

ARTICLE / INVESTIGACIÓN

Study the antioxidant of *Matricaria chamomilla* (Chamomile) powder: *In vitro* and *vivo*

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Abstract: Oxidative stress is oxidative damage caused by free radicals and reactive oxygen species (ROS). These ROS can cause oxidative damage to cellular components, including membrane lipids, receptors, enzymes, proteins, and nucleic acids. It would eventually lead to cell apoptosis and the appearance of certain pathological conditions. This work investigates the antioxidant potentials of chamomile extract *in vitro* by evaluating the extract activity to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH), also *in vivo* by investigating its effects on oxidative stress-induced rats by assessing the total oxidant status (TOS) and total antioxidant capacity in the radiation exposed rats with and without the treatment with chamomile extract. The results have shown that chamomile extract contains materials with antioxidant properties. The *in vitro* analyses have indicated activity to detoxify the DPPH radicals almost as powerful as pure ascorbic acid. Furthermore, rats exposed to electromagnetic radiation have shown a disturbance in the balance of oxidants and antioxidants, in which the levels of TOS were elevated while the levels of TAC were reduced. Chamomile extract has been shown to exhibit a powerful function as an antioxidant *in vivo*. It has enhanced the antioxidant capacity of rats, reduced their total oxidant status, and protected exposure to radiation.

Key words: Total antioxidant capacity, peach fruit, rats, DPPH, total oxidant status.

Introduction

Oxidative stress is a term used for oxidative damage caused by free radicals and reactive oxygen species (ROS)¹. The stability of the ROS is shallow, and therefore they are highly reactive, which can cause oxidative damage to cellular components, including membrane lipids, receptors, enzymes, proteins, and nucleic acids². This oxidative effect would eventually lead to cell apoptosis and the appearance of certain pathological conditions^{3,4}. Nevertheless, ROS are generally produced in the living system to perform an essential role in signaling as second messengers⁵.

The term ROS includes a wide range of oxygen-containing species such as hydroxyl radical (.OH), superoxide anion (O²⁻), hydrogen peroxide (H₂O₂), nitric oxide (NO.) and other species⁶. The mitochondrial electronic transport chain produces some of these species generally upon aerobic metabolism⁷. Other sources of ROS include NADH oxidases, xanthine oxidoreductase, arachidonic acid cascade enzymes, etc.^{3,8,9} which all increase the level of ROS under certain pathological conditions¹⁰. Other sources that can increase the level of ROS in the living systems are exogenous and include smoke, radiation, and other pollution¹¹.

To detoxify the oxidative damage of ROS and free radicals, the living system includes a synergistic defense system called antioxidants. The antioxidant materials can reduce

ROS's oxidative damage and eliminate their toxicity by different mechanisms¹². These antioxidants are classified as endogenous, like superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), and uric acid, in addition to others³. While the other sources of antioxidants introduced by diet¹³, most notably plants. The plant contains various antioxidant materials like vitamins C and E, carotenoids, polyphenols, coenzyme Q10, and flavonoids¹⁴⁻¹⁶.

Chamomile is anti-inflammatory¹⁷, anticancer¹⁸, antioxidant¹⁹, anti-diarrheal²⁰, neuroprotective²¹, anti-allergic²², and antibacterial²³. It also has heart-health benefits. In preclinical studies using skin and ovarian cancer models, medicinal herbs have been shown to have potential growth-inhibitory effects^{16,23}. In cancer cells, chamomile has been found to promote apoptosis. Terpenoids -bisabolol is the primary constituent in chamomile essential oil²⁴. We have aimed to investigate the antioxidant potentials of chamomile extract *in vitro* by evaluating the extract activity to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH), also *in vivo* by investigating its effects on oxidative stress-induced rats.

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Materials and methods

Preparation of chamomile-extract

At a nearby market, I purchased dried chamomile flowers. The flowers were macerated for two days at 4°C after being extracted with 70% ethanol and macerated for 10 grams. The product was then filtered as well as concentrated at 40°C under lower pressure. A 30 mg/mL solution was created using 50 % ethanol.

In vitro antioxidant test

The activity of chamomile extract to scavenge DPPH was determined in a spectrophotometric method¹². A series of concentrations (in methanol) each of ascorbic acid, bulk curcumin, and curcumin nanoparticles were prepared (10, 20, 40, 80, and 100 µg/mL). A weight of 0.36 g of DPPH was dissolved in 4 mL methanol. 0.15mL of the DPPH solution was mixed with 3mL of each prepared concentration and with deionized water as control. The tubes were allowed to stand in the dark for 30 minutes, then the absorbance of each tube was determined at 517 nm. The activity of each material was calculated from the following equation:

$$\% \text{ activity} = (\text{ADPPH} - \text{A}_{\text{test}}) / \text{ADPPH}$$

In vivo antioxidant test

The antioxidant activity was determined by determining the total antioxidant capacity (TAC), and total oxidant status (TOS) in rats induced oxidative stress. The experiment was included 21 male rats, which divided into three groups; i) The first group was under the stimulating of oxidative stress by exposure to electromagnetic radiation, and they were supplemented with 10mLs of chamomile extract daily; ii) the second group was contained 7 rats who exposed to electromagnetic radiations without the supplement of chamomile extract, and iii) control group which contained 7 rats without exposing to electromagnetic radiation nor supplemented with chamomile extract.

The experiment took 2weeks until analysis, and the levels of TOS and TAC were determined by Erel's method²⁵.

Results

The IC₅₀ is the concentration of the material at which it would exhibit a 50% inhibition of the free radicals (DPPH). In Figure 1, the activity of ascorbic acid to scavenge DPPH radicals is shown. Ascorbic acid has been used as a standard, and it gave an IC₅₀ of 27.26µg/mL in methanol which was less than a previously reported value²⁶. On the other hand, chamomile extract solution (Figure 2) has shown a higher IC₅₀ value than ascorbic acid, although the value represents extreme antioxidant activity. This activity of chamomile as an antioxidant is agreed with a previous study^{27,28}.

The rats were conditioned in the same conditions and fed a fat-free diet. After two weeks, the blood of rats was analyzed for TOS and TAC levels, and the results were processed statistically by analysis of variances (ANOVA) test.

Discussion

In this investigation, we evaluated the beneficial effects of TOS and TAC on the management of oxidative stress endogenously in rats treated with chamomile extract.

Figure 3 shows the levels of TOS equivalents to H₂O₂, in which the level of TOS was elevated significantly (P<0.05) in exposed untreated rats compared to the control. On the other hand, the rats treated with chamomile extract showed non-significant (P>0.05) differences in TOS level compared to the control.

Figure 4 shows the TAC levels in examined rats. The TAC level was reduced significantly in exposed untreated rats compared to the control. In contrast, the exposed rats treated with chamomile extract have shown similar results compared to control and higher than untreated rats.

These observations indicate the powerful antioxidant activity of chamomile extract in the management of oxidative stress endogenously, as well as in protecting the body from exogenous pollution (radiation). The antioxidant activity of chamomile is attributed to the phytochemicals that are found in the plant.

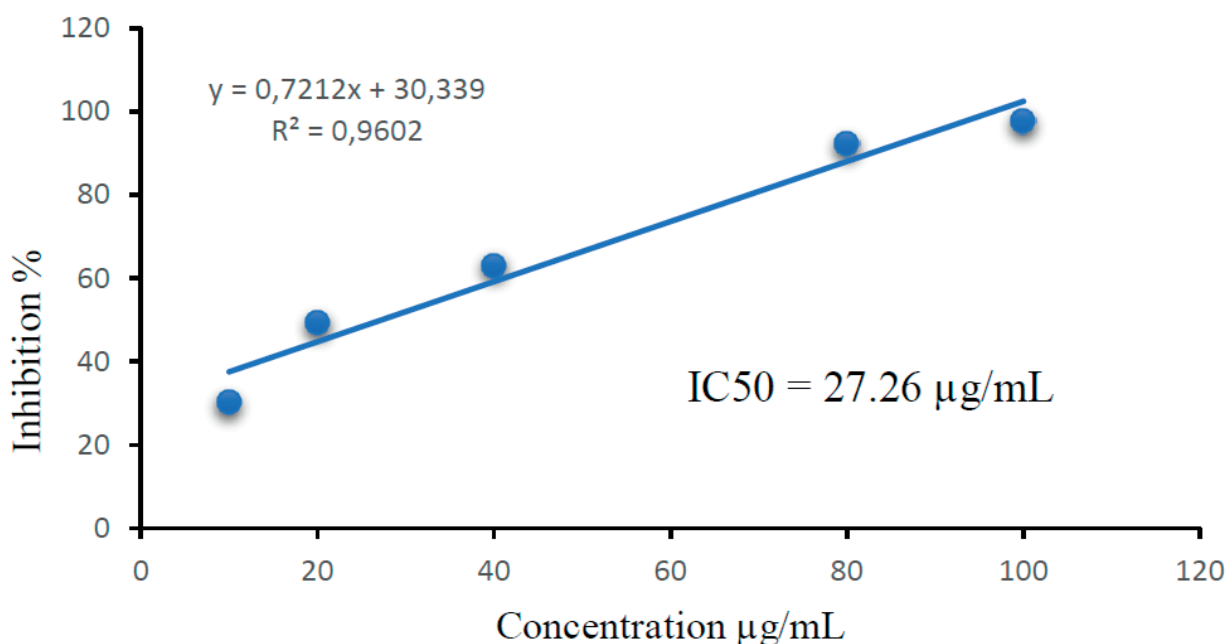


Figure 1. Ascorbic acid inhibition% against DPPH.

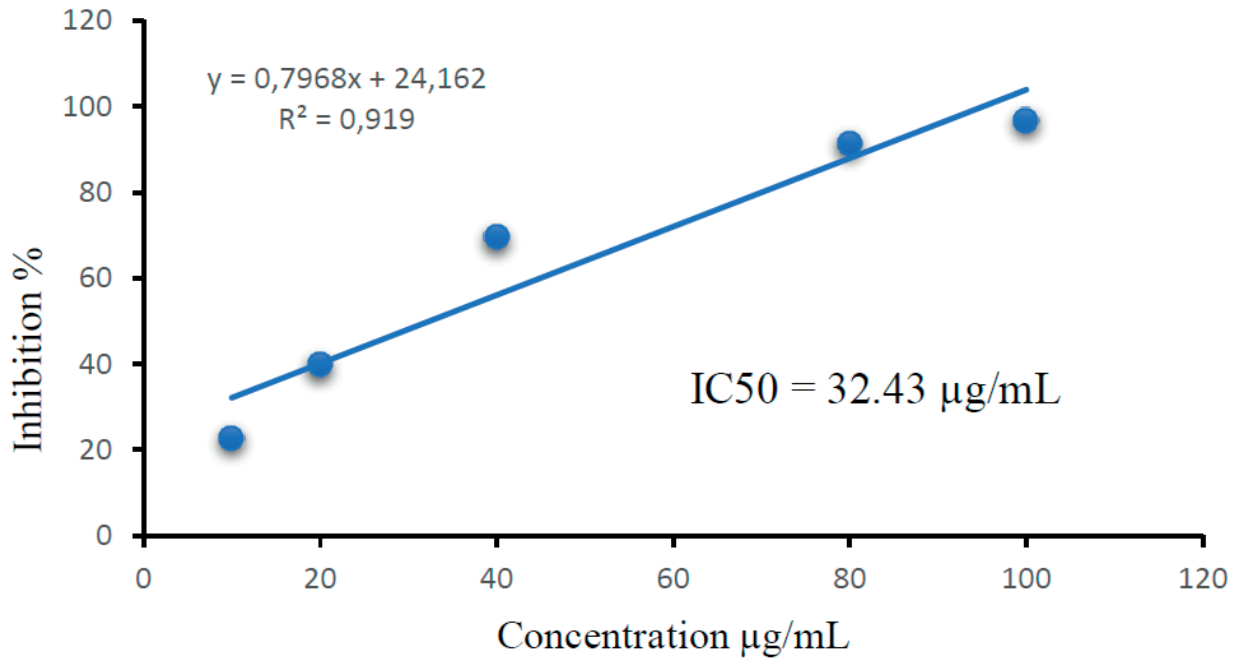


Figure 2. Chamomile extract inhibition% against DPPH.

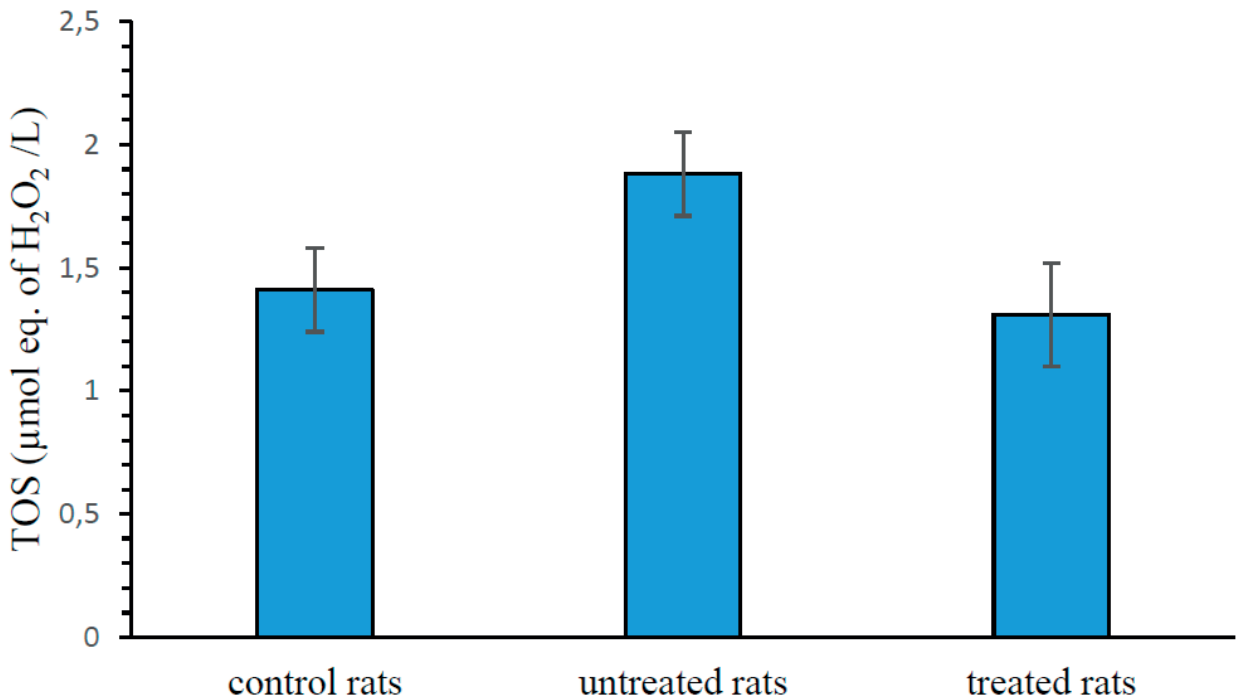


Figure 3. The levels of TOS in examined rats. $P < 0.001$ according to ANOVA.

Conclusions

The results have shown that chamomile extract contains materials with antioxidant properties. The *in vitro* analyses have indicated activity to detoxify the DPPH radicals almost as powerful as pure ascorbic acid. Furthermore, rats exposed to electromagnetic radiation have shown a disturbance in the balance of oxidants and antioxidants, in which the levels of TOS were elevated while the levels of TAC were reduced. Chamomile extract has shown to exhibit a powerful function as an antioxidant *in vivo*. It has enhanced the antioxidant capacity of rats, reduced their total oxidant status, and protected exposing from radiation.

Author Contributions

Ibtesam Y. Alja'afreh: Conceptualization, Writing – original draft. Raafat M. Alaatabi: Visualization. Haidar Abdulkareem Almashhadani: Validation and Writing – review & editing. Faten Essam Hussain Aldoghachi: Resources. Mustafa mudhafar: Investigation. Mustafa M. Kadhim: Formal analysis. Falah Hassan Shari: Software and Formal analysis.

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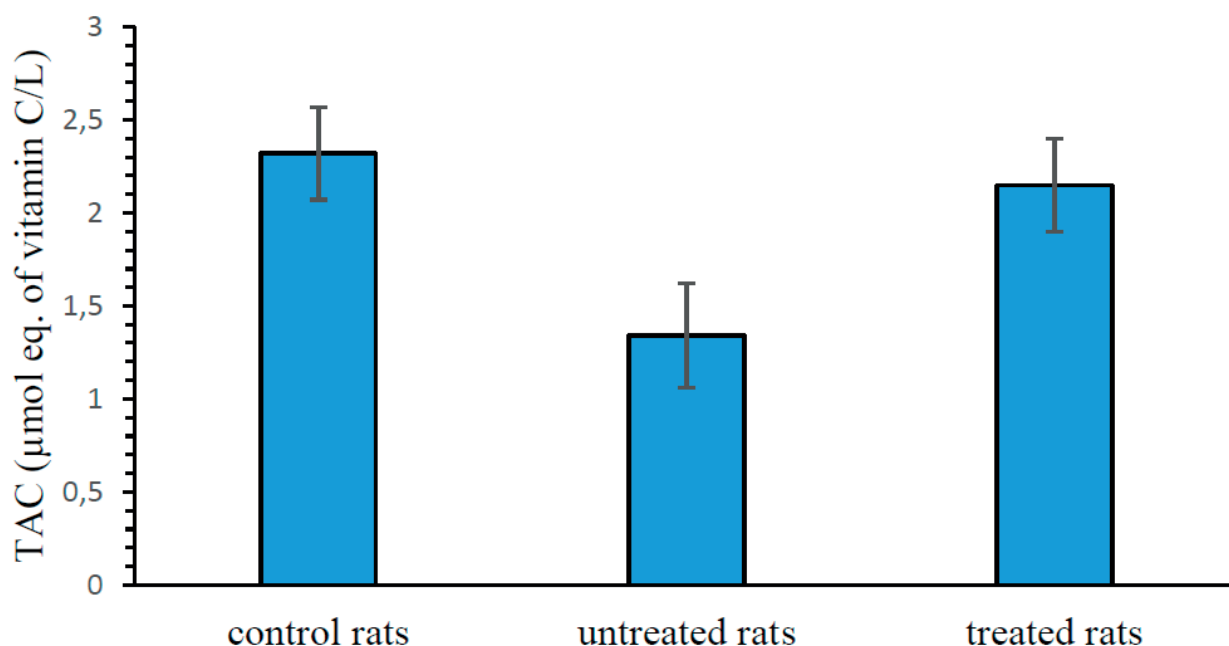


Figure 4. The levels of TAC in examined rats. $P < 0.001$ according to ANOVA.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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