The protective effect of the aqueous extract of parsley (Petroselinum sativum) seeds on experimentally – induced oxidative stress in rats

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Summary

In this study, the potential protective effect of aqueous extract of parsley (Petroselinum sativum) seeds against hydrogen peroxide (H_2O_2) – induced oxidative stress in male rats was assessed. Three groups of male albino rats were randomly divided (n=7) and were handled for twenty-eight days as follows: rats in group I served as control; animals in group II were provided with drinking water containing 0.5% H_2O_2 and those in group III received orally 8 mg/100 gm B.W. of aqueous extract of parsley seeds plus 0.5% H_2O_2 in drinking water. After four weeks experimental period, a significant increase in lipid peroxidation products (MDA), and decrease in glutathione (GSH) concentrations were observed in plasma, kidney, liver and heart tissues of H_2O_2 treated animals as compared with the control group. These biomarkers (GSH and MDA) are interrelated and indicate the occurrence of oxidative stress. Plasma total cholesterol (TC) concentration was significantly

increased in H2O2 treated rats. By administration of aqueous extract of parsley along with H_2O_2, plasma and tissue GSH levels were significantly increased while the elevation in MDA level was diminished in plasma and different tissues examined. A decrease in plasma cholesterol concentration was recorded in H_2O_2 and parsley treated group as compared with the control one and H_2O_2 treated groups. These results indicate that aqueous extract of parsley have hypocholesterolemic and antioxidant effect.

Introduction

Maintenance of normal cell functions in the presence of oxygen largely depends on the efficacy of the tissue protection against free radical (FR)-mediated oxidative stress. FRs or other reactive oxygen species (ROS), like hydroxyl radical (OH), super oxide anion (O_2), and hydrogen peroxide (H_2O_2), are continuously produced during the normal cellular metabolism (e.g. mitochondrial respiration). Other in vivo sources of ROS are phagocytic and bactericidal activities of polymorph nuclear leukocyte and alveolar macrophages (1,2). They are also produce upon exposure to external stimuli like cigarette smoking, ionization, and air pollutants such as ozone (3,4).

Over production of ROS cause oxidative damage to biological molecules, especially DNA, lipids, proteins, and

basement membranes and leads to cell and organ dysfunction (5,6).

Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have directed most of the attention on the naturally occurring antioxidant like vitamins E,C and flavonoids, which are present in many fruits and vegetables (7). Currently, there are accumulating evidence from studies showing relationship between the intake of some medicinal plants like, Olive (Olea europaea) leaves and Trichonella foenum graceum and the treatment of many FR mediated disease (8,9).

Parsley (Petroselinum sativum) belonging to the umberlliferae family is a well-known spice and vegetable. The characteristic constituents are: flavonoids (apigenin), essential oil (apiol), cumarines and vitamin C (10,11). Most of the studies focused on the effect of parsley on kidney, blood and digestion (12,13), non questioned the antioxidant effect of the plant. To this aim this paper is dedicated.

Materials and Methods

Experimental design:

Three to four months old male rats were randomly divided into three groups each of seven. Group one received regular pellets diet and served as control group; group two were

subjected to ad libitum supply of drinking water containing 0.5% H_2O_2; group three received 0.5% H_2O_2 in drinking water and 8 mg/100 gm B.W. of aqueous extract of parsley which was given daily by oral intubation. Blood samples were drawn prior to administration of H_2O_2 or parsley and at the end of twenty-eight days experimental period. Plasma samples were analyzed for measuring TC, GSH, and MDA concentrations. Pieces of kidney, liver and heart were removed for estimation of MDA and GSH levels as indexes of lipid peroxidation and thereafter, as biomarkers for oxidative stress (14).

Biochemical analysis:

All blood specimens were collected (by heart puncture)after overnight fasting for 12-14 hours. Plasma TC concentration was determined by using standard enzymatic assay (Biomeriux Vitek, Inc. USA). Whereas, glutathion and MDA level in the plasma and tissue were measured following the procedure described earlier (15,16). Aqueous extract of parsley seeds was prepared as described by Harborn, et al., (17).

Differences between experimental groups were evaluated using either one-way or two-way analysis of variance (ANOVA)(18). For all analysis, a P value of < 0.01 was considered to be significant.

Results

The effects of the aqueous extract of parsley seeds and 0.5% H_2O_2 on plasma TC and the biomarkers of oxidative status (GSH and MDA) in tissue and plasma are shown in tables 1,2 and 3.

Tissues GSH concentration:

Exposure of animals to 0.5% H2O2 in drinking water for twenty-eight days led to significant decrease ($P<0.01$) in GSH level (µmol/gm wet tissue) of liver, kidney and heart relative to untreated (control) rats (Table-1). The table also reveals that oral intubations of the aqueous extract of parsley in combination with H_2O_2, significantly increased ($P<0.01$) hepatic and renal GSH concentrations of treated animals as compared with H_2O_2 treated rats. Moreover, a tendency toward increase in cardiac GSH concentration appeared in parsley plus H_2O_2 treated animals when compared with H_2O_2 treated one. Yet, the differences were statistically non significant ($P>0.01$) (Table-1).

Tissues MDA concentration:

The present study demonstrated that rats administered 0.5% H_2O_2 for four weeks showed significant increase ($P<0.01$) in hepatic, renal and cardiac MDA concentrations (nmol/gm wet tissue) as compared with control animals (Table-2). The results also indicated a significant decrease in MDA concentrations of hepatic and renal tissue in animals received of the aqueous extract of parsley seeds in addition to H_2O_2 relative to H_2O_2-

treated rats. On the other hand, oral intubations of parsley normalized cardiac MDA content of treated rats.

Plasma TC, GSH and MDA concentrations:

While there were no significant differences ($P>0.01$) in plasma TC,GSH and MDA concentrations between groups in the pretreated period, intervention of parsley along with H_2O_2 to male rats for twenty eight days significantly increase ($P<0.01$) plasma GSH concentration and decrease significantly ($P<0.01$) plasma TC and MDA concentrations as compared with H_2O_2 treated groups (Table-3). Besides, parsley intervention normalizes plasma GSH concentration. Within the time plasma TC and MDA levels showed significant increment ($P<0.01$), while GSH concentration showed significant decrement ($P<0.01$) following intervention of H_2O_2 alone as compared with pretreated period.

Table (1): Effect of 0.5% H_2O_2, oral intubations of aqueous extract of parsley for four weeks on tissue glutathione (GSH) concentration of male rats.
(Mean values with their standard error for seven rats per group)

Groups	GSH (µmol/gm wet tissue)		
	Kidney	Liver	Heart
Control	a 2.70 ± 0.06	A 5.73 ± 0.06	a 1.55 ± 0.02
H_2O_2 treated	b 2.14 ± 0.04	B 4.61 ± 0.09	b 1.27 ± 0.05
H_2O_2+ parsley treated *	c 2.44 ± 0.06	C 4.96 ± 0.08	b 1.39 ± 0.03

*Animals received 0.5% H_2O_2 plus 8.0mg/100gm B.W. of aqueous extract of parsley.

[a,b,c] Mean values within a column with unlike superscript letters were significantly different: $P<0.01$.

Table (2): Effect of 0.5% H_2O_2, oral intubations of aqueous extract of parsley for four weeks on tissue malondialdehyde (MDA) concentration of male rats.

(Mean values with their standard error for seven rats per group)

Groups	MDA (nmol/gm wet tissue)		
	Kidney	Liver	Heart
Control	a 275.40 ± 8.02	a 240.0 ± 6.53	a 481.61 ± 9.05
H_2O_2 treated	b 511.0 ± 9.60	b 314.20 ± 7.11	b 628.31 ± 11.17
H_2O_2+ parsley treated *	c 358.76 ± 6.52	c 288.41 ± 7.54	a 507.15 ± 7.26

*Animals received 0.5% H_2O_2 plus 8.0mg/100gm B.W. of aqueous extract of parsley.

^{a,b,c}, Mean values within a column with unlike superscript letters were significantly different: $P<0.01$.

Table (3): Effect of 0.5% H_2O_2, oral intubations of aqueous of parsley for four weeks on plasma total cholesterol (TC)(mg/dl),reduced glutathione (GSH) (µmol/gm weight tissue), and malondialdehyde (MDA) (nmol/gm weight tissue) concentrations of male rats.

(Mean values with their standard error for seven rats per group)

Groups	Pre - treatment			Post - treatment		
	GSH	MDA	TC	GSH	MDA	TC
Control	A a 7.85 ± 0.07	A a 3.10 ± 0.03	A a 98.10 ± 1.76	A a 7.66 ± 0.08	A a 3.22 ± 0.04	A a 106.0 ± 1.93
H_2O_2 treated	A a 7.90 ± 0.09	A a 2.96 ± 0.05	A a 102.6 0 ± 1.83	B b 6.18 ± 0.05	B b 4.90 ± 0.07	B b 141.52 ± 2.18
H_2O_2 +parsley treated *	A a 8.0 ± 0.05	A a 3.0 ± 0.05	A a 100.75 ± 2.01	A a 7.42 ± 0.04	B c 4.06 ± 0.04	B c 119.35 ± 2.0

*Animals received 0.5% H_2O_2 plus 8.0mg/100gm B.W. of aqueous extract of parsley.

^{a,b,c}, Mean values within a column with unlike wuperscripte letters were significantly different : $P<0.01$.

^{A,B,C} Mean values within group with unlike superscript letters were significantly different : $P<0.01$.

Discussion

Administration of 0.5% of H_2O_2 in drinking water increased tissue oxidative stress as reflected by increase lipid peroxidation products (MDA) and decrease GSH concentrations of plasma, kidney, liver and heart tissues. It has been previously reported that endogenous challenge with H_2O_2 induced a case of oxidative stress manifested by decrease in the antioxidant status of the body (decrease in GSH) and increase in MDA level in different tissues in chicken (19) and rats (8). Besides, both erythrocyte and plasma of myocardial infarction patients demonstrated an intensified response toward in vitro challenge with H_2O_2 manifested by an increase in plasma MDA production and decrease in plasma GSH levels (20).

Oxidative stress induced by H_2O_2 in this study may be due to the state of hyperoxia that occur after H_2O_2 administration, followed by an increase in the production of ROS including H_2O_2 which accompanied the increase of oxidative metabolism of endogenous cholesterol (21). Moreover, the increase in MDA level, may be due to an increase in the production of FRs more than the ability of the scavenging system to remove them, due to high level of endogenous H_2O_2, leading to a defect in the antioxidant defense system with subsequent increase in the sensitivity of these tissues to oxidative stress and lipid

peroxidation (22). Recent evidence indicated that mechanisms of oxidative stress mediated by H_2O_2 injury may involve the induction of gene expression which is regulated by nuclear transcription factor-KB(NF-KB), an oxidative stress responsive transcription factors (23). These genes are found to be activated by H_2O_2 leading to higher depletion of intracellular GSH and a marked increase in oxidative stress (24).

This study also demonstrated that administration of H_2O_2 with water lead to significant increase in plasma TC concentration. In previous studies by Al-Kennany (19) and Khudiar (8), oral administration of H_2O_2 in water alone or in combination with atherogenic diet induced hypercholesterolemia and aortic lesion in chicken and rats respectively. Such studies suggested that alteration in lipid metabolism [increase in very low density lipoprotein (VLDL) and low density lipoprotein (LDL)] is the most important possible factors responsible for the hypercholesterolemic effect of H_2O_2.

The effect of aqueous extract of parsley on biomarkers of oxidative stress was studied in this experiment in order to demonstrate any antioxidant in vivo characteristic of parsley. The results showed that oral intubations of parsley along with H_2O_2 supplementation to male rats for four weeks, exerted hypochlesterolemic and antioxidant effects manifested by significant decrease in cholesterol and increase in GSH level of plasma renal, hepatic and cardiac tissues. The MDA levels of

plasma and previous tissues were significantly decreased followed parsley treatment in comparison with untreated rats. The hypolipidemic effect of parsley was reported for the first time in our previous experiment [unpublished data (25)] where such antihypercholesterolemic characteristic was suggested to be due to upergulation of lipoprotein receptrors manifested by decrease in plasma VLDL-cholesterol and LDL-cholesterol concentration, and suppression of hepatic cholesterol concentration. Elevation of plasma high density lipoprotein cholesterol concentration was suggested to be another possible pathway.

The present study showed that intervention of parsley with H_2O_2 to male rats prevented accumulation of MDA and increased the level of GSH in plasma and different tissues examined. Intracellular and extracellular concentration of antioxidant is important in protection against oxidative stress (26). Several reports have indicated that consumption of dietary flavonoids enhanced the level of antioxidant activity and inhibit lipid peroxidation by scavenging FRs and / or by chelating metal ions (27). Besides, vitamin C is considered as an important water soluble antioxidants in the body and one of the natural antioxidants which prevent lipid peroxidation (5). The parsley used in this study contains flavonoids (28) and vitamin C (11), which might have contributed to the protective effect of parsley against oxidative stress observed here. However, further

studies will be necessary to clarify this statement. Moreover, it has been mentioned that hypecholesterolemia causes depletion of GSH level (29), accordingly, the hypocholesterolemic effect of parsley observed in this study may contribute, to the increase in GSH level and annihilation of oxidative stress.

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التأثير الواقي للمستخلص المائي لبذور المعدنوس على الإجهاد التأكسدي المحدث تجريبياً في الجرذان

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الخلاصـــــة

صممت هذه الدراسة لمعرفة التأثير الوقائي للمستخلص المائي لبذور المعدنوس في حالة الإجهاد التأكسدي المحدث في ذكور الجرذان باستخدام بيروكسيد الـهيدروجين . تـم اسـتخدام ثلاث مجاميع مـن ذكـور الجـرذان التـي قسـمت عشـوائيا (بمعـدل 7 جـرذان لكـل مجموعـة) وعوملت لمدة 28 يوما كالآتي: المجموعة الأولى (سيطرة) أعطيت الماء العادي ، المجموعة الثانية أعطيت الماء الحاوي على 0.5% بيروكسيد الهيدروجين والثالثة جرعـت عـن طريـق الفم يوميا بالمستخلص المائي لبذور المعدنوس (8 ملغم/ 100غم من وزن الجسم) بالإضافـة إلى إعطائها الماء الحاوي على 0.5% بيروكسيد الهيدروجين .

تشير النتائج إلى حدوث زيادة معنوية في ناتج بيروكسدة الدهن (المالوندالديـهايد MDA) وانخفاض معنوي في تركيز الكلوتاثايون في البلازما وأنسجة الكلية ، الكبد والقلب بعد أربعـة أسابيع من معاملة الحيوانات بماء الشرب الحاوي على 0.5% بيروكسيد الهيدروجين مقارنـة مـع حيوانـات السـيطرة . إن هذيـن المعيـارين (الكلوتاثـايون والمالوندايالديـهايد) متداخليـن ويشيران إلى حدوث الإجهاد التأكسدي . كما سـجل تركـيز الكولستيرول الكلـي فـي البلازمـا ارتفاعاً معنويا في الحيوانات المعاملة ببيروكسيد الهيدروجين . لقد كـان لإعطـاء المسـتخلص المائي لبذور المعدنوس مع بيروكسيد الهيدروجين تأثيرا وقائيا ضـد حالـة الاجـهاد التأكسـدي تمثلت بارتفاع في مستوى الكلوتاثـايون وانخفـاض فـي مسـتوى المالوندالديـهايد فـي البلازمـا ومختلف الأنسجة المدروسة . بالإضافة إلى ذلك فقد لوحظ انخفاضا في مستوى الكولسـتيرول الكلي في البلازما في المجموعة المعاملة ببذور المعدنوس وبيروكسيد الهيدروجين مقارنة مع مجموعة السيطرة والمجموعة المعطاة بيروكسيد الهيدروجين لوحده . يسـتدل مـن نتـائج هـذه الدراسة إلى التأثير الخافض للدهن والتأثير المانع للأكسدة للمستخلص المائي لبذور المعدنوس