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Phytochemical and Protective Study for The Petroselinum Sativum L. (Parsley) on The Oxidative Stress and Antioxidant in Rats

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ABSTRACT

Background: This search was carried to investigate the phytochemical of *Petroselinum sativum* L.parsley and the antioxidant effect of parsley volatile oils leaves extract on oxidative stress [MDA (Malondialdehyde enzyme), LPO (lipid peroxidation enzyme) level and antioxidant glutathione enzyme (GSH)] in adult male albino rats.

Objective: This research work aimed to study the antioxidant effect of volatile oils of parsley leaves extract against environment-induced free radicals oxidative stress in male albino rats.

Methods: 30 male adult rats were used. Rats were divided into five Groups: G1 (Control group) received only 0.9% normal saline, G2 received *P.sativum* L volatile oils extracts 50 Mg/ Kg B.W, G3- received *P.sativum* L volatile oils of extracts 100 mg/kg B.W, G4- received *P.sativum* L volatile oils of extracts 200 mg/kg B.W, G5- received *P.sativum* L volatile oils extracts 250 mg/kg B.W volatile oils of extracts. The extract was injected (i.p.) in some rats for 20 days and 30 days and 60 days. Then blood samples were collected for measuring MDA, LPO and GSH levels in experimental groups and compered with control.

Results: Results indicated that MDA and LPO levels were significantly decreased at concentrations of 50, 100, 200 and 250 mg/kg B.W., while GSH levels significantly increased at the same concentrations compared to the control group.

Conclusions: There was a dose-dependent marked decrease in the levels of MDA and LPO and a dose-dependent marked increase glutathione in male rats treated with parsley extract.

Keywords: Petroselinum sativum L, Phytochemical, Oxidative stress, Antioxidant.

INTRODUCTION

Herbalists utilize different kinds of plants called medicinal plants (alternative plants). These plants are thought to be a rich source of compounds with secondary metabolisms that are active and can be used in the creation and development of medications [1]. An active secondary metabolite is any substance that can be found in plants, including polysaccharides, flavonoids, terpenoids, and phenol [2].

Volatile oils (essential oils) can be extract from different parts of *Petroselinum sativum* as a leaves, seeds and flowers, of the same plant with different percentages [3]. The Mediterranean region of Europe is where *P. sativum L.,* sometimes known as parsley, first appeared. Its leaves are tripinnate and oblong, and its roots range in thickness from thin to thick, fusiform tuberous [4]. *P. sativum* L. name commons (parsley) is a species of Umbellifera family, which plays an important role in the food, cosmetic, and pharmaceutical industries as well as in importing goods [5].

P. sativum L. seeds contains 2-8% volatile oils and the major constituents are alpha-pinene, apiol, myristicin and tetramethoxyally. Additionally, it contains 13–22% fixed oils, with petroselinic acid making up the majority of the composites. Linoleic, palmitic, myristic, myristolic, oleic, stearic, and 7-octadecenoic acids are present in minor levels [6]. Parsley contain the the components of vitamin B12, vitamin C, beta carotene, and fatty acids [7]. Additionally, parsley contains several phytochemical compounds including myristicin, apiole and protein [8].

Free radicals or oxidative stress are results of regular cellular metabolism, but there are a number of circumstances that might upset the equilibrium between the cellular defense system and reactive oxygen species (ROS) generation [9, 10].

By defending against cellular free radicals, repairing oxidative stress damage, and preventing its accumulation, antioxidant defense mechanisms can reduce the damage caused by oxidative stress. Free radicals and ROS cause cellular damage, and antioxidant enzymes serve a critical protective function in preventing this damage [11].

Under typical circumstances, ROS causes lipid, DNA, and protein oxidation [12]. Under the majority of oxidative stress conditions, MDA is recognized as a sign of peroxidation damage to cells. Lipid peroxidation is caused by the interaction of membrane lipids with ROS and free radicals [13]. The antioxidant system serves as the body's first line of defense against free radical damage and is crucial for wellbeing and maximum health [14].

Antioxidant is classified into two major groups:

1- Enzymatic antioxidant

2- Non-enzymatic antioxidant.

The body's first line of defense against oxidative stress and ROS is made up of the enzymatic antioxidants superoxide dismutase enzyme (SOD) and catalase enzyme (CAT), peroxidase, and glutathione. Non-enzymatic antioxidants include vitamins C, E, A, and K, flavonoids, alkaloids, and minerals among others [15].

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MATERIALS AND METHODS

1- Plant: Parsley from the city of Karbala. Iraq at the floral stage on October 3, 2022. The plant was air dried, ground into a powder using a mechanical grinder, and used immediately [16].

2- Soxhlet Extraction: 60 gram of parsley a thimble, along with 160 ml of 70% hexane in flask for 22 hours. A rotary evaporator was then used to evaporate the extract at 45^0C [17, 18].

The law in equation is used to estimate the percentage. No (1) [19, 20].

$$\text{Yield (wt.\%)} = \frac{\text{Weight of Oil produced}}{\text{Weight of Seed powder used}} \times 100\%$$

3: Screening study for secondary m

3-1: Saponins: The presence of saponins was revealed by the long-lasting foam that formed when the aqueous solution of oil extract was stirred in a test tube [21, 22].

3-2: Phenols: Perform a lead acetate test after adding 0.5 ml of 1% lead acetate solution to 5 mg of parsley oil extract, precipitate was produced.

3-3: Glycosides: Aqueous NaOH solution was added after 0.5 mg of parsley oils extract had been dissolved in 1 ml of water, producing the color yellow.

3-4: Tannins: 5ml of oil extract were cooked at 80-100 °C for 10 minutes in a water bath device after being mixed with distilled water. After filtering, 5 drops of 1% ferric chloride were added to the mixture to give it a dark green color [23].

3-5: Alkaloids: Wagner reagent, a solution of potassium iodide and iodine, was combined with the extracted filtrate to produce a reddish-brown precipitate [24].

3-6: Flavonoids: 1.5ml of 50% methanol was added to 4 mL of extract. 6 drops of concentrated HCl were added after mixing, and the resulting mixture was heated with magnesium metal till red color was produced. Red colors have flavonoids in them [25].

4- Experimental design:

30 male adult rats, weighing 174-312 GM were utilized in this investigation. They were kept in an animal house at a temperature of 25 ºC, in a relatively controlled environment, and fed. Rats were divided into five groups (G).

- G1: The control group received only 0.9% normal saline.
- G2: received 50 mg/kg (B.W) of *P.sativum* L volatile oils extracts.
- G3- injection 100 mg/kg B.W *P.sativum* L volatile oils of extracts.
- G4- injection 200 mg/kg B.W *P.sativum* L volatile oils of extracts.
- G5- injection 250 mg/kg B.W volatile oil of extracts.

They were injected 20 days and 30 days and 60 days.

5: Oxidant Levels Analysis

5-1: Malondialdehyde (MDA) µmol/ L:

Principle: The concept of this procedure was based on the reaction of Thiobarbituric acid (TBA) with Malondialdehyde (MDA) [26].

5-2: Lipid Peroxidation Analysis (µmol/ L): Egg-yolk homogenates were utilized as a lipid-rich medium, and the formation of lipid peroxide was assessed using a modified Thiobarbituric- reactive type's test [27].

5-3: Glutathione (GSH) Assay (µmol/ L).

Tris Buffer Solution:

To create a concentration of 50 mmol, 900 ml of distill water was mixed with tris hydroxyl methyl aminoethan. To create 1 L of distill water, 0.0292 g of ethylenediaminetetraacetic acid (EDTA) was added. The final step was to introduce an acidic substrate. The solution was kept in the fridge until use [28].

Ethical consent: Approval was obtained from Clinical Laboratories Department, Applied Medical Science College, Karbala University.

Statistical analysis

Data were expressed as mean ± SD and were tested for statistical significance using one way analysis of variance (ANOVA). P values of 0.05 and less was considered significant. LSD was performed by using SPSS for windows version SPSS, Inc., Chicago, Illinois.

RESULTS

1- Extracted volatile oil percentage: The percentage of oils for parsley gave the volatile oil extract's height percentage (2.33%), according to table (1).

Table (1): Extracted volatile oil percentage

extracted	Extracted volatile oil percentage
parsley*s*	2.8/120g *100 = 2. 33%

2- Chemical compounds screen: The active secondary metabolism molecules in the parsley extract were identified using the results of the chemical compounds screen investigation. Table (2) showed that while saponin and tannins had negative results, phenol, glycoside, alkaloid, and flavonoid had favorable results.

Table (2): Secondary metabolism screen of *parsley* oils

Chemical Compounds	extract
Saponin	-
Glycoside	+
Phenol	+
Tannins	-
Flavonoid	+
Alkaloid	+

3- Stress Oxidant Analysis

In table (3), the MDA enzyme of groups G2, G3, G4 and G5 of male rats showed a significant decrease (1.89, 2.19, 1.67 and 1.11µmol/l respectively) compared to control (2.27 µmol/l) at the 20th day, while at the 30th, G2, G3, G4 and G5 showed a significant decrease (2.06, 2.01, 1.49 and 1.29 µmol/l respectively) compared to control (2.31µmol/l.).

Table (3): MDA enzyme percentage in rat groups treated with the study concentrations

Day	Groups	Mean
20	**Control**	$\mathbf{2.27 \pm 0.19}$
	G2	$\mathbf{1.89 \pm 0.26}$
	G3	$\mathbf{2.19 \pm 0.16}$
	G4	$\mathbf{1.67 \pm 0.29}$
	G5	$\mathbf{1.11 \pm 0.11}$
Total		**1.83±0.2**
30	**Control**	**2.31±0.17**
	G2	**2.06±0.24**
	G3	**2.01±0.17**
	G4	**1.49±0.24**
	G5	**1.29±0.21**
Total		**1.42±0.21**
LSD		0.17

In table (4), LPO enzyme of groups G2, G3, G4 and G5 showed a significant decrease (19.71, 15.20, 12.66 and 12.00 µmol/l respectively) compared to control (22.29 µmol/l) at the 20th day. At the 30th day G2, G3, G4 and G5 showed a significant decrease (20.29, 16.81, 16.17and 14.58 µmol/l respectively) compared to control (22.29 µmol/l).

Table (4): LPO enzyme percentage in rat groups treated with the study concentrations

Day	Groups	Mean
20	**Control**	**22.29±7.41**
	G2	**19.71±3.73**
	G3	**15.20±3.22**
	G4	**12.66±7.39**
	G5	**12.00±4.34**
Total		**16.37±5.21**
30	**Control**	**22.29±7.41**
	G2	**20.29±7.23**
	G3	**16.81±5.89**
	G4	**16.17±2.19**
	G5	**14.58±5.52**
Total		**18.03±5.65**
LSD		5.12

In table (5), GSH level of groups G2, G3, G4 and G5 illustrated significant increases (2.75, 2.92, 3.34 and 3.87 µmol/l respectively) compared to control (2.29 µmol/l) at the 20th day , while at the 30th day G2,G3,G4 and G5 show significant increases (2.33, 2.79, 3.01 and 3.99µmol/l respectively) compared to control (1.47µmol/l).

Table (5): GSH enzyme percentage in rat groups treated with the study concentrations

Day	Groups	Mean
20	**Control**	**2.29±7.41**
	G2	**2.75±0.79**
	G3	**2.92±1.39**
	G4	**3.34±1.19**
	G5	**3.87±0.98**
Total		**3.03±2.35**
30	**Control**	**1.47±0.90**
	G2	**2.33±0.98**
	G3	**2.79±0.91**
	G4	**3.01±0.62**
	G5	**3.99±0.62**
Total		**2.72±0.81**
LSD		0.79

DISCUSSION

Family Umbelliferae has a variety of volatile oils components, which are natural product in plants [29].

The present study showed that volatile oils are impartment good rich source for secondary metabolism active compounds that have antioxidant activities that may be due to the presence of highly active essential oils that scavenge free radicals, peroxides and triplet oxygen [30]. Flavonoid compounds, to which apigenin belonged, are regarded as excellent free radicals scavenging agents [31], and inhibitors of LPO [32, 33]. The observed increase in glutathione enzyme concentration after flavonoids (apigenin) administration suggests that they have protective mechanism in response to Cadmium-induced free radicals generation and also indicated that flavonoid may be associated with decreased free radicals mediated tissue injury. The depression in MDA enzyme supports this conclusion of preventing oxidative stress damage due to ROS and suppress their formation. The antioxidant role of crude parsley extract, or its flavonoid apigenin and their free radicals scavenging activities were also confirmed by many authors. By scavenging the free radical activity of the free radicals oxidative stress, the active chemicals operate as a rich source of antioxidants that can be employed to reduce biological oxidative stress and prevent cellular damage [34], a cellular or extracellular antioxidant action that inhibits the activity of xanthine oxide, which converts its product to xanthine dehydrogenase [35].

CONCLUSION

Treatment of male rats with parsley extract decreased the levels of MDA and LPO and increased glutathione level.

RECOMMENDATIONS

Development of new antioxidants from alkaloid and flavonoid which can be used against the oxidative stress. Separation of alkaloid and flavonoid and test the secondary metabolism compounds by gas chromatography GC/MS technique.

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