

RESEARCH-ANIMAL

Almond oil improves the levels of some trace elements and antioxidant status in mice exposed to oxidative stress

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Abstract

Objectives: To evaluate the concentrations of some trace elements and the antioxidant status in male mice exposed to oxidative stress by carbon tetrachloride and then treated by either almond oil or vitamin C.

Methods: The animal study was conducted in January 2020 at the College of Nursing of the University of Thi-Qar and the Kut Technical Institute, Middle Technical University, Baghdad, Iraq, and comprised adult male mice. They were divided randomly into four equal groups and treated for 21 days. Mice in group I received sunflower oil 1ml per mice, mice in group II were injected 0.3% carbon tetrachloride intraperitoneally, mice in group III received 0.3% carbon tetrachloride plus oral intubation of vitamin C 300mg/kg body weight daily, and mice in group IV were intubated with 2.26g/kg body weight of almond oil plus 0.3% carbon tetrachloride daily. Serum and liver homogenate were used to measure the levels of trace elements and the antioxidant status. Data was analysed using SPSS version 20.

Results: There were 24 mice; 6(25%) in each on the 4 groups. Mice in group II showed a significant decrease in zinc, magnesium and phosphorus levels, and significant elevation in calcium levels ($p<0.05$). Mice in groups III and IV showed a significant difference in trace elements compared to group II ($p<0.05$). Almond oil enhanced the antioxidant status and was more active than vitamin C ($p<0.05$).

Conclusions: Almond oil was found to have beneficial and pharmacological effects against oxidative stress.

Keywords: Carbon tetrachloride, Antioxidants, *Prunus dulcis*, Ascorbic acid, Phosphorus, Fatty liver, Zinc, Vitamins, Intubation, Intratracheal. **DOI:** <https://doi.org/10.47391/JPMA.IQ-29>

Introduction

Recent studies have emphasised the important and healthy uses of flavonoids, which are active-phenolic combinations extensively found in vegetables, fruits, plant extracts, green tea, lycopene, broccoli, red bell peppers, berries, as well as almond oil (AO).¹⁻⁵ These compounds have generated a wide ranges of research areas because of their potentially therapeutic anti-inflammatory, antifungal, cardio-protective and antiviral actions.⁶⁻⁸ In addition, some of these properties are linked to their antioxidant properties that could scavenge the toxicity of free radicals (FRs).^{9,10}

Flavonoids produce a synergistic effect with other antioxidants, such as vitamins E and C, to reduce FR toxicity¹¹. FRs are constantly produced in small quantities by routine metabolism processes, and several of them have beneficial biological roles.^{12,13} But when they are produced in huge amounts, they enhance the damaging effect to different biological molecules.¹⁴ FR levels are increased in the body through the oxidative stress process. They are the result of an inequality of

antioxidants and pro-oxidants inside the living cells, which is being considered a vital cause in different chronic diseases, such as cancer, cataract, post-ischaemic reoxygenation injury, rheumatoid arthritis complications associated with aging, and cardiovascular disease.¹⁵⁻¹⁸

The trace elements have been exhibited to impact several biological, biosynthetic and biochemical processes, such as the stabilisation of the structures of both nucleic acid and proteins.¹⁹⁻²¹ Furthermore, trace elements are important in subcellular system functions, like membrane transport, mitochondria, nerve conducting, and muscular contraction.²²⁻²⁴ Moreover, copper (Cu), zinc (Zn), Zn, manganese (Mn) and selenium (Se) act as antioxidants.²⁵

The current study was planned to examine the effective role of AO in modifying the serum levels of some trace elements and antioxidant status in male mice exposed to oxidative stress by carbon tetrachloride (CCl₄) and then treated with AO and vitamin C.

Materials and Methods

The animal study was conducted in January 2020 at the College of Nursing of the University of Thi-Qar and the Kut Technical Institute, Middle Technical University, Baghdad, Iraq. After approval from the institutional ethics review board adult male Albino mice weighing 20-25g each were obtained from the animal house. They were divided randomly into 4 equal groups and treated for 21 days.

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Mice in control group I (GI) received sunflower oil 1ml per mice, mice in group II (GII) were injected 0.3% CCl₄ intraperitoneally, mice in group III (GIII) received 0.3% CCl₄ plus oral intubation of vitamin C 300mg/kg body weight daily, and mice in group IV (GIV) were intubated with 2.26g/kg body weight of AO plus 0.3% CCl₄ daily. CCl₄ and other chemicals (Sigma, St Louis, MO, USA) were used to generate a stock solution on a daily basis, AO, sunflower oil, and vitamin C were obtained from a local licensed pharmacy.

Fasting blood samples were collected at 0, 10 and 21 days of the experiment, and were used for the measurement of Zn, magnesium (Mg), Phosphorus (P) and Calcium (Ca) levels using the atomic absorption method.²⁷ Catalase, superoxide dismutase (SOD) and glutathione (GSH) antioxidant levels were measured using the cold supernatants of liver homogenates (Randox Kit, United Kingdom) as per the procedure suggested by the manufacturers.²⁸⁻²⁹ The level of malonaldehyde (MAD) was measured using the thiobarbituric acid reactive substance (TBRAS) kit (Cayman, USA) as per the procedure suggested by the manufacturers.³⁰

Data was analysed using the software SPSS version 20. Two-way analysis of variance (ANOVA) was used, with $p < 0.05$ being the marker of statistical significance.

Results

There were 24 mice; 6(25%) in each on the 4 groups. The groups showed no significant differences at baseline ($p > 0.05$). There was a significant decrease in Zn level ($p < 0.05$) in GII compared to GI, GIII and GIV, along with a significant elevation in GV at 10 and 21 days (Table 1).

There was a significant decline in Zn level in GIII compared to GI at 21 days ($p < 0.05$).

There was a significant decrease ($p < 0.05$) in mean Mg level in GII on days 10 and 21 compared to GI, GIII and GIV (Table 2). There was a significant increase ($p < 0.05$) in Ca level in GII mice at 10 and 21 days compared to GI, GIII and GIV (Table 3).

There was a significant decline ($p < 0.05$) in P level in GII and GIII at 10 and 21 days compared to GI (Table 4). There was a significant increase on day 21 in GIV mice compared to GII and GIII ($p < 0.05$).

There was a significant increase ($p < 0.001$) in MAD level in GII and a significant decline in GIII and GIV compared to GI (Table 5). There was a significant increase in GSH, catalase and SOD levels in GIII and GIV compared to GII ($p < 0.05$).

Table-1: Effect of almond oil (AO) and vitamin C on zinc (ppm) level in serum of mice treated with carbon tetrachloride (CCl₄).

Group/Time (Day)	0	10 (Days)	21 (Days)	p-value
I	2.20±0.05 Aa	2.16±0.05 Aa	2.15±0.05 Aa	NS
II	2.28±0.10 Aa	1.61±0.07 Bb	1.50±0.05 Bc	<0.05
III	2.33±0.08 Aa	2.01±0.04 Aa	1.96±0.10 Cb	<0.001
IV	2.15±0.05 Aa	2.35±0.07 Aa	2.33±0.07 Aa	NS

Values presented as means ± standard deviation (SD); n = 6 mice/group; capital letters denote the differences between groups, $p < 0.05$ and 0.001 vs. control; small letters indicate the differences within group compared to baseline; $p < 0.05$ and 0.001.

Table-2: Effect of almond oil (AO) and vitamin C on magnesium (Mg) level (PPM) in serum of mice treated with carbon tetrachloride (CCl₄).

Group/Time (Day)	0	10 (Days)	21 (Days)	p-value
I	12.19±0.22 Aa	12.05±0.08 Aa	12.05±0.24 Aa	NS
II	12.11±0.10 Aa	10.18±0.23 Bb	9.83±0.37 Bc	<0.001
III	12.26±0.32 Aa	13.51±0.19 Cb	13.33±0.30 Cb	<0.001
IV	12.05±0.09 Aa	12.56±0.30 Aa	12.90±0.33 Aa	NS

Values presented as means ± standard deviation (SD); n = 6 mice/group; capital letters denote the differences between groups, $p < 0.05$ and 0.001 vs. control; small letters indicate the differences within group compared to baseline; $p < 0.05$ and 0.001.

Table-3: Effect of almond oil (AO) and vitamin C on calcium (Ca) level (ppm) in serum of mice treated with carbon tetrachloride (CCl₄).

Group/Time (Day)	0	10 (Days)	21 (Days)	p-value
I	125.68±0.39 Aa	125.93±0.48 Aa	125.95±0.17 Aa	NS
II	125.70±0.18 Aa	121.23±0.53 Bb	119.50±0.46 Bc	<0.001
IV	125.75±0.12 Aa	126.43±1.45 Aa	126.28±0.25 Aa	NS

Values presented as means ± standard deviation (SD); n = 6 mice/group; capital letters denote the differences between groups, $p < 0.05$ and 0.001 vs. control; small letters indicate the differences within group compared to baseline, $p < 0.05$ and 0.001.

Table-4: Effect of almond oil (AO) and vitamin C on phosphorus (P) level (ppm) in serum of mice treated with carbon tetrachloride (CCl₄).

Group/Time (Day)	0	10 (Days)	21 (Days)	p-value
I	125.68±0.39 Aa	125.93±0.48 Aa	125.95±0.17 Aa	NS
II	125.70±0.18 Aa	121.23±0.53 Bb	119.50±0.46 Bc	<0.001
III	125.93±0.41 Aa	122.93±0.80 Bb	125.10±0.30 Ac	<0.001
IV	125.75±0.12 Aa	126.43±1.45 Aa	126.28±0.25 Aa	NS

Values presented as means ± standard deviation (SD); n = 6 mice/group; capital letters denote the differences between groups, $p < 0.05$ and 0.001 vs. control; small letters indicate the differences within group compared to baseline, $p < 0.05$ and 0.001.

Table-5: Effect of almond oil (AO) and vitamin C on antioxidant status of mice treated with carbon tetrachloride (CCl₄).

Group/Time (Day)	MAD (mM/L)	GSH (μmol/g)	Catalase (Unit/mg)	SOD
I	8.68±0.67 A	4.81±0.58 A	19±1.48 A	21.9±3.15 A
II	19.70±1.14 B	2.11±0.44 B	6.5±0.78 B	11.9. ±1.21 B
III	12.93±0.65 C	4.73±0.60 A	22.0±3.78 C	19.10±3.11 A
IV	10.55±0.56 D	4.93±0.50 A	22.5±3.82 C	20.28±3.33 A
P value	<0.001	<0.05	<0.001	<0.05

MAD: Malonaldehyde, GSH: Glutathione, SOD: Superoxide dismutase. Values presented as means ± standard deviation (SD); n = 6 mice/group; capital letters denote the differences between groups vs. control, $p < 0.05$ and 0.001 .

Discussion

The current study showed that the injection of CCl₄ caused significant decrease in some trace elements, such as Zn, Mg and P, with significant elevation in Ca, compared to the control group.

These results showed oxidative stress induction by CCl₄ which could have been because of a variety of reasons.³¹

The possible oxidation of GSH and nicotinamide adenine dinucleotide phosphate (NADPH) by Reactive Oxygen Species (ROS) via GSH redox cycle causes depletion of endogenous antioxidants, including GSH, leading to decrease in antioxidant / pro-oxidant ratio.³² Any trouble in nutritional equilibrium of trace elements thus decreases efficacy of antioxidant status and rises sensitivity of organism to the damage caused via FRs. High loss of trace elements could be contributing to the decreased defence mechanism.³³

The significant decreased in Zn and P could have been due to the antioxidant properties of these elements in the body, while Zn and iron elements have an important role as co-factors of some enzymes. Also, Zn is a co-factor of cytoplasmic Cu-Zn SOD enzymes that decrease the levels of these elements due to the detoxification of harmful oxygen species. In addition, Zn and Cu contribute to antioxidant defences.³⁴ One study reported that Zn and Se are antagonised to oxidative stress.³⁵ Other studies showed that Zn causes an inhibition of lipid peroxidation.³⁶

Zn stabilizes the structure of SOD activity, and increases the concentration of superoxide radicals, which could increase the formation of superoxide radicals in proximity to mitochondria.³⁷

The increase in Ca level could enhance the activation of various Ca-dependent degenerative enzymes, like

phospholipase, proteases and endonuclease, which may contribute to cell death.³⁸ In contrast, low dose of oxidative stress in Vitamin C and AO group stimulates the enzyme activity of protein kinase that is marked by increase oxidation-reduction.³⁹

The decrease of Mg ions due to the antioxidant properties and any deficiency in Mg level are linked to increased oxidative stress.⁴⁰⁻⁴² Additionally, rich diet with Mg may exert a cardiac protection effect by reducing ration involving total plasma cholesterol, triglyceride and ameliorated high-density lipoprotein (HDL).⁴³ The current study noted a significant decline in P level in GΠ and GШ at 10 and 21 days compared to the control group. This was because of damage to the kidney by CCl₄ that led to decrease in vitamin D synthesis, causing difficulty in absorption from the kidney.⁴⁴

In addition, the current results showed the protective effect of AO and vitamin C. Vitamin C is the most powerful antioxidant, which is critical for appropriate antioxidant safety as it decreases lipid peroxidation in the cell membrane.⁴⁵ The high content of different phytochemical compounds, such as flavonoids (catechins) in AO decreases lipid peroxidation level and protects cell membrane fluidity, keeping the internal environment of trace element constant.⁴⁶

It has been reported that AO contains manifold higher concentration of potassium, iron, vitamin C and organic acid as well as biologically active plant phenolic compounds.⁴⁷ Also, AO is a very good source for phosphorus, vitamin B1 (thiamine), vitamin B2 (riboflavin) and vitamin B3 (niacin) and is high in isoflavone.⁴⁸ Anthocyanins have been demonstrated in laboratory experiments to have the potential to inhibit oxidative stress suspected to be at the origin of heart disease, cancer and another chronic diseases.⁴⁹

Conclusions

A protective effect of AO against toxicity of CCl₄ was found in mice. Besides, AO can be used as preventive treatment for patients suffering from chronic diseases.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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