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The influence of different concentrations of aqueous green tea extract on methotrexate induced haematological toxicity in rats

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Abstract:

Methotrexate (MTX), a folic acid antagonist is widely used for the treatment of a variety of tumors. In the present study, the possible protective effect of aqueous green tea extract (AGTE) in methotrexate-induced haematotoxicity was investigated.

Four main groups of white Albino rats were used: control group, (MTX) group, following a single dose of MTX (20 mg/kg, i.p.) saline was administered for 5 days. (AGTE) group, was treated with 1.25% concentration of AGTE only for 12 days and the (MTX+AGTE) group, in this group rats received different concentrations of AGTE (0.625, 1.25 and 2.5%), as their sole source of drinking water, 7days before and 5 days after MTX treatment.

MTX induced significant decreases in RBC, Hb, Hct, WBC and platelets. Whereas increased MCV and MCH. Moreover, the concentrations of AGTE play a major role.

The best protective effects of AGTE treatment were observed at the concentration 1.25% While 0.625% and 2.5% AGTE had no protective effects on haematological parameters.

Keywords: Methotrexate; haematotoxicity; Aqueous Green tea extract.

تأثير تراكيز مختلفة من المستخلص المائي للشاي الأخضر ضد سميه الدم المستحثة بواسطه عقار الميثوتريكسيت في الجرذان.

الخلاصة:

الميثوتريكسيت (MTX) مضاد حمض الفوليك ويستخدم على نطاق واسع لعلاج مجموعة متنوعة من الأورام في هذه الدراسة، تم التحقق في امكانيه التأثير الوقائي للمستخلص المائي للشاي الأخضر (AGTE) في سميه الدم التي يسببها الميثوتريكسيت.

استخدمت اربع مجموعات رئيسية من جرذان البيضاء البينو: مجموعة السيطرة، ومجموعة (MTX) بعد جرعة واحدة من MTX (20 ملغم/كغم،داخل البريتون). تم اعطاء المحلول الملحي الطبيعي لمدة 5 أيام، مجموعة (AGTE) تم اعطاوها تركيز 1.25 ٪ من AGTE لمدة 12 يوما فقط، و مجموعة (AGTE +MTX)، تلقت الجرذان تراكيز مختلفة من AGTE (0.625، 1.25 و 2.5 ٪) كمصدر وحيد للشرب لمده 7ايام قبل و5 أيام بعد جرعة MTX. سبب ميثوتركسيت انخفاض كبير في RBC، HB ، HCT، WBC والصفائح الدموية. في حين زادت MCV وMCH ، علاوة على ذلك يبدو ان تراكيز AGTE تلعب دورامهما بذلك.

أفضل تاثير وقائي للمستخلص المائي للشاي الاخضركان بتركيز 1.25 ٪ بينما تركيز 0.625 ٪ و 2.5 ٪ AGTE ليس لديه آثار وقائية على الادله الخاصه بمكونات الدم.

مفاتيح الكلمات: ميثوتريكسيت , سمية الدم ,المستخلص المائي للشاي الاخضر.

Introduction:

Methotrexate (MTX), a structural analogue of folic acid, is widely used as a chemotherapeutic drug in the treatment of various malignancies and inflammatory diseases [1].

The efficacy of methotrexate is often limited by severe side effects and toxic sequelae [2]. Regarding the mechanisms of these side effects, several hypothesis have been put forward, among which oxidative stress is noticeable [3], and also as an

antifolate metabolite by reversibly inhibiting the enzyme dihydrofolate reductase, affecting purine / thymidylate and thus DNA synthesis and cell proliferation [4].

Green tea, made from the dried leaves of (*Camellia sinensis*, Theaceae) is one of the most popular beverages consumed around the world [5].

Numerous experimental and epidemiological studies support the health benefits of green tea consumption, including chemo preventive properties [6], anti-inflammatory effects [7] and antioxidants that scavenge free radicals to protect cells in normal and pathological states [8].

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)) [9].

The aim of this study was to examine the influence of aqueous green tea extract on the rat hematological parameters after methotrexate intoxication.

Materials and Methods:

Evaluation of haematological parameters blood samples with anti-coagulant EDTA were analyzed for hematological parameters [red blood cell (RBC) counts, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), white blood cell (WBC) counts and platelet numbers] by using Auto hematology analyzer (Mindray BC 2800,China).

Preparation of Aqueous Green Tea: Extract (AGTE):

Aqueous green tea extract (AGTE) was freshly prepared on daily basis by soaking for 10 minutes 0.625gm, 1.25gm and 2.5gm, respectively of green tea leaves in 100 ml of distilled water whose temperature was 90^{o}C. Solutions then filtered to obtain the final 0.625%, 1.25% and 2.5%, respectively of AGTE [10].

The aqueous extracts were substituted for water as the sole source of drinking fluid in the 1.25% AGTE alone treated group and in the combination group animals.

Experimental protocol:

Thirty white Albino rats of both sexes, weighing 200-250g were used in this study; the rats 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.

The groups of animals selected which served as control or those treated with MTX alone were allowed access to tap water *ad libitum*; the remainder groups of animals that were utilized in this study were allowed access to specific concentrations of AGTE as their sole source of drinking fluid.

The rats were randomized and divided into six groups of five animals each. Methotrexate was administered to a group of rats in a dose of 20 mg / kg, i.p., for one day.

Following this dose, saline i.p was administered for 5 consecutive days [11]. AGTE alone, at a concentration of 1.25%, was given to third group of animals for 12 consecutive days. Another three groups of rats received different concentrations (0.625, 1.25 and 2.5%) of AGTE, as their sole source of drinking water, 7 days before and 5 days concomitant with methotrexate [10].

Statistical analysis:

Data were expressed as the mean ± SEM, and the differences between the mean values were calculated using unpaired Student's t-test. Comparisons among treated groups were made using analysis of variance (ANOVA). P-values less than 0.05 were considered significant for all data showed in the results [12].

Results:

As shown in Table 1, methotrexate treatment caused diminished numbers of red blood cells and significant reductions in hemoglobin, hematocrit, white blood cells and platelets counts while increased mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) values ($p<0.05$) compared to control group.

Oral administration of 1.25% AGTE alone to rats for 12 days, produced non significant changes in haematological parameters ($p>0.05$) compared to control group.

Rats treated with an oral concentrations (0.625%, 1.25% and 2.5%) of AGTE 7 days prior to and 5 days after single intraperitoneal injection of methotrexate (20 mg/kg) resulted in significant difference in RBC, MCH, Platelets compared to control group ($p< 0.05$).

Concerning Hb and Hct 1.25% of AGTE produced significant difference while 0.625 % and 2.5% produced non significant difference compared to control group.

Rats treated with an oral concentrations (0.625% and 2.5%) of AGTE 7 days prior to and 5 days after single intra-peritoneal injection of methotrexate (20 mg/kg) resulted in non significant difference in RBC, MCV, MCH, WBC compared to MTX group ($p>0.05$); but MTX+1.25% group showed significant difference compared to methotrexate group ($p<0.05$) as shown in (Table-1).

A non-significant difference ($P>0.05$) was observed in the levels of Hct of rats treated with (0.625%, 1.25% and 2.5%) AGTE 7 days prior to and 5 days after MTX compared to methotrexate groups, but there were significant differences ($P<0.05$) concerning HB levels in animals treated with (0.625%, 1.25% and 2.5%)% AGTE compared to methotrexate group as shown in (Table-1).

Table1-1 Showed that, increasing the concentrations of AGTE produced no significant differences among the different concentrations on Hct and platelets level compared to MTX group.

Table1-1: The hematological analysis in the control, AGTE, and methotrexate-injected rats treated with either (0.625%, 1.25%and 2.5%) AGTE or saline.

	Control (n=5)	MTX + Saline(n=5)	AGTE 1.25%(n=5)	MTX + AGTE 0.625%(n=5)	MTX + GTE 1.25%(n=5)	GTE 2.5%(n=5)
Red blood cells (RBC) ($\times10^6$ cells/µl)	7.95+0.17	6.38± 0.19 * a	7.96± 0.18	6.5± 0.25 * a	7.33± 0.13 * b	6.75± 0.13 * a
Hemoglobin (gm/dl)	13.42± 0.25	11.08± 0.23 * a	13.88± 0.11	12.78± 0.34 b	12.34± 0.37 * b	12.74± 0.2 b
Hematocrit (Hct) (%)	41.36± 0.53	34.8± 0.56 * a	42.24± 1.1	34.88± 4.14 a	37.3± 1.09 * a	39.68± 0.99 a
Mean corpuscular volume (MCV) (fL)	49.22± 1.38	61.05± 1.45 * a	52.4± 0.49	59.24± 0.74 * a	53.56± 1.13 * b	60.84± 1.49 * a
mean corpuscular hemoglobin(MCH) (pg)	16.02± 0.71	18.94± 0.32* a	17.78± 0.13	19.34± 0.09 * a	17.94± 0.17 * b	18.92± 0.11 * a
Total white blood cells (WBC) ($\times10^3$ cells/µl)	10± 0.63	4.04± 0.41* a	10.1± 1.14	3.52± 0.4* a	8.76± 1.15 b	5.38± 1.07* a
Platelets ($\times10^3$ cells/µl)	400.8± 29.23	143± 17.48* a	428.8± 18.05	150.2± 21.51* a	190.2± 12.94* a	166.2± 18.58* a

- Data are presented as mean ± SEM, n= number of animals, *P<0.05 with respect to control group.
- Non-identical superscripts (a and b) among (MTX, MTX+ 0.625% AGTE, MTX + 1.25% AGTE, MTX + 2.5 % AGTE) groups considered significantly different, P<0.05.

Discussion:

In the present study, the alteration in the hematological parameters is in agreement with *Göksel Şener et al*. [11] and *Mustafa Çetiner et al*. [3] who showed that methotrexate caused changes in the blood indices of rats.

This may be related to depletion of reduced folates due to inhibition dihydrofolate reductase by methotrexate [13].

Folate deficiency adversely affects several biochemical pathways, including the activated methyl cycle, the synthesis of biogenic amines, purine and pyrimidine metabolism. These metabolic alterations are responsible for both the therapeutic and the toxic effects of MTX [14].

Kremer et al. [15] demonstrated that MTX caused significant reduction in the antioxidant enzyme levels, sensitizing the cells to reactive oxygen species (ROS).

Erythrocytes are particularly susceptible to oxidative damage as a result of high polyunsaturated fatty acid content in their membranes and high concentration of oxygen and haemoglobin, the latter being a potentially powerful promoter of oxidative processes [16].

ROS reactions with haemoglobin destabilise the haeme and globin structure and release free iron ions that play significant role in the generation of ROS. Any change either in the generation of endogenous ROS or the cellular antioxidant reserves can alter the corresponding oxidative DNA modifications resulting in the perturbations of cellular activities [17].

The deleterious consequences of the above mentioned actions have stimulated studies on the mechanisms of action of biologically relevant natural antioxidants such as polyphenolic compounds. One of such potentially health promoting beverages is green tea.

Our results showed that Hb levels and platelets counts in the methotrexate group showed significant decrease in comparison with the control groups, MTX +1.25% AGTE stimulated the elevation of Hb and platelets levels after methotrexate treatment , but statistically still non significant different compared to methotrexate groups.

Treatment of rats with different concentrations (0.625% and 2.5%) of AGTE orally 7 days prior to and 5 days after MTX, resulted in a non significant change in RBC, MCV ,MCH,WBC while 1.25% AGTE resulted in a significant increase in levels of these parameters compared to MTX group suggesting that they can attenuate methotrexate-induced damage to the hematopoietic system.

This may be due to insufficient concentrations of AGTE at 0.625%, pro-oxidant or antifolate effect of AGTE at high concentrations (2.5%) and antioxidant effect of AGTE at 1.25%.

These results are consistent with those observed in other reports suggesting that EGCG is a powerful antioxidant *in vitro* capable of protecting fresh isolated human erythrocyte membrane lipids or proteins against oxidative damage The protective effects of EGCG in this system was either due to scavenging peroxides before attacking membranes and / or due to blocking the oxidation of membrane lipids [18].

Moreover, *EWA et al.* [19] demonstrated the protective effects of orally administered green tea extract solutions due to its antioxidant properties against ethanol, acetaldehyde, and tert-butyl hydroperoxide-induced oxidative damage on rats' erythrocytes.

It was demonstrated that, tea catechins, including EGCG possesses both pro-oxidant and antioxidant activities because of its unique ability for auto-oxidation, increased production of reactive oxygen species *in vitro* [20] and *in vivo* [21]. And acts as a hydrogen donor [22] .

Furthermore, esterbonded gallate catechins from green tea, such as epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG), are potent *in vitro* inhibitors of several DHFRs at concentrations found in the serum and tissues of green tea drinkers (0.1–1.0 μM)[23].

This observation is further supported by the evident chemical and structural analogy of tea catechins to some classical antifolic agents e.g. trimethoprim and methotrexate [24].

At high concentration 2.5% AGTE may decrease bioavailability of folic acid and this is consistant with other studies [25].

Thus, conflicting data were obtained concerning these respects. Studies with AGTE are quite interesting, because of its dual characteristic behaviour.

Conclusion:

Haematoprotective strategies may offer new approaches to prevent chemo-therapy induced haematotoxicity. From the present results, it can be concluded that pretreatment with different concentrations of aqueous green tea extract prevented toxicity of methotrexate not in a concentration dependent manner and 1.25%

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