). Blood was obtained from each animal via intra-cardiac puncture and put in EDTA-containing tubes for analysis of selected indices [hemoglobin (Hb), hematocrit (HCT), mean corpuscular Hb concentration (MCHC), mean corpuscular hemoglobin (MCH), red blood cell (RBCs) counts, total white blood cell (WBCs) counts and platelet counts] utilizing auto-haematology analyzer (Mindray BC 2800). The measure of mean corpuscular volume (MCV) is attained by multiplying a volume of blood by the proportion of blood that is cellular HCT, and dividing that product by the number of RBCs in that volume [16].

The significance of differences between two mean values was calculated using unpaired Student's t-test. Descriptive data were analyzed depending upon the one way Analysis of Variance (ANOVA) to compare the results among the various groups. Data were expressed as mean \pm SEM. P-values less than 0.05 ($P < 0.05$) were considered significant for the data presented in the results of the current study.

RESULTS

Single IP injection of 20mg/kg MTX to rats caused significant decrease ($P < 0.05$) in levels of Hb, HCT, MCHC, MCH, reduction in numbers of RBCs and total WBCs; while significant ($P < 0.05$) increase in platelets count was observed compared to negative control group. Tables 1 and 2.

Oral administration of 1.25% AGTE alone to rats for 5 days produced non-significant differences ($p > 0.05$) in levels of [Hb, HCT, MCV, MCHC, and RBCs]; while, significant differences ($P < 0.05$) were observed in levels of [MCH, WBCs, and platelets counts] compared to negative controls as shown in tables 1 and 2.

Rats received different concentrations of AGTE (0.625, 1.25 or 2.5%), as their sole source of drinking water during MTX treatment, and continued for 5 days, produced significant ($P < 0.05$) alterations in some blood indices compared to MTX-treated. There was a significant increase ($P < 0.05$) in the level of Hb in groups of rats co-administered either 1.25, or 2.5% AGTE with MTX compare to MTX-treated animals. Haemoglobin (Hb) levels in the previously-mentioned groups being non-significantly different ($P > 0.05$) with that of negative control. While, in 0.625% AGTE group, there was non-significant ($P > 0.05$) in the level of Hb compared to MTX-treated rats. Table 1.

Rats orally-administered with either (1.25%, or 2.5%) of AGTE during MTX treatment, and continued for 5 days, produced significant increase ($P < 0.05$) in levels of HCT compared to MTX-treated rats; while, non-significant difference in the level of HCT in group of rats administered 0.625% AGTE during MTX treatment, and continued for 5 days, was observed compared to MTX-treated animals. Table 1.

Concerning MCV, significant increase in such blood index was produced in rats orally-administered 0.625% and 1.25% AGTE concentrations; while, significant decrease in MCV was observed in the animals orally-administered 2.5% AGTE concentration during MTX treatment, and continued for 5 days compared to MTX-treated rats.

Concerning MCHC, rats orally-administered with either (0.625%, or 1.25%) of AGTE during MTX treatment, and continued for 5 days, produced non-significant differences ($P > 0.05$) in such concentration; while, a significant difference ($P < 0.05$) in MCHC in rat administered 2.5% AGTE during MTX treatment, and continued for 5 days, compared to MTX-treated rats. Table 1.

There were the significant increase ($P < 0.05$) in MCH index in groups of rats orally-administered with either (0.625%, 1.25% or 2.5%) AGTE during MTX treatment, and continued for 5 days, compared to MTX-treated rats. Table 1.

Concerning RBCs counts, rats orally-administered with 2.5% AGTE during MTX treatment, and continued for 5 days, produced

significant increase ($P < 0.05$) in such count compared to MTX-treated and negative control rats. Moreover, there was a significant increase ($P < 0.05$) in such counts in rats orally-administered with 1.25% AGTE during MTX treatment, and continued for 5 days compared to MTX-treated rats. The RBCs counts were non-significantly ($P > 0.05$) different compared to that of negative control animals. While, no significant differences ($P > 0.05$) in RBCs counts in rat administered 0.625% AGTE during MTX treatment, and continued for 5 days, compared to MTX-treated rats. Table 2.

There were significant decrease ($P < 0.05$) in total WBCs and platelets counts in rat administered either 0.625, 1.25 or 2.5% AGTE during MTX treatment, and continued for 5 days, compared to MTX-treated and to negative control groups, as shown in table 2.

DISCUSSION

Data obtained from the current study showed that there were alterations in blood indices-induced by MTX in rats manifested by decline in all the studied corpuscular indices and blood cells counts (except platelets counts, where an elevation in platelets number were observed in the present study in contrast to that observed by other investigators) [17].

The alterations in the blood indices-induced by the drug may be related to the depletion of tetrahydro folic acid (THFA) as a consequence of the inhibitory property of MTX on dihydrofolate reductase (DHFR) [18] leading to inhibition of purine and pyrimidine metabolism, which in turn causing inhibition of DNA and RNA synthesis [19], limiting cell division, hindering of erythropoiesis [20].

It has been reported that such metabolic alterations are responsible for both the therapeutic and the toxic effects of MTX [21]. Moreover, the toxic effects of the drug was attributed to its action on the S-phase of the cell-cycle and by this action, it affects tissues with high turnover such as bone marrow [22]. Therefore, the risk involved with the use of MTX on the haematological system is high. One of the most common adverse effects caused by MTX is the reduction in WBCs counts and thus predisposition to infection. Additionally, it has been reported that MTX produced a significant reduction in the antioxidant enzyme levels and thus sensitizing cells to ROSs [23].

Erythrocytes are particularly susceptible to oxidative damage as a result of high polyunsaturated fatty acid (PUFA) content in their membranes and high concentration of oxygen and Hb, the latter being a potentially powerful promoter of oxidative processes [24].

The alterations in the above mentioned blood indices induced by MTX may possibly be influenced by the administration of green tea, which is one of the most popular beverages consumed around the world and have various characteristics behavior [25-27].

The results of the current study showed that significant increase in the levels of Hb, HCT, MCHC, and RBCs in group of rats treated with 1.25% AGTE alone; also, there were marked increases in total WBCs and platelets counts compared to both negative control and MTX-treated animals. The elevation in the previously-mentioned blood indices after 1.25% AGTE might be related to the antioxidant properties of the extract catechines on haematopoietic cells [28]. Such concentration of AGTE, (1.25%) utilized in the current study produced comparable beneficial antioxidant effects to that observed by others [14].

Depending on the concentration of AGTE that was utilized, the present study revealed that the administration of 0.625, 1.25, or 2.5% of AGTE during MTX treatment, and continued for 5 days, produced an improvement in some blood indices (Hb, HCT, MCHC, MCH, and RBCs); and deleterious impacts on others (WBCs, and platelets counts). A striking result obtained from this study showed that an elevation in MCV (an indicator of hematologic toxicity), is manifested only after the administration of either 0.625% or 1.25% of AGTE during MTX; but not observed after that of 2.5% AGTE with

MTX, where the latter combination produced a reduction in MCV compared to MTX-treated and to both 1.25% AGTE alone-

treated and control groups, this may be attributed to unknown mechanism.

Table 1: Blood indices (Hb, HCT, MCV, MCH, and MCHC) levels among groups of rats, ((negative control, methotrexate (MTX)-treated, 1.25% aqueous green tea extract (AGTE) alone, and AGTE concentrations administered (0.625, 1.25 or 2.5%) during MTX treatment)), and continued for 5 days.

| Blood Index | Negative Control (n=6) | MTX-treated (n=6) | AGTE 1.25%(n=6) | MTX plus AGTE 0.625%(n=6) | MTX plus GTE 1.25%(n=6) | MTX plus AGTE 2.5%(n=6) |
|---|---------------------------|---------------------------|---------------------------|----------------------------|----------------------------|----------------------------|
| Hemoglobin (gm/dl) | 13.04 ± 0.23 ^a | 11.7 ± 0.23 ^{*b} | 13.5 ± 0.18 ^a | 12.25 ± 0.24 ^{*b} | 14.5 ± 0.27 ^{*c} | 14.4 ± 0.2 ^{*c} |
| Hematocrit (HCT) (%) | 43.1 ± 0.8 ^a | 38.6 ± 0.56 ^{*b} | 42.0 ± 1.1 ^a | 37.6 ± 1.1 ^{*b} | 48.3 ± 1.09 ^{*c} | 44.4 ± 1 ^a |
| Mean corpuscular volume (MCV) (fL) | 58.24 ± 0.54 ^a | 57.61 ± 0.55 ^a | 55.92 ± 0.67 ^a | 61.43 ± 0.61 ^{*b} | 63.72 ± 0.58 ^{*b} | 47.74 ± 0.57 ^{*c} |
| mean corpuscular Hb (MCH) (pg) | 18.3 ± 0.2 ^a | 17.8 ± 0.12 | 17.7 ± 0.13 ^{*b} | 19.9 ± 0.09 ^{*c} | 18.8 ± 0.16 ^a | 20.3 ± 0.22 ^{*c} |
| Mean corpuscular Haemoglobin concentration (MCHC) (g/L) | 32.2 ± 0.8 ^a | 31.6 ± 0.91 ^{*b} | 32.6 ± 0.75 ^a | 31.0 ± 0.63 ^b | 30.1 ± 0.9 ^b | 32.6 ± 1.18 ^a |

Data are presented as mean ± SEM, n= number of animals, *P<0.05: significant difference with respect to control group, Values with non-identical superscripts (a, b, and c) among different groups considered significantly different, P<0.05, MTX-treated = methotrexate-treated rats, 1.25% AGTE= 1.25% aqueous green tea extract alone.

Table 2: Blood cells counts among groups of rats, ((negative control, methotrexate (MTX)-treated, 1.25% aqueous green tea extract (AGTE) alone, and AGTE concentrations administered (0.625, 1.25 or 2.5%) during MTX treatment)), and continued for 5 days.

| Blood Index | Negative Control (n=6) | MTX-treated (n=6) | AGTE 1.25%(n=6) | MTX plus AGTE 0.625%(n=6) | MTX plus GTE 1.25%(n=6) | MTX plus AGTE 2.5%(n=6) |
|---|----------------------------|--------------------------|-----------------------------|-----------------------------|----------------------------|---------------------------|
| Red blood cells (RBC) (×10 ⁶ cells/μl) | 7.4 ± 0.17 ^a | 6.7 ± 0.19 ^{*b} | 7.51 ± 0.19 ^a | 6.12 ± 0.25 ^{*b} | 7.58 ± 0.15 ^a | 9.3 ± 0.15 ^{*c} |
| Total white blood cells (WBC) (×10 ³ cells/μl) | 9.24 ± 0.54 ^a | 7.5 ± 0.41 ^{*b} | 13.4 ± 0.9 ^{*c} | 5.7 ± 0.65 ^{*d} | 3.0 ± 0.23 ^{*e} | 5.26 ± 0.57 ^{*d} |
| Platelets (×10 ³ cells/μl) | 438.5 ± 20.32 ^a | 450 ± 18.8 ^{*b} | 428.8 ± 18.05 ^{*c} | 151.2 ± 19.55 ^{*d} | 123.0 ± 17.8 ^{*e} | 112 ± 15.67 ^{*f} |

Data are presented as mean ± SEM, n= number of animals, *P<0.05: significant difference with respect to control group, Values with non-identical superscripts (a, b, c, d, e, and f) among different groups considered significantly different, P<0.05, MTX-treated = methotrexate-treated rats, 1.25% AGTE= 1.25% aqueous green tea extract alone.

It has been reported that, although green tea and its constituents, catechins possess antioxidant properties and have health benefits, but, they led to increase production of reactive oxygen species (ROS) and increased intracellular oxidative stress *in vitro* [26] and *in vivo* [27]. Besides, the ester-bonded gallate catechins from green tea, such as EGCG and ECG, are considered as potent *in vitro* inhibitors of several DHFRs at concentrations found in the serum and tissues of green tea drinkers (0.1–1.0 M); and consumption of large amounts of green tea could decrease the activity of dihydrofolate reductase enzyme (DHFR) [25, 29-30].

CONCLUSION

According to the results obtained from this study, one can conclude that AGTE alone has an opposite impact on selected blood indices measured in this study compared to that produced by MTX. Furthermore, marked impacts in RBCs, WBCs, and platelets counts were observed in a group of rats administered AGTE during MTX compared to MTX-treated group. This study provide the first evidence concerning the impacts of the concentrations of AGTE utilized in this study (0.625, 1.25, or 2.5%) administered during MTX, and thus, it warned about the administration of different concentrations of AGTE with MTX when it is therapeutically utilized, due to marked alterations in haematological indices. Further studies are needed to confirm the finding of the current study.

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CONFLICT OF INTERESTS

Declared None

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