

Effect of some plant oils and garlic on lipids of rats fed on high cholesterol diet

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Abstract

The present work was to evaluate the effect of ginger, garden rocket oils (8%) and garlic powder (2%) on plasma lipid profile and the activity of some antioxidant enzymes of rats fed on high cholesterol diet. Thirty-six male rats were divided into 6 groups; first group fed on basal diet and served as control. Second group fed on a basal diet with 3.5% cholesterol. The other four groups fed on the high cholesterol diet supplemented with the tested oils alone and with the addition of garlic powder for 30 days. Nutritional parameters, plasma total lipids, total cholesterol, triglycerides, high and low-density lipoproteins, their ratio and the activity of some antioxidant enzymes were measured. In conclusion, the tested oils and garlic powder were effective in reducing the plasma lipid parameters and in restoring the activity of the antioxidant enzymes. The present work confirmed the health benefits of ginger and garden rocket oils with the addition of garlic in lowering the plasma lipid profile and enhancing the cells antioxidant state, when using a high cholesterol diet.

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Keywords

High cholesterol diet
Garlic
Plant oils
Lipid profile
Antioxidant enzymes
Rats

Introduction

Hypercholesterolemia is a risk factor for the development and progression of atherosclerosis and cardiovascular diseases. A high cholesterol diet is a major environmental contributor to unbalanced lipid metabolism and associated with an increase in prevalence of coronary heart disease (Libby, 2008). Some studies have demonstrated that lowering plasma total cholesterol, low-density lipoprotein cholesterol and increasing high-density lipoprotein cholesterol are useful in preventing the risk of cardiovascular disease (Agbedana and Akanji, 1988).

Herbs were used for various medicinal purposes, including the treatment of hyperlipidemia. The oil from garden rocket leaves (*Eruca sativa*) contains some polyunsaturated fatty acids such as α -linoleic acid (0.2%) and linolenic acid (0.1%) that have a good effect in controlling the effects of hyperlipidemia in rats (Miyazawa *et al.*, 2002). Vardavas *et al.* (2006) analyzed the fatty acids content of the fresh leaves of garden rocket oil and they reported that the amount of the total fat was 114 mg/100g fresh weight, the saturated fat level was 41.5%, monounsaturated fatty acids concentration was 49.6% and the polyunsaturated fatty acids level was 8.9% of total fat contents. The ω -3 fatty acids level was 44.7 mg/100g fresh weight and ω -6 fatty acids level was 11.7 mg/100 g fresh weight.

Ginger (*Zingiber officinale Roscoe*) oil has an effective role on lipid profile during the use of

high cholesterol diet. Ginger has many bioactive compounds that might be potentially useful in the treatment of various diseases including hypercholesterolemia (Afzal *et al.*, 2001; Anonymous, 2003; Shanmugam *et al.*, 2011). It contains up to 9% lipids or glycolipids and contains up to 3% essential oil, accounting for 20 – 25% of the oleoresin. Gas chromatography/mass spectrometry identified 66 compounds in the essential oil of ginger Zhou *et al.* (2006). Recently, a study done by Williams and Lamprecht (2008), detected some sulfonated compounds including 4-gingesulfonic acid and shogasulfonic acids in ginger. Ginger could lower cholesterol levels by reducing cholesterol absorption in blood and liver (Bhandari *et al.*, 1998).

Garlic (*Allium sativum*) is a bulb that belongs to the family Liliaceae and provides a helpful role via its polyunsaturated fatty acids in lowering the bad lipid profile that resulted from the high cholesterol diet. Some studies done by Jain *et al.* (1993), Watkins (2002) and Santhosha (2013) reported the ability and the evidence on the role of garlic in reducing plasma cholesterol in humans. Some authors demonstrated that ginger; garden rocket and garlic have strong anti-oxidant properties and have an effective role in preventing the lipid peroxidation (Ali *et al.*, 2008; Tsai *et al.*, 2012; Guarrera and Savo, 2013). Whereas, some authors explained that some herbs may have a role in producing the reactive oxygen species (ROS) and enhancing the activity of some

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antioxidant enzymes including superoxide dismutase (SOD), catalase and glutathione peroxidase (Borek, 2001; Williams and Lamprecht, 2008). The present work was to evaluate the healthy effect of ginger and garden rocket oils (8%) with and without powder of dried garlic (2%) on lipid profile and the activity of some antioxidant enzymes in rats fed on a high cholesterol diets.

Materials and Methods

Materials

Fresh garlic (*Allium sativum*) was purchased from the local market, Cairo, Egypt. Pealed, sliced, and dried in a hot air oven (40°C), then ground to a fine powder and kept in polyethylene bags. Garden rocket, leaves oil (*Eruca sativa*) and the ginger, rhizome oil (*Zingiberis officinale Roscoe*) were purchased from the local herbal market, Cairo, Egypt.

Diets and animals experiment

Sixty- three Sprague Dowley male rats, weighed from 80 to 120 g were divided into six groups each had 6 rats. High cholesterol diet and the tested diets were prepared according to Bulur *et al.* (1995). Diets composition and the number of rat groups were presented in Table 1. Mineral and vitamin mixtures in the basal diet were prepared according to the method of Reeves *et al.* (1993). Water and food were provided ad-libitum for a period of 30 days. Food intake and body weights were measured weekly. At the end of the experiment, rats were weighed, fasted over night, and then blood was withdrawn by heart puncture under slight diethyl ether anesthesia in heparinized tubes. Plasma was separated after the whole blood centrifugation at 3500 rpm for 15 minutes.

Nutritional and biochemical parameters assays

At the end of experiment, total food intake, final rats' body weights and body weights gain were recorded. Food efficiency ratio was calculated according to this equation: food efficiency ratio = body weight gain/food intake (Proll *et al.*, 1998). Total plasma cholesterol was determined according to Richmond (1973), plasma high-density lipoprotein (HDL) cholesterol was determined according to the method used by Lopes (1977), plasma low-density lipoprotein (LDL) cholesterol was determined according to the method of Assmann *et al.* (1984). Plasma total lipids were determined according to the method used by Draven and Schmite (1964). Plasma triglycerides were determined according to the method of Fossati and Prencipe (1982). The oxidation state of rats was measured by determining

the activities of plasma superoxide dismutase (SOD), catalase, and the level of the plasma malondialdehyde (MDA) (as a parameter for the lipid peroxidation) using the methods of Roth and Gilbert (1984), Aebi (1984) and Ohkawa (1979), respectively.

Statistical analysis

Results expressed as means \pm standard errors of means (SEM). Comparison between the means (was done using a one-way ANOVA, followed by Duncan Multiple Range Tests for all variables (Rukhin, 2012). Differences between groups were considered significant at $p < 0.05$.

Results

Body weight gain, total food intake and food efficiency ratio

The results in Table 2. show that the rat group number 5 (fed on garden rocket oil) was the lowest value in the body weight gain than all of the other tested groups. In addition, the rat group number 2 that fed on the high cholesterol diet gave the highest values for the body weight gain and in total food intake. The rat group number (1) that fed on the basal diet recorded the lowest value of total food intake; all of the other tested diets were close to the rat group that fed on the basal diet relative to body weight gain values except that fed high cholesterol diet. For the food efficiency ratio, the rat group number (5) that fed on the garden rocket oil was recorded significantly ($P < 0.05$) the lowest value than the rat groups fed on the other tested diets.

Changes in plasma total lipids

The effects of dietary treatments on plasma total lipids are presented in Table 3. When garden rocket oil or ginger oil added, there was a significant ($P < 0.05$) effect on decreasing the total lipids levels from 870 mg/dl in rat group fed on high cholesterol diet to 435 mg/dl and 705 in rat groups fed on garden rocket or ginger oils respectively. The addition of 2% of garlic powder led to another decrease in total lipids to 380 mg/dl and 373 mg/dl in rat groups fed on garden rocket or ginger oils, respectively.

Changes in plasma total cholesterol

As presented in Table 3. adding ginger and garden rocket oils (8%) was effective in reducing the total cholesterol level from 104.9 in the rat group fed on the high cholesterol diet to 74.2 mg/dl in rats that fed on the garden rocket oil and to 81.3 mg/dl with rat group that fed on ginger oil. When garlic powder was added with the tested oils, the decreased levels were

Table 1. The composition of the tested diets (g/100g) and the number of the rat groups.

Ingredient	Number of the rat groups**					
	1	2	3	4	5	6
Casein	14	14	14	14	14	14
Cellulose	4	4	4	4	4	4
Sucrose	5	5	5	5	5	5
*minerals mixture	3.5	3.5	3.5	3.5	3.5	3.5
*Vitamins mixture	1	1	1	1	1	1
Cholic acid	-	0.5	0.5	0.5	0.5	0.5
Cholesterol	-	3.5	3.5	3.5	3.5	3.5
Corn oil	1	1	-	-	-	-
Ginger oil	-	-	8	8	-	-
Garden rocket oil	-	-	-	-	8	8
Garlic	-	-	-	2	-	2
Starch	71.5	67.5	60.5	58.5	60.5	58.5
Total	100	100	100	100	100	100

*Vitamin and mineral mixtures used according to Reeves et al., (1993).

** (1) Control diet, (2) high cholesterol diet (3.5%), (3) high cholesterol diet with ginger oil, (4) high cholesterol diet with ginger oil and garlic(2%), (5) high cholesterol diet with garden rocket oil and (6) high cholesterol diet with garden rocket oil and garlic.

Table 2. Body weight gain, total food intake and food efficiency ratio of rats fed on tested diets

Group*	Body weight gain** (g)	Total food intake** (g)	Food efficiency ratio**
1	79.2 ± 7.8 ^{bc}	410 ± 3.5 ^d	0.193 ± 0.002 ^{ab}
2	109.6 ± 8.5 ^a	536 ± 0.5 ^a	0.204 ± 0.002 ^a
3	72.2 ± 2.0 ^{bc}	462.4 ± 8.6 ^b	0.156 ± 0.001 ^{bc}
4	88.2 ± 4.0 ^b	459.8 ± 6.0 ^b	0.192 ± 0.001 ^{ab}
5	68.0 ± 6.7 ^c	449 ± 11.2 ^{bc}	0.151 ± 0.001 ^c
6	72.2 ± 0.9 ^{bc}	431.4 ± 10.4 ^{cd}	0.167 ± 0.001 ^{abc}

*Numbers of rat groups were described in table 1.

** Values are means ± SEM, any two means have the same letter in the same column did not significantly different at $P < 0.05$

much better; the level in rat groups fed on garden rocket oil and garlic was 55 mg/dl and in rat group fed on ginger oil and garlic was 76.9 mg/dl.

Changes in plasma triglycerides

Changes in the plasma triglycerides levels of rats fed on different tested diets are explained in Table 3. The rats groups number 3 and 5 that fed on high cholesterol diet with ginger and garden rocket oils, respectively were recorded significant ($P < 0.05$) decrease in the triglycerides levels (84.3 mg/dl and 82.7 mg/dl, respectively) than the rat group number 2

(fed on high cholesterol diet) that recorded 129.8 mg/dl. Adding garlic powder with the ginger and garden rocket oils recorded significant ($P < 0.05$) decrease in the plasma triglycerides levels and the values were 74.2 mg/dl, 76.3 mg/dl, respectively.

Changes in plasma HDL- cholesterol

In the rat groups that fed on the high cholesterol diet supplemented with garden rocket or ginger oils, the plasma HDL- cholesterol levels were significantly higher than the rats fed on the high cholesterol diet. No significant ($P < 0.05$) differences were found

Table 3. Plasma total lipids, total cholesterol and triglycerides of rats fed on tested diets

Group*	Total lipids** (mg/dl)	Total cholesterol** (mg/dl)	Triglycerides** (mg/dl)
1	776.6 ± 97.5 ^{ab}	85.6 ± 2.0 ^b	72.4 ± 6.2 ^b
2	870 ± 117.5 ^a	104.9 ± 11.3 ^a	129.8 ± 14.4 ^a
3	705.2 ± 88.0 ^b	81.3 ± 5.9 ^b	84.3 ± 13.6 ^b
4	373.4 ± 156 ^c	76.9 ± 4.0 ^b	74.2 ± 4 ^b
5	435 ± 85.5 ^c	74.2 ± 9.0 ^b	82.4 ± 11.4 ^b
6	379.5 ± 65.6 ^c	55.0 ± 7.4 ^c	76.3 ± 1.0 ^b

* Numbers of rat groups were described in table 1.

** Values are means ± SEM, any two means have the same letter in the same column did not significantly different at $P < 0.05$

Table 4. Plasma HDL, LDL and LDL / HDL ratio of rats fed on the tested diets

Group*	HDL-cholesterol** (mg/dl)	LDL - cholesterol** (mg/dl)	LDL / HDL Ratio**
1	22.0 ± 2.0 ^b	36.5 ± 4.7 ^b	1.7 ± 0.07 ^b
2	17.8 ± 3.9 ^a	57.2 ± 8.5 ^a	3.2 ± 0.04 ^a
3	21.2 ± 0.8 ^b	38.3 ± 4.6 ^b	1.8 ± 0.03 ^b
4	23.9 ± 0.9 ^b	35.2 ± 4.1 ^b	1.5 ± 0.05 ^b
5	21.8 ± 1.3 ^b	36.6 ± 3.9 ^b	1.7 ± 0.03 ^b
6	24.8 ± 4.1 ^b	34.5 ± 6.6 ^b	1.4 ± 0.02 ^b

between the rats fed on the basal diet and the rats fed on diets with tested oils (Table 4.).

Changes in plasma LDL -cholesterol

In Table 4. the rats fed on high cholesterol diet supplemented with the tested oils showed a significant ($P < 0.05$) decrease on the levels of LDL-cholesterol than rats fed high cholesterol diet without the addition of the tested oil. The addition of 2%

garlic powder led to another significant ($P < 0.05$) decrease in the LDL than rats fed on high cholesterol diets alone.

Changes in plasma LDL-cholesterol / HDL-cholesterol ratio

As shown in Table 4. when garlic powder 2% added to the high cholesterol diets supplemented with different oils the LDL / HDL ratio was decreased

Table 5. Activities of the plasma Catalase and Superoxide Dismutase (SOD) and the level of the plasma malondialdehyde (MDA) of rats fed on tested diets

Group*	Catalase** U/ml	SOD ** U/ml	Malondialdehyde ** nmol/ml
1	642.8 ± 42.5 ^{ab}	288.1 ± 18.0 ^{ab}	5.7 ± 2.0 ^c
2	710.0 ± 100.4 ^a	301.7 ± 15.1 ^a	9.7 ± 1.1 ^a
3	636.8 ± 79.3 ^{ab}	293.7 ± 9.1 ^a	6.9 ± 1.1 ^{ab}
4	660.2 ± 123.3 ^{ab}	294.0 ± 7.0 ^a	7.3 ± 0.9 ^{bc}
5	672.6 ± 29.9 ^a	274.1 ± 3.1 ^b	8.1 ± 0.8 ^{ab}
6	553.0 ± 34.8 ^b	213.4 ± 11.6 ^c	6.9 ± 1.2 ^{bc}

* Numbers of rat groups were described in table 1.

** Values are means ± SEM, any two means have the same letter in the same column did not significantly different at $P < 0.05$

from 3.8 to 1.8 and 1.7 in rats fed on ginger and garden rocket oil, respectively. Another significant ($P < 0.05$) decrease was found in the plasma LDL / HDL ratio between rats fed on high cholesterol diet alone and the rats fed on the high cholesterol diet with the addition of tested oils and garlic, the ratios are 1.5 and 1.4 (in ginger and garden rocket oil, respectively).

Oxidation state

The activities of the antioxidant enzymes including, catalase, and superoxide dismutase (SOD), and the level of plasma malondialdehyde are presented in Table 5. The rat group that fed on high cholesterol diet recorded the highest value for activities of the tested antioxidant enzymes and the level of the plasma malondialdehyde (MDA) compared with the rat group that fed on the basal (control) diet. The adding of garden rocket and the ginger oils to the high cholesterol diet was of help in restoring the activities of the tested antioxidant enzymes and in the plasma malondialdehyde (MDA) level. In addition, garlic powder showed more pronounced effect with garden rocket oil than the oil alone $P < 0.05$.

Discussion

The purpose of this experiment was to investigate the healthy effects of some plant oils (ginger and garden rocket) with and without the addition of garlic

powder, on plasma lipids profile and the activity of some antioxidant enzymes in rats fed on high cholesterol diet. Both of the tested oils and garlic are rich in some essential fatty acids and some bioactive compounds such as sulfur peptides that provide a healthy effect in fat metabolism and in scavenging of free radicals. These effects are strongly indeed this time especially in some developing countries that suffering from several diseases that caused by eating unbalanced diet that rich in saturated fats. Our results in this study confirm the healthy effect of the tested oils with or without adding garlic powder for reducing the body weight gain. Adding the ginger oil and garden rocket oil with and with out garlic led to significant decrease in the body weight gain and food efficiency ratio compared with the rat fed on high cholesterol diet alone. That confirmed the health benefits of the tested materials that rich on some essential oils that can help in burning fats and in weight loss. Ginger oil that used in the present work is a great way for weight loss, it has the ability to reduce the cholesterol level and it also stimulates cholesterol conversion to bile acids so it can increase bile extraction. All these factors led to weight loss. Also, it helps in preventing the body from producing fat cells, as well as decreasing in cells size by releasing fats from the cells (Onu and Aja, 2011). The essential fatty acids in the garden rocket oil have the similar effect in reducing the body weight (Al and Araki, 2013). In rat group that fed on the high cholesterol

diet supplemented with the tested oils and garlic, the food efficiency ratios were decreased because the body weight gain was decreased and the food intake was increased. So, according to the equation: food efficiency ratio = body weight gain/food intake, the food efficiency ratios were decreased. The decreasing effect of the tested oils and garlic in the levels of the body weights gain and the food efficiency ratios in the present study were in agreement with a study done by Onu and Aja (2011), their results showed that garlic and ginger produced significant reducing effects on body weight gain, food intake and food efficiency ratio in rabbits.

Also, our tested oils and garlic with the certain addition (8% for both of ginger and garden rocket oils and 2% for garlic) provided a significant decreasing in the level of plasma total lipid, total cholesterol and triglycerides in rat groups that fed on these tested diets than the rat group fed on the high cholesterol diet. These effects related to the healthy effect of the tested materials that rich on omega fatty acids and some bioactive compounds in reducing the of lipid profile biosynthesis. For example, garden rocket oil can induces turning of serum lipid profile, also prevent the changes of triglycerides (Guarrera and Savo, 2013). For example, Garlic helps to reduce cholesterol by inhibiting the cholesterol biosynthesis enzymes and reducing the cholesterol absorption in blood and liver (Bhandari *et al.*, 1998). In addition, Sanghal *et al.* (2012) confirmed the reducing effect of using garlic and ginger oil on the levels of the total lipids of rats. A study carried out by Chetty *et al.* (2003) reported that the addition of garlic powder to rats fed on high cholesterol diet with some oils that rich in omega fatty acids such as olive oil or corn oil reduced the total cholesterol level by 50%. The present results for the lipid parameters are in agreement with Chetty *et al.* (2003) results that confirmed the significant effect of garlic powder in reducing the lipid profile especially the triglycerides levels in rats. In general, Ahmed and Sharma (1997) conducted an experiment using rats fed on ginger oil and garlic powder and they evidenced that the combination of garlic and ginger for 4 weeks led to a significant decrease in serum total lipids and total cholesterol. In addition, a study done by Thomson *et al.* (2002) confirmed a significant reduction in serum cholesterol of rats fed on ginger extract. Bhandari *et al.* (1998) also confirmed the lowering effect of ginger in the serum cholesterol and triglycerides levels in rats. Also, Al and Araki (2013) studied the effect of the garden rocket oil on rats' plasma lipid profile and they found that the garden rocket oil provided a good effect in reducing the rat lipid profile; their results are

in agreement with the present results.

In the present experiment, adding both of the tested oils and garlic to the high cholesterol diet led to a significant increase in the plasma HDL-cholesterol. Whereas, plasma LDL-cholesterol and the LDL / HDL ratios were significantly decreased, these results also confirmed the health benefits of the present tested oils and garlic in reducing the plasma level of LDL-cholesterol that reduces the coronary artery disease. The role of these oils and garlic is possible because of the there effect in blocking the cholesterol biosynthesis by inhibiting the activity of the cholesterol biosynthesis enzymes. Whereas, the plasma HDL cholesterol level was significantly ($P < 0.05$) increased in rat fed on the diets supplemented with ginger oil and the ginger oil with garlic. The studies of Chetty *et al.* (2003) and Sanghal *et al.* (2012) were in agreement with the decreasing role of the tested oils and garlic in the rats' plasma level of LDL-cholesterol in our study. Another study indicated that a combination of garlic and ginger is more effective in reducing the LDL level and enhancing the values of the HDL (Ahmed and Sharma, 1997). The activity of the antioxidant enzymes catalase and SOD and the level of the plasma malondialdehyde (MDA) as a parameter for the lipid peroxidation, were evaluated in the rat groups that fed on the tested oils with and without adding garlic. The highest significant level of these antioxidant parameters was in the plasma of rat group that fed on the high cholesterol diet, the values were (710 U/ml for catalase , 301 U/ml for SOD and 9.7 nmol/L for MDA), that because of the role of the high cholesterol level in the activation of the lipid oxidation rate and the production of the free radicals that led to activate the plasma antioxidant enzymes to help the body in releasing of these free radicals in this rat group that fed on the high cholesterol diet. When the tested oils alone or with garlic were added to the high cholesterol diet, the levels of the plasma antioxidant parameters were significantly decreased (especially in the diets supplemented with garden rocket oil alone or with garlic, the values were 672.6, 553 U/ml for catalase, 274.1, 213.4 U/ml for SOD and 8.1, 6.9 nmol/L for MDA in the diet supplemented with garden rocket oil alone or with the addition of garlic, respectively) , that because of the health benefits of the tested oils and garlic that rich in some omega fatty acids, selenium (in garlic) and some natural antioxidant compounds that are effective in scavenging the free radicals and in protecting the cells against the oxidative damage. In addition, some studies done by Wei *et al.* (1998), Helen *et al.* (1999); Arivazhagan *et al.* (2000) and Kumaraguruparan *et al.* (2005) confirmed the effective role of using garlic

in decreasing the oxidative cell damage.

Conclusion

The present study proved that using of ginger and garden rocket oils at the level 8% can provide a good nutritional values evidenced by accepted rate of growth and food efficiency ratio. In addition, these tested oils are promoting the antioxidant power in the body that protects from free radical damage. The tested oils are also reducing the levels of the total cholesterol, total lipids, and the low-density lipoprotein (LDL-cholesterol) thus reducing the bad effect of these lipids in rats fed on high cholesterol diet. The addition of garlic powder (2%) was much helpful to improve that healthy effect. The study also, introduces the garden rocket oil as a good source for omega fatty acids and can be used as a healthy oil in our Mediterranean dishes to reduce the use of the saturated fats and oils that commonly consumed and are the reason for several chronic diseases.

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