EFFECT OF GARLIC ON ATHEROSCLEROSIS IN RABBITS

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ABSTRACT

The present study was performed on rabbits for studying the possible role of garlic powder as an antilipidaemic agent in the prevention of hyperlipidaemia and atherosclerosis. The animals were kept on atherogenic diet showed a deleterious change in lipid profile than the normal control one which was clearer in the significant elevation of serum cholesterol, triacylglycerol (TAG), low-density lipoprotein (LDL), and total saturated free fatty acids, while high-density lipoprotein (HDL), and total unsaturated free fatty acids levels were decreased. On the other hand the rabbits kept on atherogenic diet and supplemented with garlic powder showed significantly better lipid profile. It is suggested that, garlic powder is an important determinant of serum lipid level, which is an antilipidaemic agent against the pathogenesis of atherosclerosis.

Key Words: *Garlic, lipid profile, malondialdehyde, hypercholesterolemia, atherosclerosis, free fatty acid fractionation.*

INTRODUCTION

The most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 mg/g in fresh garlic or 30 mg/g dry garlic. The general composition of garlic is about 65% water, fructose-containing carbohydrates, sulfur-containing compounds, proteins, free amino acids, vitamins, minerals, lipids and fatty acids. Garlic was relatively safe, cheap herbs drug used for treatment (**Lawson 1993 & Lawson 1998**). Atherosclerosis is a chronic disease that is caused by damage to the arterial wall from inflammation as well as fibro-fatty deposits. This condition also involves several cell types, particularly smooth muscle cells, monocyte-derived macrophages, T-lymphocytes and platelets (**Schwartz et al., 1993**). Atherosclerosis is considered as one of the principal causes of morbidity and mortality worldwide. The development of atherosclerosis, identification of its risk factors and remedies for its complications has been elucidated in recent decades (**Bitzur et al., 2005**). Dietary therapy is the first step in the treatment of atherosclerosis. This intervention involves the inclusion in the diet of materials such as soluble fibers, soy proteins and garlic, which may lower lipid concentrations to desired targets without the use of drugs (**Srinivasan and Srinivasan 1995**). A meta-analysis of 13 trials showed that, garlic tablets significantly reduced total blood cholesterol levels (**Stevinson et al., 2000**).

The objectives of the present investigation were:

a) Estimation of serum total lipid, total cholesterol, triacylglycerol, high density lipoprotein-cholesterol (HDL_C), low density lipoprotein-cholesterol (LDL_C) and Very low density lipoprotein (VLD_C).
b) Determination of the antioxidant activity in blood and tissue in different rabbit grouping.
c) Determination of the free fatty acid fractionation in serum by gas liquid chromatography.
d) Histopathological examination.

MATERIALS AND METHODS

Animals:

For performing the present study forty white male New-Zealand rabbits of one month old which were used in the experimental investigation of this study. Rabbits were obtained from laboratory animal research center faculty of agriculture, Mansoura University. The rabbits were randomly divided into four main equal groups:

Group I: (Control group)

Comprised ten rabbits, kept on basal balanced diet. The feeding was carried out for 30 days.

Group II: (Garlic group)

This group included ten rabbits, kept on basal balanced diet supplemented with 2% Garlic powder for 30 days.

Group III: (Cholesterol group)

This group consisted of ten rabbits, kept on basal balanced diet supplemented with 1% cholesterol for 30 days. **(Aftab, et al., 2007)**.

Group IV: (Cholesterol +Garlic group).

This group included ten rabbits, kept on basal balanced diet supplemented with 1% cholesterol and 2% garlic powder for 30 days. **(Sanjay and Subir, 2002)**.

Collection of blood sample:

At the end of the experiment (30 days), overnight fasting blood samples were collected by vein puncture of the marginal ear vein. Approximately 6.0 ml of blood were obtained from each animal in all groups in a clean and dry centrifuge tubes, then allowed to coagulate at room temperature for 30 minutes.

The collected blood samples were centrifuged at 3000 rpm for 15 minutes to separate clear serum samples. The separated serum samples were kept at -20°C for determination of the following biochemical parameters:

1- Total lipid concentration (TL) according to **Zollner and Kirsch, (1962)**.
2- Total cholesterol concentration (TC) according to **Allain et. al., (1974)**.
3- Triacylglycerol concentration (TAG) according to **Foessati and Prencipe, (1982)**.
4- High density lipoprotein - cholesterol concentration (HDL_C) according to **Lopez -Virella et al., (1977)**.
5- Low density lipoprotein - cholesterol calculation (LDL_C) according to **Friedwald et. al., (1972)**.
6- Very low density lipoprotein - cholesterol calculation (VLDL_C) according to **Vance and Vance, (1996)**.
7- Malondialdhyde concentration (MDA) according to **Draper and Hadley, (1990)**.
8- Free fatty acid fractionation according to

Radwan, (1978).

These parameters were determined by using commercial kits from (Biodiagnostic, Dokki, Giza, Egypt).

Dissection of experimental rabbits:

Tissue sampling:

Heart, liver and large blood vessels were taken from each group after had been sacrificed at the end of the experiment for determination of:

tissue Malondialdhyde concentration (MDA) according to **Yoshioka et al., (1982)**.

Histopathological finding:

Large blood vessels were fully immersed in formalin 40% and stored in containers for histopathological examination, according to **Woods and Ellis (1994)**.

RESULTS & DISCUSSION

The obtained results showed that the serum levels of total lipids, total cholesterol, TAG, LDL_C, VLDL_C, and saturated free fatty acids were increased while values of HDL_C and unsaturated fatty acids were decreased in animal kept on basal balanced diet supplemented with 1% cholesterol. On the other hand, animals kept on basal balanced diet supplemented with 1% cholesterol and 2% garlic powder showed lower levels of total lipids, total cholesterol, TAG, LDL_C, VLDL_C, and saturated free fatty acids, and higher levels of HDL_C, and total unsaturated free fatty acids when compared with control group. These findings were in agreement with a previous study conducted by **Mirhadi et al., (1991)** who stated that, male rabbits, when fed a cholesterol-rich diet, showed an increase in the levels of cholesterol and total lipids in plasma, aorta and liver. Moreover, the same study showed that, garlic supplementation to cholesterol rich diet significantly decreased cholesterol and total lipids levels. The cholesterol-lowering effect of garlic is due to decreased hepatic synthesis of cholesterol, while the triacylglycerol-lowering effect is due to inhibited fatty acid synthesis as shown in rat hepatocytes in vitro **(Yeh and Yeh, 1994)**. Our results were in agreement with study of **Elmahdi et al., (2008)** who reported that, fresh crushed garlic is effective in lowering cholesterol levels; hence, it can play a role in the prevention of atherosclerosis. In addition, the results also agree with those of **Sheia et al., (2005)** who reported that adding aqueous extract of raw garlic with 1% cholesterol to rabbits' diet significantly decreased plasma total cholesterol and LDL-C.

Initiation and progression of atherosclerosis is very much associated with oxidation of LDL cholesterol. When endothelial cells were incubated with Sallylcysteine, the major sulfur-containing compound of garlic, the oxidized LDL-mediated membrane damage was prevented. In addition, loss of cell viability and lipid peroxidation were also reduced. These findings indicate a protection to vascular endothelial cells against injury caused by oxdized LDL cholesterol **(Ide and Lau 1997)**. **Brousseau et al., (2004)** reported that, the increase in HDL-C level is usually attributed to allicin, which significantly altered the distribution of cholesterol among HDL and LDL subclasses by a mechanism, of reduction of VLDL level, with a secondary decrease in apo D activity which results in less transfer of HDL-C to VLDL acceptor particles.

The MDA levels in blood serum, liver, heart

and blood vessel were significantly increased in animal fed cholesterol rich diet compared with control group. Garlic supplementation significantly lowered MDA levels. This is due to the fact that Garlic is a potent oxidants' scavenger, as it inhibits lipid peroxidation and inflammatory prostaglandins. Garlic also reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA. Garlic has been shown to inhibit LDL oxidation (Borek, 2006).

Our results showed that, the serum total saturated fatty acids were increased, while total unsaturated fatty acids were decreased in animals fed cholesterol rich diet. These findings were reversed in garlic supplemented group. **Hanumantha et al., (2005)** reported that, the level of serum and liver tissue fatty acids (saturated, mono and poly unsaturated) such as palmitic acid, stearic acid, Oleic acid, linoleic acid, arachidonic acid and linolenic acid monitored by gas chromatography were considerably altered in acetaminophen intoxicated animals (produce hyperlipidemia) when compared with control animals.

From the obtained results we could be concluded that feeding garlic powder (2%) has a hypolypedemic effect against atherosclerosis.

Table I: Effect of garlic feeding on of serum lipid profile and MDA (mg/dl).

	Group I	Group II	Group III	Group IV
Total lipid	316.04 ± 13.66^{c}	349.1 ± 16.84^{c}	1493.61± 108.14^{a}	1056.01± 117.94^{b}
Total cholesterol	114.23± 8.72^{c}	126.1 ± 9.41^{c}	819.7 ± 55.33^{a}	565.76 ± 64^{b}
Triacylglycerol	59.7± 4.19^{c}	73.2 ± 7.69^{c}	259.8 ± 40.13^{a}	177.4± 30.87^{b}
HDL_C	20 ± 1.3^{b}	28.7 ± 4.7^{b}	23.4 ± 2.3^{b}	51.3 ± 6.2^{a}
LDL_C	82.3 ± 8.81^{c}	82.86± 9.41^{c}	744.33 ± 51.17^{a}	479.03 ± 57.18^{b}
VLDL_C	11.9 ± 0.83^{c}	14.6± 1.54^{c}	52 ± 8.03^{a}	35.4 ± 6.18^{b}
MDA	11.8 ± 1.8^{c}	14.9 ± 1.6^{c}	43.3 ± 7.1^{a}	25.4 ± 4.2^{b}

Means (±SE) with the same letter in each row are not significantly differed ($p > 0.05$).

Table II: Effect of garlic feeding on malondialdehyde (MDA) in rabbits tissues (nmol / gm).

	Group I	Group II	Group III	Group IV
Liver MDA	12± 1.5^{c}	16.42 ± 0.6^{c}	57.14 ± 8.7^{a}	37 ± 6.5^{b}
Heart MDA	10.96± 0.64^{c}	13.94 ± 1.7^{c}	27.06 ± 1.4^{a}	16.88 ± 2.8^{b}
Blood vessel MDA	18.36± 2.2^{c}	21.46 ± 5.6^{c}	54.4± 7.3^{a}	40.62 ± 3.2^{b}

Means (±SE) with the same letter in each row are not significantly differed ($p > 0.05$).

Table III: Effect of garlic feeding on total saturated and unsaturated fatty acids:

	Total saturated fatty acids	Total unsaturated fatty acids
Group I	46.9 ± 0.7^{a}	53.04 ± 0.7^{c}
Group II	41.8 ± 1.8^{c}	57.11 ± 1.8^{b}
Group III	64.3 ± 0.9^{a}	33.07 ± 0.8^{d}
Group IV	36.5 ± 0.3^{d}	66.5 ± 0.3^{a}

Means (±SE) with the same letter in each column are not significantly differed ($p > 0.05$).

Fig.(1) Effect of garlic feeding on serum total lipid, total cholesterol, triacylglycerols and MDA in experimental groups of rabbits.

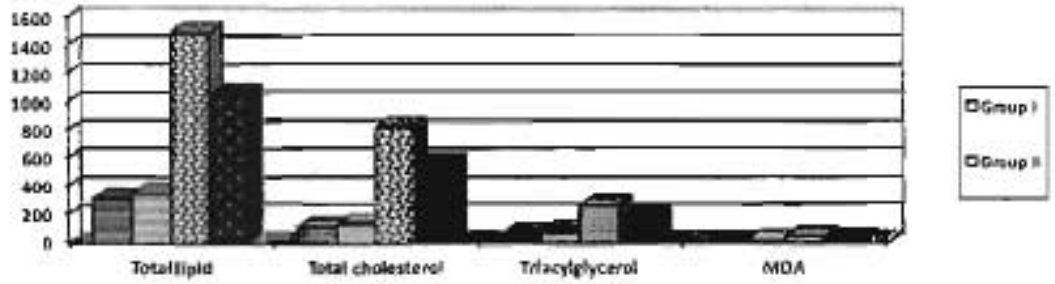


Fig.(2) Effect of garlic feeding on serum lipoprotein fractionations in experimental groups of rabbits.

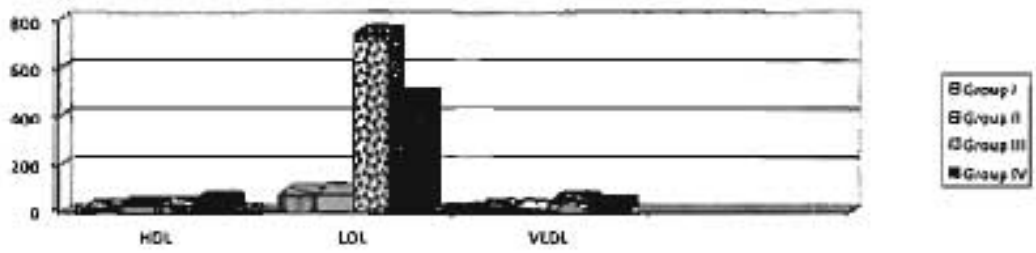


Fig.(3) Effect of garlic feeding on liver, heart and blood vessels tissue MDA in experimental groups of rabbits.

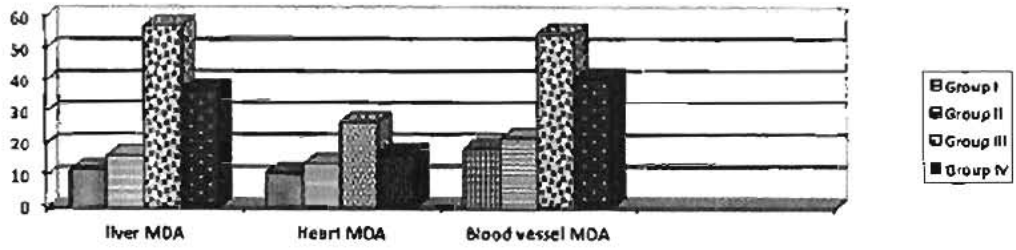
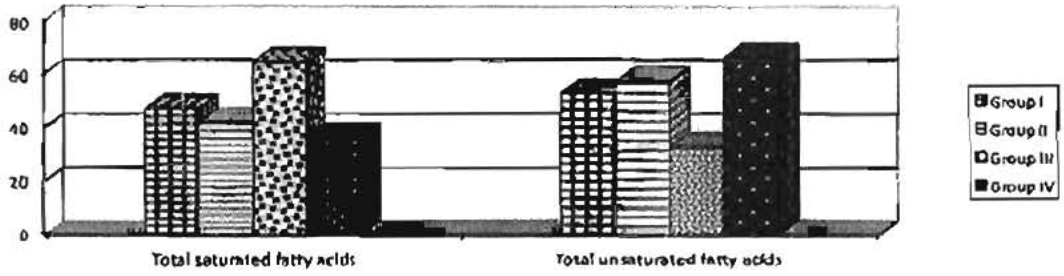


Fig.(4) Effect of garlic feeding on serum saturated and unsaturated fatty acids in experimental groups of rabbits.



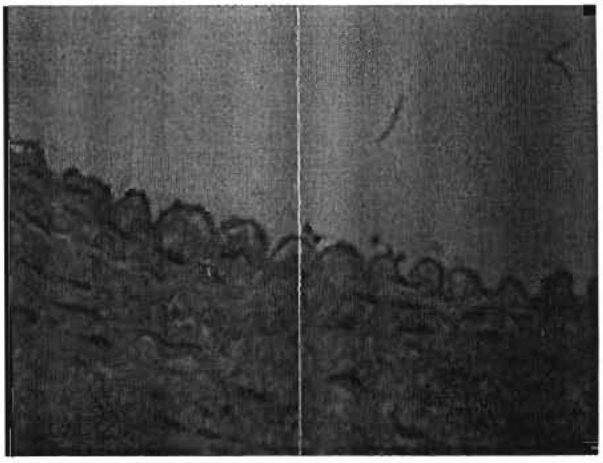


Fig. (5): Exhibits wall of aorta of Group II). The aortic wall structures were intact, the intima was intact.

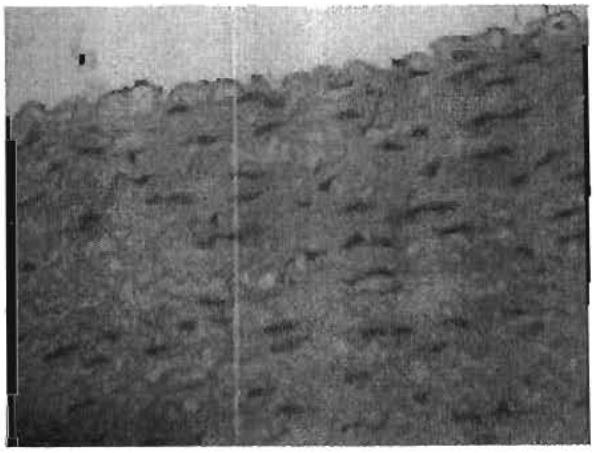


Fig. (6): Reveals wall of aorta of Group (II) showing intact tunica intema and tunica media.

Fig. (7): Represents wall of aorta of Group (III) showing marked destruction of tunica intema and macrophages accumulate cholesterol esters (foam cells) in subendothelial and in tunica media.

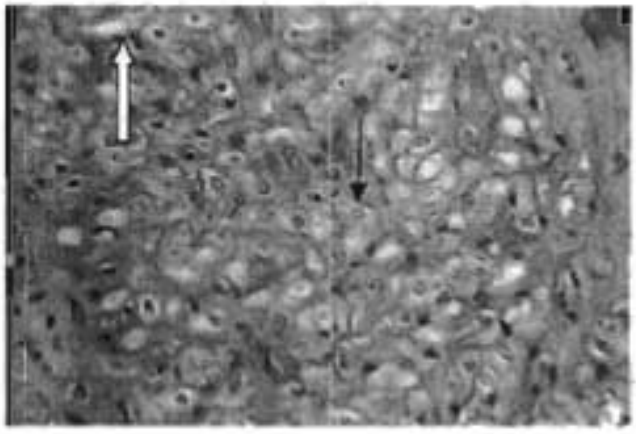


Fig. (8): Exhibits wall of aorta of Group(III) by high power of the previous slide to show foamy histiocytes in tunica media (arrow) and free cholesterol crystal (thick arrow).

Fig. (8): Indicates wall of aorta of Group IV showing intact tunica intema, with few foam cells.

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الملخص العربى

تأثير الثوم على تصلب الشرايين فى الأرانب

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تبحث هذه الدراسة تأثير مسحوق الثوم على تصلب الشرايين فى الأرانب من خلال البيانات المستمدة من الدراسات العلمية المتعددة التى استخدم فيها العديد من مركبات الثوم ومستحضراته، والتى جاءت بنتائج متفاوتة، وهى تؤكد على الحاجة إلى وضع مواصفات معيارية لمنتجات الثوم المتوافرة، للسماح بمقارنة أفضل بين نتائج البحوث، لاستخلاص النتائج حول التأثيرات المفيدة للثوم فى تصلب الشرايين.

بعد إنتهاء فترة التجربة تم تجميع عينات الدم والأنسجة (الكبد، القلب والأورطى) من جميع الحيوانات لقياس المالون داى الدهيد، الأحماض الدهنية، الدهون الكلية، الكوليسترول الكلى، الجليسريدات الثلاثية والبروتينات الدهنية عالية وقليلة الكثافة فى المصل بالإضافة إلى قياس نسبة المالون داى ألدهيد فى أنسجة الكبد، والقلب، والأورطى وكذلك عمل قطاع عرضى فى أنسجة الأورطى.

أوضحت النتائج أنه اختلفت معنوياً تأثير إضافة مسحوق الثوم على مستوى الدهون الكلية والكوليسترول الكلى والبروتينات الدهنية قليلة الكثافة التى زيادتها تسبب تصلب الشرايين كذلك الأحماض الدهنية المشبعة بالدم والمالون داى الدهيد بالتناقص عن المجموعة الغير معالجة بالثوم ومن الناحية الأخرى اختلفت معنوياً بالزيادة البروتينات الدهنية عالية الكثافة والأحماض الدهنية الغير مشبعة وهما من عوامل الأمان لتقليل فرص الإصابة بتصلب الشرايين لذلك توصى الدراسة باستخدام الثوم فى الأطعمة لتقليل الأضرار الناتجة عن تكون الشوارد الحرة وأيضاً لتقليل الإصابة بتصلب الشرايين.