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Effect of volatile oils extracted from orange peels *Citrus sinensis* and silver nanoparticles manufactured from it on the concentration of glutathione (GPX) and Levels of oxidative stress Malondialdehyde (MDA) and Lipid peroxides (LPO) in the serum of male rats

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Abstract---Citrus fruits are used for the prevention of many chronic diseases such as cancer, diabetes, and cardiovascular diseases. The leaves and fruits of the citrus genus contain antioxidants. The oil of the family plants has uses in folk medicine, The results also showed a significant increase in the concentration of glutathione peroxidase (GPX) when treated with orange peel extract at a concentration of 450 mg/kg and 1 ml/kg of hydrogen peroxide, which amounted to $64.77 \pm 1.3 \mu\text{mol/L}$ Compared to With the treatment that was dosed only with 1 ml/kg of hydrogen peroxide H_2O_2 as its concentration level was $43.69 \pm 4.1 \mu\text{mol/L}$, there were also significant differences for the treatment when dosed with 0.6 mg/kg with nanosilver and 1 mm/kg of peroxide. The hydrogen peroxide amounted to $57.32 \pm 6.2 \mu\text{mol/L}$, while the concentration of malondialdehyde (MAD) decreased significantly when treated with orange peel extract at a concentration of 450 mg/kg and 1 ml/kg of hydrogen peroxide, reaching $0.390 \pm 0.056 \mu\text{mol/L}$ (representing the lowest concentration of MAD levels) compared to the treatment dosed with only 1 ml/kg of hydrogen peroxide of $0.757 \pm 0.021 \mu\text{mol/L}$, there was a significant decrease for the dosing treatment of 0.6 mg/kg with nanosilver + 1 mm/kg hydrogen peroxide, which was $0.510 \pm 0.028 \mu\text{mol/L}$, While the level of lipid peroxide (LPO) concentration, it showed a significant decrease when treated with orange peel extract at

a concentration of 450 mg/kg and 1 ml/kg of hydrogen peroxide, which amounted to $3.95 \pm 0.27 \mu\text{mol/L}$ (which represents the lowest concentration of LPO levels).) compared to the treatment that was dosed only with 1 ml/kg of hydrogen peroxide amounting to $7.13 \pm 0.22 \mu\text{mol/L}$, as well as a significant increase when dosed with 0.6 mg/kg of nano silver + 1 mm/kg hydrogen peroxide, which amounted to $5.79 \pm 0.22 \mu\text{mol/L}$.

Keywords---*Citrus*, D-limonene, Silver nanoparticles, LPO, GPX, MAD.

Introduction

Citrus essential oils have been classified as generally recognised as safe (GRAS) due to their wide spectrum of biological activities such as antimicrobial (Fisher and Phillips, 2008), antifungal (Chutia, et al. 2009), antioxidant (Rehman, 2006), anti-inflammatory and anxiolytic (De Moraes et al. 2006) . Previous studies related to the essential oil of *C. sinensis* from different locations focused on the exfoliating oil contains a percentage limonene (94.7%)It is a natural antioxidant, the antioxidants are molecules that are able to slow down or prevent the oxidation of other molecules, and they protect the cells of the body from free radicals, thus maintaining the health of the body and the regularity of the functions of its various organs (Yıldız, 2010). D-limonene is a colorless liquid at room temperature

The structural {1-methyl - 4 (1-methylethenyl cyclohexane)} is a natural compound monocyclic monoterpene (Figure 1) and the main component of the oil extracted from Citrus peels (Sun ,200 ; Hadi and Aljanaby, 2022) with chemo preventive and anti-tumor activities with odor lemon like and is a major constituent in several citrus oils (orange, grapefruit, lemon and limetta etc.). due to its pleasant citrus aroma, D-limonene is used widely as a flavor , fragrance additive in perfumes, foods, beverages, chewing gum and soaps. Being stellar solvent of cholesterol, d-limonene Use clinically to dissolve cholesterol-containing gallstones. fruit peels have volatile oil composed of d-limonene, gamma-aminobutyric acid, d-linalool and N-acetyl octopamine. limonene contains sedative effects and antianxiety (Carvalho Freitas and Costa, 2002). Oxidized glutathione is an antioxidant enzyme, containing selenium, and by the effect of this enzyme to rid the body of toxicity caused by hydrogen peroxide H_2O_2 by 90% and organic peroxide by 70%, this enzyme can also reduce the oxidation of lipids in cell membranes (Zulekha, 2017; Medhat and Aljanabay, 2022). Fats are a heterogeneous group of compounds that have several functions in the body, the most important of which is a source of energy in the body of the organism. It also enters the composition of cell membranes and nervous tissues (Murray *et al.*, 2000)) and thus The occurrence of a defect in the antioxidants leads to an increase in the level of lipid peroxidation and a decrease in the activity of oxidative glutathione (Moustafa et al., 2001). The fats are mainly divided into two groups: polar and non-polar. Apolar, and the triglycerides stored in adipose tissue are usually non-polar, which is the main energy store in mammals, that the occurrence of oxidative stress causes direct damage to fats due to high levels of free radicals, especially hydroxyl radical, which leads to the oxidation of unsaturated fatty acids and the formation of lipid peroxides (Ayala *et al.*, 2014).

Materials and Methods

Extraction of volatile oils

The fruits peel of *C. sinensis* and *C. limon* were collected from the farms of Babylon Governorate in Iraq, Fresh fruits were peeled with a razor Carefully to prevent damage to the oil glands. I have been put 100 g of peels in 200 ml distilled water into a Clevenger ap-paratus. The temperature of the device was set at 95Co for 3 hours. (Davidowski and Di Marco, 2009). After obtaining the volatile oils, they were sent to the Al-Razi Center for a medical examination GC/MS.

Experimental groups

The experiment rats were divided into eight main groups, each group included five rats, as as follows:

- First group: 5 rats were orally dosed with extract of peels of type C fruit. *sinensis* (the proportion of the compound D limonene 90.41%) at a concentration of 150 mg / kg per day with hydrogen peroxide H₂O₂ at a concentration of 0.5% at a dose of 1 ml / kg per day for 35 days.

- The second group: 5 rats were dosed orally with extract of peel fruits type *C. sinensis* (the proportion of the compound D limonene 90.41%) 300 mg / kg daily with hydrogen peroxide H₂O₂ at a concentration of 0.5% and at a dose of 1 ml / kg daily for 35 days.

- The third group: 5 rats were dosed orally with *C* fruit peel extract. *sinensis* (the proportion of the compound D limonene 90.41%) 450 mg / kg daily with hydrogen peroxide H₂O₂ at a concentration of 0.5% and at a dose of 1 ml / kg daily for 35 days.

- Fourth group: 5 rats were orally dosed with biosynthetic nanosilver with aqueous extract of *C* fruit peels. *sinensis* water at a concentration of 0.1 mg/kg daily with 1 ml/kg hydrogen peroxide H₂O₂ at a concentration of 0.5% daily for 35 days.

- Fifth group: 5 rats were orally dosed with nanosilver biosynthesized with aqueous extract of type *C* scales. *sinensis* aqueous at a concentration of 0.3 mg/kg daily with 1 ml/kg hydrogen peroxide H₂O₂ at a concentration of 0.5% daily for 35 days.

- Sixth group: 5 rats were orally dosed with nanosilver biosynthesized with aqueous extract of type *C* scales. *sinensis* at a concentration of 0.6 mg/kg daily with 1 ml/kg of hydrogen peroxide H₂O₂ at a concentration of 0.5% daily for 35 days.

- Seventh group: 5 rats were given orally administered 1 ml/kg of hydrogen peroxide H₂O₂ at a concentration of 0.5% daily for 35 days.

- The eighth group: It represents the control group, and it was dosed orally with tap water for 35 days.

Study and biochemical blood parameters

Using the examination kit supplied by the Chinese company (BT LAB) Bioassay Technology Laboratory.

Results and Discussions

Table (1) indicated that there was a significant decrease in the concentration of glutathione GPX when treated with hydrogen peroxide H_2O_2 (G7), which amounted to $43.69 \pm 4.1 \mu\text{mol} / \text{liter}$ (which represents the measurement of the concentration level at the level of glutathione GPX 450 ml / kg and 1 ml / kg of hydrogen peroxide (G3) reaching $64.77 \pm 1.3 \mu\text{mol/L}$ in the presence of Significant differences for treatment (G6) when dosing with 0.6 mg/kg with nano silver + 1 mm/kg hydrogen peroxide amounted to $57.32 \pm 2.6 \mu\text{mol/L}$ compared with treatment (G7) of $43.69 \pm 4.1 \mu\text{mol} / \text{L}$. Table (1) also shows a significant increase in the level of the concentration of Malone Dialdehyde (MAD) when treated with hydrogen peroxide H_2O_2 (G7), which amounted to $0.757 \pm 0.021 \mu\text{mol/L}$ (which represents the highest concentration of MAD levels) compared to the control treatment (G8), which amounted to $0.603 \pm 0.017 \mu\text{mol/L}$, while its concentration decreased significantly when treated with orange peel extract at a concentration of 450 mg/kg and 1 ml/kg of hydrogen peroxide (G3). $0.390 \pm 0.056 \mu\text{mol/L}$ (representing the lowest concentration of MAD levels) compared to the treatment that was dosed only with 1 ml/kg of hydrogen peroxide (G7).), and the table shows that there were significant differences for treatment (G6) when dosed with 0.6 mg / kg with nano silver + 1 mm / kg hydrogen peroxide, which amounted to $0.501 \pm 0.028 \mu\text{mol} / \text{L}$ compared to parameter (G7) of $0.757 \pm 0.021 \mu\text{mol/L}$. Table (1) shows a significant increase in the level of LPO lipid peroxide concentration when treated with hydrogen peroxide H_2O_2 (G7) as it reached $7.13 \pm 0.22 \mu\text{mol/L}$ (which The highest concentration of lipid peroxide levels (LPO) compared to the control treatment (G8) was $7.13 \pm 0.22 \mu\text{mol/L}$, while its concentration decreased significantly when treated with orange peel extract at a concentration of 450 mg/kg. And 1 ml/kg of hydrogen peroxide (G3) was $0.27 \pm 3.95 \mu\text{mol/L}$ (representing the lowest concentration of LPO levels) compared to the treatment that was dosed only with 1 ml/kg of hydrogen peroxide (G7), and the table shows that there were significant differences for treatment (G6) when dosed with 0.6 mg/kg with nano silver + 1 mm/kg hydrogen peroxide, which amounted to $0.22 \pm 5.79 \mu\text{mol/L}$ compared to the treatment (G7). G7) of $0.22 \pm 7.13 \mu\text{mol/L}$. Glutathione oxidized GPX and markers of oxidative stress (MDA and LPO) have a direct relationship (Akinmoladun *et al.*, 2017). Kamal and others (2013), as they noticed a significant difference in the antioxidant activities when they studied the volatile oils extracted from three species of the genus Citrus, namely C. sinensis and C. paradisi and C. reticulata, and it also agrees with the findings of Al-Snafi (2016) and with the findings of Zou *et al.* (2016), explaining the high oxidized glutathione (GPX) and the decrease in both malondialdehyde (MDA). and LPO lipid peroxidase for treatments dosed with orange peel extract because it contains a large percentage of the active compound D-Limonene (90.41%), as mentioned by Tao *et al.* (2019) and Sarjorno *et al.* (2019). The active compound, D-Limonene, has the ability to break down and prevent the formation of reactive oxygen species (ROS), such as hydrogen peroxide, H_2O_2 .

Table (1): Effect of treatment with orange peel extract type *C. sinensis* L. in the levels of oxidized glutathione GPX and markers of oxidative stress (MDA and LPO) in the serum of male rats

NO	Dose concentrations	Arithmetic mean of the coefficients + standard error		
		GPX ($\mu\text{mol/L}$)	MAD ($\mu\text{mol/L}$)	LPO ($\mu\text{mol/L}$)
G1	150 mg/kg orange peel extract +1 ml/kg H ₂ O ₂	51.87 \pm 4.2	0.626 \pm 0.019	5.72 \pm 0.22
G2	300 mg/kg orange peel extract +1 ml/kg H ₂ O ₂	58.06 \pm 0.9	0.496 \pm 0.048	5.33 \pm 0.32
G3	450 mg/kg orange peel extract +1 ml/kg H ₂ O ₂	64.77 \pm 1.3	0.390 \pm 0.056	3.95 \pm 0.27
G4	0.1 mg/kg Silver nanoparticles +1ml/kg of H ₂ O ₂	51.26 \pm 2.7	0.506 \pm 0.029	5.40 \pm 0.22
G5	0.3 mg/kg Silver nanoparticles +1ml/kg of H ₂ O ₂	50.59 \pm 1.6	0.546 \pm 0.021	5.55 \pm 0.22
G6	0.6 mg/kg Silver nanoparticles +1ml/kg of H ₂ O ₂	57.32 \pm 2.6	0.501 \pm 0.028	5.79 \pm 0.22
G7	1ml/kg of H ₂ O ₂	43.69 \pm 4.1	0.757 \pm 0.021	7.13 \pm 0.22
G8	Control (tap water)	69.56 \pm 2.5	0.603 \pm 0.017	6.25 \pm 0.22
LSD 0.05		6.21	0.083	0.60

Conclusions

The results proved that the dosing of the experimental animals with the active compound D-Limonene at a concentration of 450 mg/kg led to a significant increase in the concentration of oxidized glutathione GPX and a significant decrease in the concentration of each of Malone Didehyde (MAD) and lipid peroxide. The results proved that the dosing of experimental animals with biosynthetic silver nanoparticles from the aqueous extract of orange peels at a concentration of 0.6 mg/kg led to a significant increase in the concentration of oxidized glutathione GPX and a significant decrease in the concentration of each of Malone Dialdehyde (MAD) and peroxide fat LPO .

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