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Synergistic Immunosuppressive Activity of Chamomile Flower (*Matricaria Chamomilla L.*) Extracts and Methotrexate *in Vivo*

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Abstract

Chamomile (*Matricaria chamomilla*), a common herb used as a traditional medicine for centuries, was used to treat a variety of human ailments, although its anti-inflammatory action is unknown.Therefore, the purpose of this study was to investigate theimmunosuppressive effects of chamomile flowers methanolic and aqueous extractswith and without methotrexate (MTX) in male albino rats (*Rattus rattus*).Thirty-six male rats were divided into six groups (6 rats for each), G1(normal saline), G2(MTX), G3(methanolic extract of *Matricariachamomilla*), G4(aqueous extract of *Matricariachamomilla*) , G5(methanolic extract of *Matricariachamomilla* and MTX) , and G6(aqueous extract of *Matricariachamomilla* and MTX).All the groups wereprimed for 30 days, then sacrificed on day 31and blood samples was taken to estimate the immunological biomarkers including ; CD4 , CD28 and CD80.. In the normal state (G1) , chamomile extractswere showed a minor inhibitory responses; nevertheless, the levels of CD_4in G5 and G6 groupswas considerably lower than the control group, whereas there was no significant differences in the levels of these parameters in the other groups when compared to the control group. It could be concluded that the methanolic and aqueous extracts ofchamomile flowers alone not revealed immunosuppressant activity but have some synergistic potentials with MTX.

Keywords:*Methotrexate, Chamomile Flower Extract,Immunosuppressive, Inflammation.*

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Introduction

Methotrexate (MTX) is a drug that widely used in the treatment of leukemia, lymphoma, solid tumors, psoriasis, and rheumatoid arthritis ,it is an antimetabolite and antifolate agent with antineoplastic and immunosuppressant activities.MTX binds to and suppresses the enzyme dihydrofolate reductase, causing purine nucleotide and thymidylate synthesis to be inhibited and, as a result, DNA and RNA synthesis to be inhibited[1].Plant extracts typically offer many medicinal applications due to their bioactive richness and have been used since ancient times to treat the health problems of people, in recognition of this fact, the World Health Organization (WHO) has worked to support their use in health systems and recently issued guidelines on the safety, effectiveness and quality of herbal medicines [2]. From thousands of years, chamomile has been used in herbal medicines, common in ancient Egypt, Greece and Rome, in 26 countries, the chamomile medication is included in the pharmacopeia and it is a part of many modern medicinal preparations, unani, and homeopathic remedies [3]. Today, an average daily intake of more than one million cups of tea [4,5], because extract of *chamomilla* has a large range of constituents, this can have antioxidant , anti-inflammatory, moisturizing and emollient properties that may be used in topical formulations though exhibits various pharmacological behaviors, such as anti-inflammatory, anti-cancer, stress and depression management, anti-allergic and others . The principal components of this plant include the α-bisabololterpenoids and their oxides and azulenes, including chamazulene. The flowers of *M. chamomilla*contain the blue essential oil from 0.2 to 1.9%, hence the use of extract also contributes to the various benefits to the skin [6,7]. Literatures evidence indicates that standardized chamomile extracts contain 1.2 percent apigenin in 50 percent alcoholic extracts, whereas aqueous extracts contain low free apigenin concentrations but high, apigenin-7-glucoside (APG) levels [8] .

Materials and Methods

1. Chemicals

MTX injection 50 mg/5ml manufactured by KOCAK FARMA -Turkey. Methyl alcohol (98%) was obtained from El-Nasr Pharmaceutical Chemicals Co., “ADWIC” (Egypt).

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2.Preparation and extraction of the chamomile plant materials

A. Preparation of Methanolic Extract

Dried chamomile (*Matricaria chamomilla*) flowers seeds was purchased from Cairo, Egypt. Eighty grams of dried chamomile flowers (ground) were transported into 1 L Erlenmeyer flasks, then 800 mL of 80% (80 : 20, methanol: water) were added to the samples, extraction was carried out using an shaker at a room temperature for 8 hours, they were filtrated through filter paper (What man No.1), then the extract were evaporated at 45℃by using an oven and stored at -4℃ until use [9].

B. Preparation of Aqueous Extracts

Dried chamomile flowers were weighed and crushed to powder with a marblepestle and mortar, and a 5% (w/v) suspension was prepared in a flask by adding hot boiled water. The flask was then placed on a shaker (200 rpm) for 4 h, and the temperature was maintained at 37 °C, after being shaken, the flask was brought to room temperature, and the suspension was filtered through a series of Whatman filters and finally passed through a 0.22 μm filter (Millipore, Billerica, MA), the filtered aqueous extract was freeze-dried and stored at -20 °C until it was used [5].

3. Preparation of MTX Concentration

The concentration of MTX that used for priming theexperimental rats was 10 mg / kg (1000 mg)

4. Laboratory Animals

The current study was conducted on thirty six male rats (*Rattus rattus*) their weight ranged from 200–300gm. Rats were housed in controlled condition of temperature (25± 3C) and 12 hours (light – dark cycle) and fed commercial pellets and tap water / *ad libitum* maintained in the animal house of College of Science, University of Babylon .The rats were divided into six groups (6 rats for each),including ; Group I(G1): six rats received oral daily dose of distilled water (2 ml/ kg) for 30 successive days this group served as negative control. Group II(G2): six rats received single oral daily dose of methanolic extract of chamomile flowers (200 mg / kg) dissolved in 1 ml distilled water given by oral gavage. Group III(G3): six rats received single oral daily dose of aqueous extract of chamomile flowers (200 mg / kg) dissolved in 1 ml distilled water given by oral gavage. Group IV(G4): six rats received single oral daily dose of methanolic extract of chamomile flowers (200 mg / kg) dissolved in 1 ml distilled water given by oral gavage and on day 28 the animals given single IP dose of methotrexate (10mg / kg). Group V(G5)0: six rats received single oral daily dose of aqueous extract of chamomile flowers (200mg/kg) dissolved in 1 ml distilled water given by oral gavage and on day 28 the animals given single IP dose of methotrexate (10mg/ kg). Group VI(G6): six rats received single oral daily dose of 1

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ml distilled water given by oral gavage and on day 28 the animals given single IP dose of methotrexate (10mg / kg).Then all the animals were anesthetized by chlorophorm and sacrificed on day 31.

5.Collection of blood samples

At the end of the experimental period, animals were fasted overnight, they were anesthetized by chloroform and sacrificed. About 5 ml of the blood was collected directly by heart puncture with sterile syringes, then were put in gel tubes for 30 minutes at room temperature to allowing clotting, then centrifuged at 3000 g for 15 min at 4 °C where the clear sera were separated and kept in eppendrof tubes for estimating the levels of immunological biomarkersCD4, CD28 and CD80 .

6. Estimating the levels of immunological parameters by ELISA Assay

The sera levels of CD4, CD28 and CD80 was evaluated by commercially available ELISA kits, according to the manufacturer's instructions (MyBioSource, USA).

7.Statistical Analysis

Prior to perform the statistical analysis, all data were checked for normality using Anderson–Darling test and the transformation was made when the data were abnormal distributed ones.General linear model (GLM) was used to assess the potential changes among studied groups in which the type of group was fitted as a fixed factor and values of studied blood variables were fitted as response factor in the GLM model. In addition, Tukey test was further made to evaluate the significant changes among studied groups when the P values of GLM appeared significant. The results were expressed as a mean ± standard error and P value equal or less than 0.05 were considered statistically significant.Statistical analyses were conducting using paired Minitab for Windows (version 17 ; Minitab Inc). $p \leq 0.05$ were considered as significant[10].

Results

The levels of CD28 and CD80in current study did not show any significant differences compared to the control group and also among groups ($P>0.05$) However, the administration of chamomile flower extract alone revealed insignificant changes in these parameters when compared to control rats, except in rats that pre-administrated with chamomile flower methanolic extract was showed significant decrease in the level of CD_4 compared to that observed in the control group, while there were no significant changes between other groups comparing to the normal healthy rats , as shown in table 1 and figures 1, 2 , 3 .

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Table 1: The statistical outcomes of the immunological parameters in treated and control groups.

Immune Parameter	G1, Mean±SE	G2, Mean±SE	G3, Mean±SE	G4, Mean±SE	G5, Mean±SE	G6, Mean±SE	Group's Effect
CD4	1471.8^{a}±95.7	1033.0^{ab}±74.4	1386^{ab}±116	780^{b}±136	1412^{a}±169	1202 ^{ab}±200	$F_{5,28}$= 3.53 P=0.013
CD28	3.83 ^{a} ±0.65	2.45 ^{a} ±0.17	2.74 ^{a} ±0.27	6.07 ^{a} ±2.15	2.46 ^{a} ±0.19	3.24 ^{a} ±0.69	$F_{5,28}$= 1.28 P>0.05
CD80	833.0 ^{a} ±79.1	560.0 ^{a} ±54.8	909 ^{a} ±214	657 ^{a} ±129	749.6 ^{a}±74.0	664.2 ^{a} ±59.4	$F_{5,28}$= 1.44 P>0.05

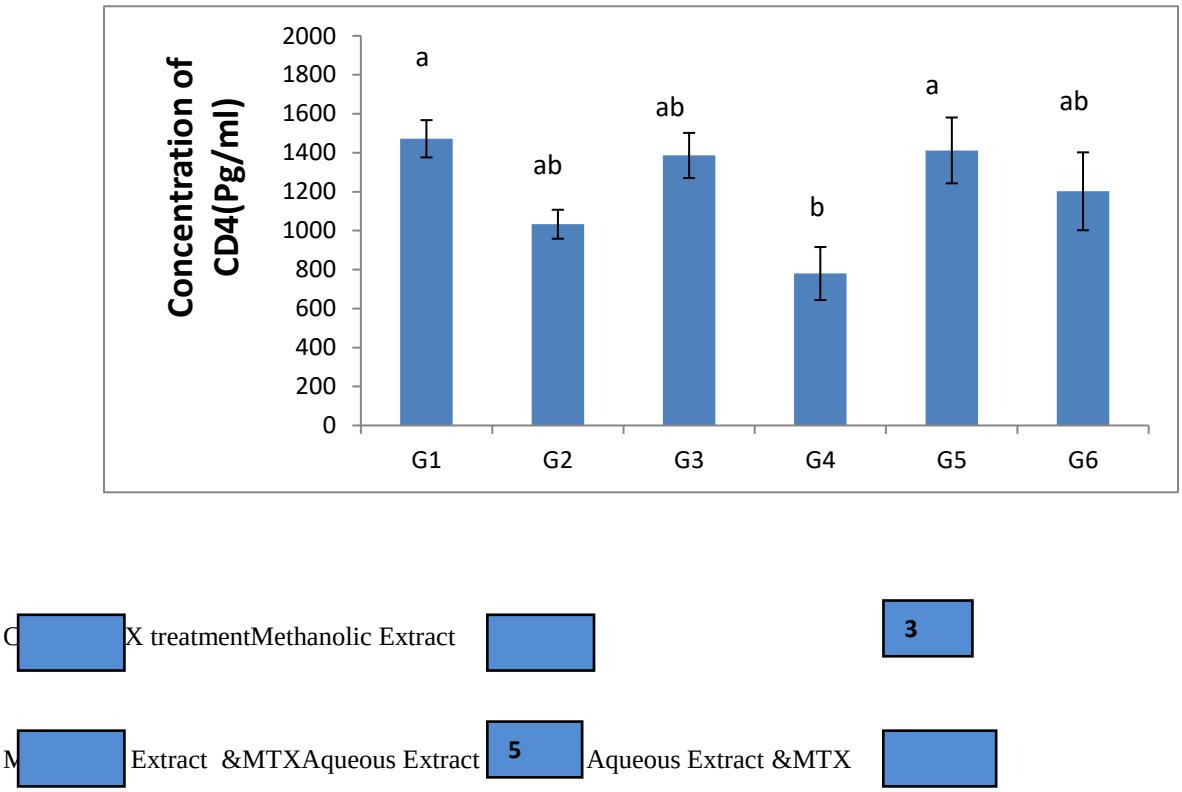
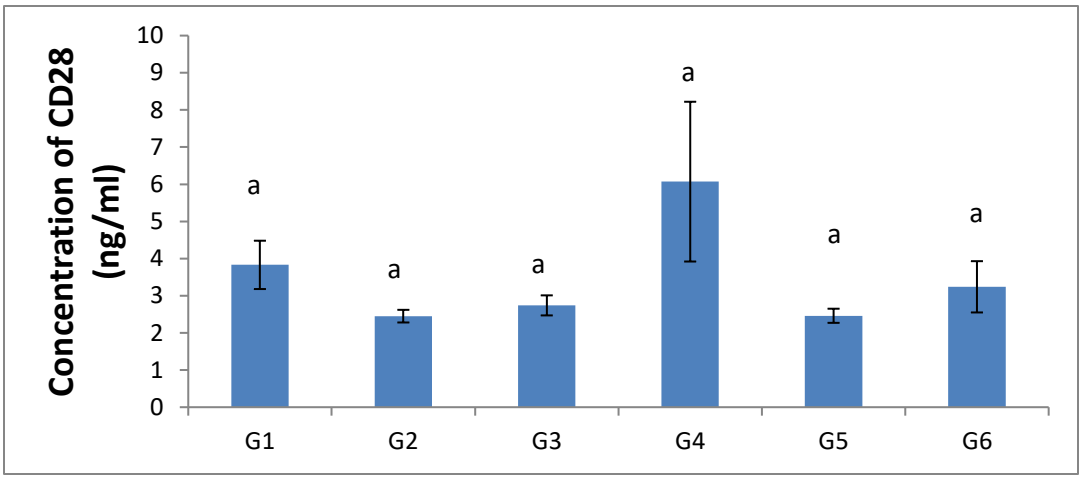


Figure (1) : The concentration of CD_4 in treatment and control groups of rats.

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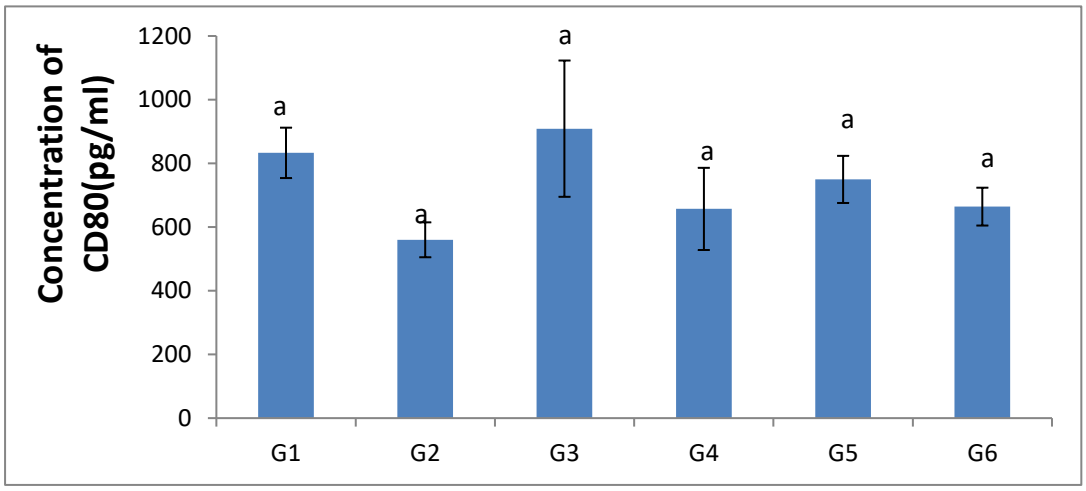
ControlMTX treatmentMethanolic Extract

G1G1 G2G2 G3G

Methanolic Extract &MTXAqueous Extract Aqueous Extract &MTX

G4G4 G5G G6G6

Figure (2) : The concentration of CD_{28} in treatment and control groups of rats.



ControlMTX treatmentMethanolic Extract

G1 G2 G3

Aqueous Extract Methanolic Extract&MTX Aqueous Extract &MTX

G4 G5 G6

Figure (3) : The concentration of CD_{80} in treatment and control groups of rats.

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Discussion

MTX is immunosuppresses treatment altered the pattern of IL-17, IFN-γ, IL-4, IL-21 and CD_{4+}T-lymphocytes development[11]. In current study there were no significant differences in the levels of CD_{4+}T-lymphocytes in MTX experimental animals groups and in alone chamomile extraction groups, except the chamomile flower methanol .The chamomile preparations made from *Matricaria chamomilla* are one of the most common herbal remedies available today and therapeutic preparations for its made up of a variety of biologically active compounds that have anti-inflammatory properties, such as essential oils and flavonoids, from the major flavonoids are apigenin, quercetin, and luteolin, which have anti-inflammatory properties through various mechanisms ,apigenin inhibits the formation of pro-inflammatory cytokines, while luteolin inhibits the release of nitric oxide (NO), prostaglandin E2, and the expression of inducible NO synthase and cyclooxygenase-2, both of which are involved in anti-inflammatory activity, owever, also additional components of MC preparations play a role in the plant's anti-inflammatory actions through different pathways may be useful in reducing rheumatoid arthritis inflammation and pain[12].In animal models, MTX's effects have been attributed to MTX-induced anti-inflammatory adenosine, whose production is mediated by CD39 and CD73 ectoenzymes[13,14].

In conclusion, in part through their different mechanisms ofaction, chamomile flower methanolic extract showed synergistic effects with single low dose MTX and significantly suppressed CD_4 compared to the value observed in the control group, while there were no significant changes between other groups(MTX alone, chamomile flower extract alone) comparing to the control group, in addition , the levels of CD28, and CD80 did not show any significant changes compared to the control group and also among groups.

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