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Effect Of Parsley On Weight Reduction And Antioxidant Enzymes In Overweight And Obese Women Subjects

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Abstract: The aim of this study was to evaluate effect of parsley on weight loss and antioxidant enzymes in overweight and obese women subjects. A number of 150 women subjects were classified as follow: group (1) 30 normal volunteers women with BMI<25 Kg/m^2 was considered as a control group, group (2) 30 overweight women with BMI between 25-30 Kg/m^2 eating one cup/ day parsley in addition their weight reduction diet, group (3) 30 obese women with BMI>30 Kg/m^2 eating one cup/day parsley in addition their weight reduction diet, group (4) 30 overweight women with BMI between 25-30 Kg/m^2 eating only their weight reduction diet, group (5) 30 obese women with BMI>30 Kg/m^2 eating only their weight reduction diet. The weight reduction diet is containing the same types and quantities for all participants in groups 2, 3, 4 and 5 with exchange during the week days along the study period. Age (35-60 years) with no prior history of chronic diseases was selected from outpatient clinics in Assiut University Hospitals after their agreement for the participation in the study. Information on age, weight, height was collected. Antioxidant enzymes including superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPX) and catalase (CAT) were assessed in the groups at the beginning of the study and after 2 months of intervention. The investigation was approved by the ethics committee at Medicine Faculty, Assuit University, Assuit, Egypt. Results showed that the high content from

protein, total carbohydrates, fiber, vitamins (A, C and E) and minerals (K, Na, Ca, Fe, Mn and Zn) in parsley. A significant ($P \leq 0.05$) difference was observed between the weight and TSF in groups 4 and 5 of the participants before and after the study period except BMI. While the high significant ($P \leq 0.01$) increasing was observed of the participants before and after intervention in weight and triceps skin fold (TSF) only. The activity of SOD was increased in the all groups after study period when compared with before ($P \leq 0.01$) in groups 2 and 3 and ($P \leq 0.05$) in groups 4 and 5 but still lower than the control group. The results showed a high significant decrease ($P \leq 0.0001$) in the GR, GPX and catalase activities in groups 2 and 3 after intervention except GR ($P \leq 0.05$) in group 3. In the present study there was a slight increase in groups 4 and 5. In conclusion, a weight loss by parsley besides weight reduction diet can reduce the weight and BMI as well as increasing the antioxidant enzymes activities in overweight and the obese subjects.
Keywords: parsley, nutritional value, anthropometric measurements, superoxide dismutase, glutathione peroxidase and catalase

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Introduction

Parsley (*Petroslimum crispum*) or garden parsley is a species of Petroselinum in the family *Apiaceae*. It can be used as a herb, spice, or as vegetable. It is also known for its use against obesity because it is low in calories and high in fiber (Rakhi *et al.,* 2012). It is a nutritious addition to a weight-loss diet. Parsley is rich with an antioxidant arsenal that includes luteolin, flavonoid that searches out and eradicates free radicals in the body that cause oxidative stress in cells (Nabila, 2012). It is widely used in Middle Eastern cooking. Green parsley is used frequently as a garnish on rice, potato, fried chicken, fish, lamb, steaks, and goose as well in meat or vegetable stews (including shrimp creole, beef bourguignon, goulash, or chicken paprikash dishes (Meyer *et al.,* 2006). Parsley is a source of flavonoid and antioxidants especially apigenin, folic acid, luteolin, vitamins (C, K, K, A and tocopherol) and minerals (Mg, Ca and K). Apigenin, one of the main flavonoids in parsley showed strong antioxidant effects, increasing the activities of antioxidant enzymes and in turn decreasing the oxidative damage to tissues (Kolarovic *et al.,* 2010).

Half a tablespoon (a gram) of dried parsley contains about 10.7 µg of α-carotene and 6.0 µg of lycopene as well as 82.9 µg of lutein+zeaxanthin and 80.7 µg of β-carotene (Jett, 2007; Nutritional Data, 2013 and Zhai *et al.,* 2015). Furthermore, two tablespoons of parsley contain 16% of the recommended daily allowance (RDA) of vitamin C and over 12% of the RDA of vitamin A which represent two powerful antioxidants. Parsley and parsley juice are a great source of natural β-carotene, chlorophyll, essential fatty acids, folic acid and iron (Nabila, 2012). These components of fresh parsley leaf scavenge superoxide anion *in vitro* and hydroxyl radical in addition to protecting against ascorbic acid-induced membrane oxidation, where lipid oxidation is a major cause of food quality deterioration. Supplementation of diets with fresh parsley leaf can increase antioxidant capacity of rat plasma and decrease oxidative stress in humans (Zhang *et al.,* 2006).

Obesity, which is defined as having a body mass index (BMI) of 30 kg/m^2 and above, has been on the increase in in both developed and developing countries in recent decades (Rakhi *et al.,* 2012 and DeBoer, 2013). It is widely accepted that both genetic and environmental factors (such as diet) can predispose individuals to the development of obesity (Pi-Sunyer, 2002). Obesity is recognized as a risk factor for insulin resistance, which can lead to major diseases, such as high blood pressure, arteriosclerosis, cardiovascular diseases and type 2 diabetes (Tataranni and Ravussin, 2002). Obesity pathogenesis is accompanied with the increase of oxygen derived free radicals (Zwirska *et al.,* 2003). Recent studies show that obesity results in oxidative stress even in the absence of other risk factors including cardiovascular diseases and therefore plays a role in development of the above-mentioned diseases (Speakman and Mitchell, 2011). Likewise, adipose tissue was found to be an independent factor to produce oxidative stress (Fernandez-Sanchez *et al.,* 2011). Researches on human have shown that an increase in weight can reduce the antioxidant capacity of plasma (Karaouzene *et al.,* 2011). Some studies have shown that antioxidant enzymes increase in obesity (Koboyasi *et al.,* 2010).

The role of oxidative stress and reactive oxygen species (ROS) in the pathophysiology of obesity has been recently the focus of many investigations (Vincent and Taylor, 2006; Atabek *et al.,* 2004; Vincent *et* *al.*, 2007). Oxidative stress occurs when redox homoeostasis within the cell is altered. The enzymes, glutathione reductase (GR), glutathione peroxidase (GPX) and catalase (CAT) act as important endogenous antioxidants enzymes which play a crucial role in neutralizing the deleterious effects of peroxides (Flora, 2007).

Even though a number of studies in the literature have shown the presence of oxidative stress in obese individuals from other ethnic groups, little information is available on the activity of GPX, GR and CAT in obese individuals. As oxidative stress can be modulated by nutritional, genetic and environmental factors (Flora, 2007). Therefore, the present study aimed to investigate the effect of parsley on weight reduction and antioxidant enzymes in overweight and obese women subjects.

Materials, Subjects and Methods

Materials

The fresh parsley (*Petroslimum crispum*) leaves were purchased from the local market in Assiut city, Assiut Governorate, Egypt. All chemicals, reagents and solvents were of analytical grade and purchased from Al-Gomhoryia Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Subjects

This study included 150 volunteers subjects in divided into 5 equally groups: group (1) 30 normal women with BMI <25 Kg/m^2 was considered as a control group, group (2) 30 overweight women with BMI between 25-30 Kg/m^2 eating one cup/day parsley in addition their weight reduction diet, group (3) 30 obese women with BMI >30 Kg/m^2 eating one cup/day parsley in addition their weight reduction diet, group (4) 30 overweight women with BMI between 25-30 Kg/m^2 eating only their weight reduction diet, group (5) 30 obese women with BMI >30 Kg/m^2 eating only their weight reduction diet, aged 35-60 years, with no prior history of chronic diseases were selected from outpatient who visited a diet therapy clinic in Assiut University Hospitals after their agreement for the participation in the study. Researcher advised participants in groups 2 and 3 that can be eaten or insert parsley in salads, fish, meat, chicken, potato and rice dishes. Information on age, weight, height, TSF was collected through questionnaires. Superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPX) and catalase (CAT) were assessed in the groups at the beginning of the study and after 2 months intervention. All subjects are apparently healthy and free of any disease that may alter the oxidants and antioxidants balance. The investigation was approved by the ethics committee at Medicine Faculty, Assuit University. The participant's weight was measured with digital scale (Seca GmbH & Co.KG, Germany) and height was measured with nonstretchable tape. BMI was calculated as weight in kilogram divided by squared height in meter. Triceps skin fold (TSF) was measured with a caliper with an accuracy of 0.1 ml. TSF, BMI and weight measurement were used to intensify that weight loss was achieved after the intervention.

Methods

Chemical Analysis of fresh parsley

Moisture, protein (Micro-Kjeldahl, TN x 6.25), Fat (hexane Soxhlet extractor), fiber and ash were determined according to the AOAC, (1995). The total carbohydrate was calculated by the difference. All vitamins (A, E and C) were extracted and analyzed by HPLC techniques as follow: Epler *et al*., (1993), Hung *et al*., (1980) and Moeslinger *et al*., (1994), respectively. Potassium and sodium were determined by flame photometer. The elements (calcium, iron, manganese, zinc, and phosphor) were determined using the ICP (Inductively Coupled Plasma Emission Spectrometer) (ICAP6200). These analyzes were determined in Central Laboratory for Chemical Analysis, Faculty of Agriculture, Assiut University.

Biochemical analysis of blood sample

In order to measure antioxidants enzymes activities, 10 mL fasting blood sample was drawn from the saphenous vein and collected in EDTA tubes before and after intervention. Plasma was collected from the rest of the sample by centrifuging at 5000 rpm for 5 minutes at 4°C. SOD enzyme activity was measured according to Ransod Kit method (Cat No: SD 125, Randox-Ransod, UK). In this method SOD activity was assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine (Lohe and Otting, 1984). GPX activity was measured according to Paglia and Valentine method with modifications according to Lawrence and Burk (Lawrence and Burk, 1976). GR activity was measured according to Sauberlich method (Sauberlich, 1999). CAT activity in erythrocytes was assessed by the method described by Hugo Aebi (Aebi, 1984). In this method, activity of CAT was determined by following the decomposition of H_2O_2 in phosphate buffer pH=7.2 spectrophotometrically at 230 nm.

Statistical analysis

Results were statistically analyzed by SPSS version 10 T-test was used to assess the significance of difference between the groups. All results are presented as mean ± SD and p. value of less than 0.05 was considered significant.

Results and Discussion

Nutritional value of fresh parsley

Data in Table (1) showed the nutritional value of fresh parsley. Each 100 gram of fresh parsley contained (0.79 g) fat, (3.10 g) protein, (5.33 g) total carbohydrates, (4 g) fiber, vitamin A (400 µg), vitamin C (125 mg), vitamin E (0.60 mg), potassium (450 mg), sodium (53 mg), calcium (120 mg), iron (5.5 mg), manganese (0.14 mg) and zinc (0.99 mg). The results are in agreement with results were obtained by USDA Nutrient Database (2013) which have reported the nutritional value of fresh parsley. Also, with Pattison *et al.*, (2004).

Table (1): Nutritional value of fresh parsley (per 100g)

Component	Value
Fat	0.79 ± 0.23 g
Protein	3.10 ± 0.54 g
Carbohydrates	5.33± 1.04 g
Fiber	4±1.09 g
Vitamin A	400± 22.76 µg
Vitamin C	125± 12.84 mg
Vitamin E	0.60± 0.12 mg
Potassium	450 ±32.98 mg
Sodium	53 ± 10.76 mg
Calcium	120 ± 15.94 mg
Iron	5.55 ± 1.04 mg
Manganese	0.14± 0.05 mg
Zinc	0.99 ± 0.11 mg
Phosphorus	42.65± 5.82 mg

Each value represents the mean of three replicates ±SD.

Age and anthropometric measurements of the study subjects before and after intervention

Age and anthropometric measurements of the study subjects before and after intervention are shown in Table (2). No statistical significant difference was observed between the ages of subjects in the all groups. There was a significant (P≤0.01) difference between the weight and TSF in groups 4 a nd5 of the participants before and after the study period except BMI. The high significant (P≤0.01) increase was observed only of the participants before and after intervention in weight and TSF. The results in groups 2 and 3 may be due to content of parsley a high proportion of fiber (See Table 1). This result is in agreement with Otles and Ozgoz, (2014) and Tucker and Thomas (2009) who mentioned that an inverse relationship between dietary fiber intake and change in body weight.

Table (2): Age and anthropometric measurements of the study subjects before and after intervention

		Age (yrs)	P. value between the groups	Weight (kg)	BMI (kg/m2)	(TSF) (cm)
Group (1) (normal)		45.5 ± 15.3	N.S	62.44± 8.42	22.44 ± 1.47	4.35 ± 0.15
Group (2) (overweight) +intervention	Before	44.3 ± 16.2		79.43±5.17	26.97 ±1.47	7.01 ± 0.15
	After			72.37±6.20	24.92 ±1.52	6.70 ± 0.17
	p.value			P < 0.01	P <0.05	P < 0.01
Group (3) (obese)+ intervention	Before	46.9 ± 14.6		90.58 ± 2.37	36.12 ± 0.91	5.95 ± 0.15
	After			78.36 ± 2.21	32.41 ± 0.81	2.99 ± 0.15
	p.value			P < 0.01	P <0.05	P < 0.01
Group (4) (overweight)	Before	43.9 ± 17.39		77.43 ± 6.23	27.68 ± 1.47	4.91± 0.16
	After			69.73 ± 5.42	21.77 ± 1.56	3.53± 0.14
	p.value			P < 0.05	P < 0.01	P < 0.05
Group (5) (obese)	Before	45.3 ± 14.2		89.58 ± 3.06	34.86 ± 3.53	5.82 ± 0.15
	After			80.36 ± 2.21	32.41 ± 0.81	3.94 ± 0.17
	p.value			P < 0.05	P < 0.01	P < 0.05

Data are shown as mean ± SD. * P < 0.05 and ** P < 0.01 were considered statistically significant.

Antioxidant enzymes activities of the study groups before and after intervention

Table (3) shows mean ± SD of antioxidant enzymes activities of the study groups before and after study period. The levels of SOD were increased in the all groups after study period when compared with before ($P \leq 0.01$) in groups 2 and 3 and ($p \leq 0.05$) in groups 4 and 5 but still lower in the test subjects compared with the control group. The results showed a high significant decrease ($P \leq 0.001$) in the GR, GPX and catalase concentration in groups 2 and 3 after intervention except GR ($P \leq 0.05$) in group (3). This study is agreement with Brown *et al.,* (2009). In the present study there was a slight increase in groups 4 and 5. This could be because of the lower amount of weight reduction compared with groups 2 and 3. This result is in agreement with Ahmed *et al.,* (2016) who found a positive correlation between vitamin C intake status and SOD activity after intervention. Vitamin C as an antioxidant could be effective in maintaining and increasing SOD activity. We observed a significant positive correlation between vitamin E intake and GPX activity after intervention. This can be the reason for the increase in GPX activity after intervention with parsley which contains vitamins C and E (See Table 1). GR an enzyme protects the cell, including being a free radical scavenger as a primary antioxidant defense (Lee *et al.,* 2008 and Chen *et al.,* 2013). The glutathione concentration may be affected by many factors related to the life style such as non-healthy food and psychological stress, leading to decrease antioxidants level (Patki *et al.,* 2013). Its dysregulation represents one of the main factors responsible for overproduction of ROS in many other obesity associated diseases (Goyal *et al.,* 2011).

Bougoulia *et al.,* (2006) showed that the reduction of BMI from 38.5 to 30.9 is accompanied with a significant increase in the GPX enzyme activity from 22.3 to 48.9 ng/mL. The results in groups 2 and 3 after intervention agrees with Crujeiras *et al.,* (2006) who stated that total antioxidant capacity improved in those who consumed more vegetables.

Table (3): Mean ± SD of antioxidant enzymes activities of the study groups before and after intervention

	SOD	GR (U/gHb)	GPX	CAT

		(U/gHb)		(U/gHb)	(U/gHb)
Group (1) (normal)		452.1 ± 32.4	3.58 ± 1.26	44.84 ± 17.93	221.98 ± 7.95
Group (2) (overweight) + intervention	Before	429.1 ± 32.4	2.12 ± 0.77	32.45 ± 12.83	198.72 ± 6.54
	After	442.8 ± 34.7	2.48 ± 0.64	41.56 ± 10.73	209.82 ± 5.98
	p.value	P < 0.01	P < 0.01	P < 0.01	P < 0.01
Group (3) (obese) + intervention	Before	399.3 ± 30.9	2.83 ± 1.28	29.23 ± 12.46	188.33 ± 8.95
	After	443.4 ± 29.5	3.17 ± 1.25	38.42 ± 14.93	198.72± 6.54
	p.value	P < 0.01	P < 0.05	P < 0.01	P < 0.01
Group (4) (overweight)	Before	425.5 ± 27.9	3.01 ± 1.31	30.44 ± 13.93	192.42 ±2.89
	After	435.6 ± 28.7	4.26 ± 1.01	36.48 ± 13.45	198.26 ± 2.43
	p.value	P < 0.05	P < 0.05	P < 0.05	P < 0.05
Group (5) (obese)	Before	411.5 ± 29.6	2.83 ±0.28	30.98 ± 13.46	200.57 ± 3.46
	After	423.7 ± 27.8	3. 6 ± 0.38	35.89 ± 14.65	206.52 ± 4.87
	p.value	P < 0.05	P < 0.05	P < 0.05	P < 0.05

Data are shown as mean ± SD. * P < 0.05 and ** P < 0.01 were considered statistically significant.

The increased GPx activity of obese subjects may be interpreted as compensatory mechanism due to increased lipid peroxidation. Because it has been shown that GPx is more potent on a molar basis than catalase and other antioxidant enzymes to protect cells from oxidative stress, it can be hypothesized that body tends to combat stress by over expressing GPx gene as the first line of defense in obese subjects (Masoud *et al.,* 2014).

CAT is yet another free radical scavenger that is known for its detoxification action against lipid peroxides (Yu, 1994). Our results are disagree with Masoud *et al.*, (2014) who found no significant alteration in the activity of erythrocyte catalase in obese individuals when compared with controls. Also, study of Azza, (2014) disagrees with our results which did not reveal any significant differences between healthy weight volunteers and obese subjects or overweight subjects. Many reasons may explain and support these inconsistent results. The glutathione concentration may be affected by many factors related to the life style such as non-healthy food and psychological stress, leading to decrease antioxidants level (Patki *et al.*, 2013). The results showed that more weight loss causes an increase in GR, SOD and GPX activities. However, the amount of increase in GR activity after intervention was almost twice as before the intervention. Perhaps if there had been more weight loss among the participants, other enzymes would have been increased and the effects of weight loss would have been more apparent.

In conclusion, a weight loss by parsley besides weight reduction diet can reduce the weight loss and BMI which can increase of antioxidant enzymes activities in overweight and the obese subjects. We suggest further studies with more weight reduction, longer duration of intervention and different percentages of antioxidant enzymes concentration. Further studies are also required to define whether parsley alone possess the same effect, or perhaps less.

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تأثير البقدونس على إنقاص الوزن والإنزيمات المضادة للأكسدة في النساء ذات الوزن الزائد والبدينات

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الملخص:
أجريت الدراسة الحالية بهدف تقييم تأثير البقدونس على فقدان الوزن والانزيمات المضادة للأكسدة في النساء اللآتى يعانين من زيادة الوزن والبدانة. لذلك تم في الدراسة الإستعانة بـ ١٥٠ سيدة متطوعات كونت المجاميع التالية: مجموعة (١): ٣٠ سيدة متطوعة بمؤشر كتلة الجسم أقل من ٢٥ كجم/م٢ (مجموعة ضابطة)، مجموعة (٢): ٣٠ سيدة ذات وزن زائد بمؤشر كتلة الجسم ما بين ٢٥-٣٠ كجم/م٢ يتناولن (فنجان واحد/يوم) بقدونس بالإضافة إلي النظام المتبع لإنقاص الوزن، مجموعة (٣) : ٣٠ سيدة بدينة بمؤشر كتلة الجسم أعلي من٣٠ كجم/م٢ يتناولن فنجان واحد/يوم بقدونس بالإضافة إلي النظام المتبع لإنقاص الوزن، مجموعة (٤): ٣٠ سيدة ذات وزن زائد بمؤشر كتلة الجسم ما بين ٢٥-٣٠ كجم/م٢ يتناولن فقط النظام المتبع لإنقاص الوزن، مجموعة (٥): ٣٠ سيدة بدينة بمؤشر كتلة الجسم أعلي من٣٠ كجم/م٢ يتناولن فقط النظام المتبع لإنقاص الوزن. نظام إنقاص الوزن المتبع يشتمل علي نفس الأنواع والكميات لأفراد المجموعات (٢،٣،٤،٥) بالتبادل أثناء أيام الأسبوع خلال فترة البحث. كما نصح الباحثان المتطوعات في المجموعتين (٢،٣) بأنهن يستطعن أكل أو إدخال البقدونس في أطباق السلطة والسمك واللحمة والفراخ والبطاطس. كانت أعمار السيدات تتراوح بين ٣٥-٦٠ عام بدون تاريخ سابق من الأمراض المزمنة، وكن من المترددات علي العيادات الخارجية بمستشفيات جامعة أسيوط بعد موافقتهن على المشاركة في الدراسة. تم جمع معلومات عن العمر والوزن والطول وتم تقدير درجات نشاط إنزيمات سوبر أوكسيد ديسميوتيز والجلوتاثيون المختزل والجلوتاثيون بيروكسيديز والكاتاليز في كل المجموعات عند بداية الدراسة وكذلك بعد شهرين من التدخل الغذائي بالبقدونس. تمت الموافقة علي إجراء البحث من قبل لجنة الأخلاقيات في كلية الطب، جامعة أسيوط. أظهرت نتائج تحليل عينات البقدونس وجود محتوي عالي من البروتين، الكربوهيدرات، الألياف، فيتامينات أ، ج، هـ ومعادن البوتاسيوم ،الصوديوم، الكالسيوم،الحديد، المنجنيز، الزنك. كذلك لاحظنا فروق ذات دلالة احصائية منخفضة بين الوزن وسمك طبقة الدهن تحت الجلد في المجموعتين (٣،٤) قبل وبعد فترة الدراسة بينما الدلالة كانت أعلي في مؤشر كتلة الجسم. أيضا مستويات درجة نشاط إنزيم السوبر أوكسيد ديسميوتيز والتى إرتفعت كثيرا في المجموعات (٢،٣) بعد فترة الدراسة وإرتفعت بشكل أقل في المجموعتين (٤،٥) ولكن هذا الإرتفاع مازال أقل من المجموعة الضابطة. أيضا أظهرت النتائج إنخفاض ذات دلالة إحصائية في درجة نشاط إنزيمات الجلوتاثيون المختزل، والجلوتاثيون بيروكسيد والكاتاليز في المجموعتين (٢،٣) وزيادة طفيفة في المجموعتين (٤،٥). وخلصت الدراسة الي أن تناول البقدونس بالإضافة إلي نظام متبع لإنقاص الوزن يخفض كلا من الوزن ومؤشر كتلة الجسم الذي بدوره يساعد علي إرتفاع درجة نشاط الإنزيمات المضادة للأكسدة في السيدات ذات الوزن الزائد والبدينات.
الكلمات المفتاحية: البقدونس، القيمة الغذائية، المقاييس الجسمية، سوبرأوكسيد ديسميوتيز، جلوتاثيون بيروأكسيديز، كاتاليز.