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Effects of short-term garlic supplementation on lipid metabolism and antioxidant status in hypertensive adults

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Abstract:
This prospective and uncontrolled clinical study attempted to evaluate the effects of short-term supplementation with oily garlic formulation on lipid metabolism, glucose level and antioxidant status in patients suffering from primary arterial hypertension. Seventy subjects aged 30 to 60 years with primary arterial hypertension, including 38 females (mean age: 52.0 ± 8.3 years) and 32 males (mean age: 48.6 ± 8.2 years), took part in the study. In addition to receiving a standard antihypertensive pharmacotherapy they took 6 capsules of garlic preparation daily for 30 days. Before and after phytotherapy blood samples were collected to assay total cholesterol, HDL, LDL, triglycerides, lipid peroxidation products (TBARS) and vitamin A, C and E, β-carotene, glutathione and glutathione peroxidase activity, and arterial blood pressure was measured.
The analyzed garlic preparation was found to significantly lower lipid level and the level of lipid peroxidation products in the blood. It markedly increased vitamin E concentration in the serum, whereas the increases in the levels of other antioxidant vitamins and glutathione peroxidase activity proved insignificant. The product did not affect arterial blood pressure in the study subjects.
The results of this study suggest that the investigated garlic preparation may be tentatively used as an adjunct agent in treatment of arterial hypertension because of its hypolipemic and antioxidant properties.

Key words:
hypertension, oily garlic preparation, serum, lipids, antioxidants, vitamins, glutathione, glutathione peroxidase

Abbreviations: ACE-I – angiotensin converting enzyme inhibitors, BP – blood pressure, CHOL – total cholesterol, DSB – diastolic blood pressure, GPx – gluthatione peroxidase, GSH – glutathione, HDL – high density lipoproteins, LDL – low density lipoproteins, SBP – systolic blood pressure, TBARS – lipid peroxidation products reacting with thiobarbituric acid, TG – triglycerides

Introduction

Garlic (*Alium sativum*) contains biologically active compounds which exert multiple beneficial effects on human organism [16, 29]. Some reports indicate that

garlic or garlic preparations can correct lipid abnormalities and lower blood pressure in patients with hyperlipidemia and arterial hypertension [12, 24] and these data have been confirmed by recent experimental studies of Thomson et al. [26] and Haraum et al. [14]. Studies by Rahman and Billington [24] revealed, however, that plasma lipid levels can be lowered only after 2–3 months of garlic supplementation at the dose of about 7 cloves daily. The compounds found in garlic may also exert antioxidant action [3], inhibit platelet aggregation [24] and delay development of atherosclerosis [6]. All those properties of garlic have not yet been definitely validated and explained and some reports do not confirm hypotensive and hypolipemic effects of garlic, as shown by the metaanalysis of clinical and experimental research data on the effects of garlic or garlic preparations on cardiovascular risk published by Brace [5]. Beneficial effects of garlic preparations in subjects with hypercholesterolemia receiving aged garlic extract or powdered garlic for 6 months were not confirmed by Gardner et al. [13]. Similarly, Van Doorn et al. [27] did not reveal any significant effects of garlic powder taken for 3 months on inflammatory markers, endothelium function or lipid profile in overweight smokers.

Most of pharmaceutically active preparations obtained from garlic contain active ingredients and are devoid of the specific smell. Their efficacy is, however, an arguable issue and depends on the presence of specific active ingredients and the manufacturing method. This prospective, longitudinal, uncontrolled intervention study has attempted to assess the effects of short-term supplementation with oily garlic preparation on plasma lipid metabolism and antioxidant status in subjects with primary arterial hypertension.

Materials and Methods

The study protocol was approved by Bioethics Commission at Poznań University of Medical Sciences – Bioethics Commission Approval No. 346/02.

Study group

The study group of 70 patients aged 30 to 60 years with primary hypertension – 1st or 2nd stage as defined based on the criteria of *Joint National Committee* VII [8] – included 38 females (mean age: 52.0 ± 8.3 years) and 32 males (mean age: 48.6 ± 8.2 years). The subjects were receiving pharmacological treatment in the Clinic of Internal Diseases, Metabolic Disorders and Arterial Hypertension at Poznań University of Medical Sciences, Poland. All subjects were informed about the study aim, procedures and measurement methods and all signed written informed consent to take part in the study. Patients with comorbid disorders, such as ischemic cardiac disease, history of myocardial infarction, renal insufficiency, diabetes mellitus, gout or hyperlipidemia were excluded from the study.

The participants received 2 capsules of garlic preparation after meals, three times daily, for the period of 30 days, at the same time continuing their hypotensive pharmacotherapy as recommended by the physician. Over 80% of subjects received combined antihypertensive treatment of two or three drugs, i.e. angiotensin converting enzyme inhibitors (ACE-I) + diuretic (24% of patients), ACE-I + diuretic + β-blocker (23% of patients), ACE-I + diuretic + calcium channel antagonist (22% of patients) and diuretic + β-blocker (17%). Only 7% of subjects received monotherapy or the combination of all four drugs (7%).

Characteristics of the garlic preparation

The investigated garlic preparation was an oily macerate of garlic (*Allii sativi bulbus maceratio oleosa*) produced in Poland (AGROPHARM, Poland). Each capsule contained 270 mg of garlic macerate suspended in rape seed oil (1:1). According to the manufacturer, each capsule of the oily macerate contained 0.27 mg of allicin derivatives standardized for allicin. Among other sulfur compounds, the capsules contained mainly 2-vinyl-4H-1,3-dithiin and 3-vinyl-4H-1,2-dithiin (both of cyclic structure) and the excipients – glycerin, gelatin, mannitol and sorbitol.

Blood pressure measurements

Blood pressure (BP) was measured twice, before and after the treatment, using *OMRON* type 711 *Automatic IS* sphygmomanometer whose accuracy was periodically checked against a standard mercury sphygmomanometer. BP was measured always after a 5-min rest, in a sitting position and with the left arm supported at about the heart level. Two BP measurements

were performed per each visit at a 10-min interval and the mean value was recorded.

Biological material

The blood samples collected from study participants before and after garlic phytotherapy were sent for biochemical assay. About 20 ml of blood was collected in the morning (around 12 h after the last meal) from a forearm vein. Thus collected blood was placed in two tubes – one containing granulate to accelerate clotting and separate the serum (15 ml of blood) and the other with heparin sodium to obtain plasma and the hemolysate (5 ml). Total cholesterol (CHOL-C), high density lipoproteins (HDL) and low density lipoproteins (LDL), triglycerides (TG), vitamin A, C, E, β-carotene and lipid peroxidation products reacting with thiobarbituric acid (TBARS) were assayed in each blood sample. Plasma glutathione peroxidase activity (GPx) and hemolysate total glutathione (GSH) level were also assayed. Serum was stored at a temperature of –20°C and plasma and hemolysates at –70°C for no longer than 2–3 days following sampling.

Biochemical assays

CHOL-C, HDL, LDL and TG levels were assayed by enzymatic techniques based on colorimetric measurement with ANALCO reagent kits. LDL level was calculated using Friedewald's formula, i.e.:

$$\text{LDL (mg/dl)} = \text{(CHOL-C)} - \text{(HDL)} - \text{TG}/5.$$

Serum levels of vitamins in the samples were assayed by colorimetry. For vitamin C level measurement, serum had to be deproteinized with trichloracetic acid and purified with activated charcoal [21]. Vitamin E (total tocopherols) level was assayed with Emmerie-Engel's technique in ethanol-deproteinized serum following its extraction to heptane solution [15]. Vitamin A (retinol) was assayed following its reaction with antimony chloride (Carr-Price reaction) [17]. For β-carotene serum level assay, the samples were prepared in the same way as for tocopherol assay [15]. The level of lipid peroxidation products was checked with colorimetric technique developed by Ohkawa et al. [20] and modified by adding 0.1 ml of 0.1% BHT (Merck) as lipid peroxidation inhibitor [22]. GPx activity was measured using indirect technique developed by Rice-Evans et al. [25]. Total GSH was assayed based on catalytic activity of GSH in a system where it undergoes cyclic oxidation by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and reduction by NADPH [1]. Vitamin levels were assayed with Spekol 11, GPx activity was measured with Helios Beta Unican 2000 apparatus and GSH level was assayed with ELX808 reader (BIOTEK Instrument INC., Biokom).

Accuracy and precision of the techniques used to assay biochemical parameters were validated. Accuracy was assessed by analyzing the reference material supplied by *Lyotrol N* and *bioMerieux* (CHOL-C, TG) and by calculating the recovery with an inner standard (vitamins and TBARS). Precision was tested based on repeatability and reproducibility of measurement results (CHOL-C, TG). Reproducibility was checked with *NORTROL 4205 C* control serum. Accuracy was assessed through the recovery value and it ranged between 95% and 109% and the variability coefficient, the indicator of method precision, did not exceed 10%.

Statistical analysis

Statistica 6.0 (StatSoft) was used for statistical analysis of the results. The key descriptive parameters, i.e. arithmetic mean, standard deviation and percentage distribution, were calculated. Wilcoxon's test was used to establish significance of inter-group differences for dependent variables at the significance level of $\alpha = 0.05$.

Results

The results presented in Tables 1 and 2 show only a slight decrease in arterial BP after garlic phytotherapy. A nonsignificant decrease in systolic and diastolic BP of 3 mmHg could be observed in women, whereas in men there was a nonsignificant increase in both values of 2 mmHg each (Tab. 1). Garlic supplementation was found to slightly promote normalization of BP in women as revealed by an increased ratio of subjects with normal systolic and/or diastolic BP by 14% and 9%, respectively. This was paralleled by a 5% decrease in the percentage of male subjects with normal BP. The reported changes, however, did not reach statistical significance (Tab. 2).

Tab. 1. Effect of phytotherapy on blood pressure and selected biochemical parameters

Parameter	Total n = 70		Women n = 38		Men n = 32	
	I	II	I	II	I	II
SBP (mmHg)	141 ± 26	140 ± 25.7	142 ± 23.3	139 ± 22.6	146 ± 28.7	148 ± 28.2
DBP (mmHg)	89 ± 12.3	88 ± 12.4	87 ± 10.8	84 ± 10.2	93 ± 14.4	92 ± 14.1
CHOL-C (mmol/l)	5.64 ± 1.3[a]	5.13 ± 1.3[a]	5.19 ± 1.09	4.87 ± 1.04	6.14 ± 1.58[b]	5.69 ± 1.57[b]
HDL (mmol/l)	1.10 ± 0.42	1.23 ± 0.42	1.09 ± 0.35	1.26 ± 0.34	1.1 ± 0.45	1.29 ± 0.46
LDL (mmol/l)	3.55 ± 1.13[c]	3.05 ± 1.12[c]	3.54 ± 0.91[d]	2.89 ± 0.9[d]	4.16 ± 1.3	3.62 ± 1.31
TG (mmol/l)	1.57 ± 0.81	1.40 ± 0.82	1.74 ± 0.69	1.45 ± 0.68	1.43 ± 0.9	1.38 ± 0.91
TBARS (μmol/l)	4.9 ± 0.9[e]	2.8 ± 0.9[e]	3.4 ± 0.7[f]	2.4 ± 0.8[f]	4.9 ± 0.9[g]	3.1 ± 0.9[g]
Vitamin E (μmol/l)	12.8 ± 6.03[h]	15.8 ± 6.5[h]	11.8 ± 6.03[i]	14.6 ± 5.8[i]	13.2 ± 6.5[j]	16.5 ± 6.73[j]
Vitamin C (μmol/l)	38.6 ± 20.4	42 ± 21	39.2 ± 23.3	40.9 ± 26.1	37.5 ± 17.6	41.5 ± 19.9
Vitamin A (μmol/l)	1.21 ± 0.53	1.24 ± 0.55	1.19 ± 0.41	1.23 ± 0.4	1.25 ± 0.6	1.25 ± 0.61
β-caroten (μmol/l)	0.59 ± 0.38	0.68 ± 0.39	0.62 ± 0.32	0.7 ± 0.32	0.5 ± 0.43	0.68 ± 0.43
GSH (μmol/l)	60 ± 27.5	53.7 ± 27.1	58.5 ± 26.3	69 ± 25.9	59.5 ± 27.2	50.4 ± 26.6
GPx (U/l)	4.8 ± 2.5	5.8 ± 2.4	4.0 ± 1.9	4.2 ± 2.1	5.6 ± 2.7	6.4 ± 2.5

I – before phytotherapy; II – after phytotherapy; * Wilcoxon test; [a, b, c, d, e, f, g, h, i, j] – significant differences $p < 0.05$

Tab. 2. Percentage ratio of subjects within the normal range for the analysed parameters

Parameter	Norm[1]	Range	Total n = 70		Women n =3 8		Men n = 32	
			I	II	I	II	I	II
			% subjects					
SBP (mmHg)	140	> normal	58	51	61	47	51	56
DBP (mmHg)	90	> normal	51	46	46	37	56	56
CHOL-C (mmol/l)	5.2	> normal	60	51	52	42	69	62
HDL (mmol/l)	0.9	< normal	24	24	26	13	28	27
LDL (mmol/l)	4.0	> normal	35	33	33	32	53	53
TG (mmol/l)	2.3	> normal	15	12	11	8	22	19
TBARS (μmol/l)	3.0	> normal	52	41	50	32	53	53
Vitamin E (μmol/l)	11.6–16.2	< normal	38	24	48	32	29	16
Vitamin C (μmol/l)	11.4–25.6	< normal	3	1	4	3	2	0
Vitamin A (μmol/l)	0.35–1.05	< normal	0	1	0	3	0	0
β-carotene (μmol/l)	0.56–0.93	< normal	37	26	37	24	38	28
GSH (μmol/l)	60.5*	< normal	50	53	50	21	50	81
GPx (U/l)	6.43*	< normal	68	53	83	57	54	34

[1] [9]. * Calculated based on the mean values in healthy individuals

The data from Table 1 demonstrate that garlic supplementation decreased serum levels of CHOL-C, TG and LDL and elevated serum levels of HDL in hypertensive subjects, though the changes proved significant only for CHOL-C and LDL. It is noteworthy that garlic supplementation produced a slight increase of 7% to 10% in the ratio of male and female patients with normal total serum cholesterol level (Tab. 2). Study treatment failed to radically change the ratio of patients with normal serum LDL and TG levels, yet it favorably affected serum HDL levels among female subjects as the ratio of women with normal HDL level rose by 13% with the corresponding ratio among male patients remaining virtually unchanged.

TBARS data (Tab.1) confirm significantly lower serum levels of lipid peroxidation products in all female and male subjects as reflected by a smaller percentage of women with elevated TBARS serum levels (Tab. 2). The decrease in serum TBARS levels in hypertensive subjects was accompanied by the increase in serum levels of vitamins E, C, A and of β-carotene, yet the rise was significant only for vitamin E (Tab. 1). Study treatment was found to result in increased percentage of patients with normal plasma concentrations of vitamins E, C and β-carotene and the changes were most pronounced for vitamin E (Tab. 2).

Evaluation of antioxidant potential involved total GSH concentration and glutathione peroxidase activity assays. A distinct, gender-dependent effect of garlic phytotherapy was demonstrated with regard to serum GSH concentration which increased slightly among females and decreased in males (Tab. 1) and was reflected by evident increase in females and decrease in males of the percentage of subjects with normal serum concentration of GSH. Study treatment also insignificantly increased glutathione peroxidase activity in the blood (Tab. 1) and the percentage of patients with normal enzyme activity (Tab. 2).

Discussion

The first indications of hypotensive potential of garlic date back to the 1920s, yet various studies carried out thereafter have provided contradictory data. Our study has attempted to evaluate the effect of garlic supplementation on BP in patients with primary arterial hypertension and without any comorbid disorders. Similarly to our results, the results of some studies on garlic preparations have failed to document any significant effects of garlic on BP in hypertensive subjects [5], though there have been some reports indicating hypotensive potential of garlic or its preparations. Decrease in systolic and diastolic BP observed in humans and animals has been relatively small and averaged 2 to 9% [2, 5]. Durak at al. [12] and Dhawan and Jain [10] detected significant decrease in both systolic and diastolic BP in patients with arteriosclerosis or arterial hypertension following administration of garlic preparations for at least 2–4 months. Banerjee and Maulik [2] reported results of studies where oily garlic preparations produced hypotensive effect in hypertensive individuals.

As concluded by some authors, hypotensive properties of garlic may stem from the action of γ-glutamyl cysteine and fructans which inhibit the activity of angiotensin-converting enzyme, activate nitrogen oxide synthetase and control the production and action of vasoconstricting and vasodilatory substances found in vascular endothelium [3]. Banerjee and Maulik [2] believe that allicin may exhibit hypotensive properties and refer to the results of studies where oral administration of allicin to rats with arterial hypertension resulted in a marked hypotensive effect.

Our results fail to confirm a clear-cut hypotensive properties of allicin derivatives (vinyl-dithiins) which can be considered to be the key active substances in the investigated garlic preparation, though they are not so abundantly present in other commonly used garlic products, such as garlic powder, steam distilled garlic oil or fresh garlic. Cyclic structure of these substances may have a unique role in their metabolism, as other allicin dithio-allyl derivatives are linear molecules. Our results may also reflect an insufficient daily dose of the preparation or insufficient treatment duration, as the study subjects received only a minimal dose recommended by the manufacturer as a supplement to standard antihypertensive pharmacotherapy.

This study also investigated the effects of garlic preparation on lipid metabolism. Based on the changes in the levels of the analyzed parameters, the investigated garlic preparation has been shown to exert a beneficial effect on lipid metabolism. Most publications show that the effects of garlic phytotherapy are the most pronounced during the period of up to 3 months [2]. The effects of garlic and garlic preparations on lipid metabolism have already been discussed in several papers [6, 24] and the beneficial action seems to

be linked mainly to hypolipemic effects of allicin-containing products [2, 28, 31]. Reduced levels of CHOL-C, by 10–13% on average, LDL and TG, by about 13%, have been noted both in healthy individuals and in patients with hyperlipidemia and only few authors reported a significant increase in HDL.

Warshafsky et al. have found out that consumption of 1–1.5 cloves of garlic daily, which is equivalent to about 1.5 mg of allicin, can lower total serum cholesterol in patients with hypercholesterolemia by about 9% [28]. This has been confirmed by our study where garlic preparation lowered blood cholesterol level by 9.2% on average. Four months of supplementation with garlic extract in patients with elevated cholesterol level (> 200 mg/dl) and with arterial hypertension produced a significant decrease in CHOL-C, LDL and TG levels, as well as elevation of HDL as reported by Durak et al. [12]. Our study treatment resulted in slight decrease in blood TG levels in most subjects, though a highly significant decrease could be observed in patients with pre-treatment TG level three to four times higher than normal – the highly elevated levels of 400–600 mg/dl decreased to 200––250 mg/dl following garlic phytotherapy.

It seems interesting to compare the results of our study to those of Zhang et al. [31], where the authors detected a variable and gender dependent effect of oily garlic preparations on lipid parameters in healthy individuals. The authors have found out that 11 weeks of garlic phytotherapy induced a nonsignificant decrease in CHOL-C and LDL with concurrent HDL rise in women, whereas in men the effects were opposite – an increase in CHOL-C and LDL and a decrease in HDL. Our study has failed to document any such gender effect on the analyzed lipid metabolism parameters. Nevertheless, regarding HDL we have documented a more pronounced beneficial effect of garlic in females as compared to males – our garlic phytotherapy increased the percentage of subjects with normal HDL by 13% among females, though the percentage among males remained unchanged (Tab. 2).

Though the referenced studies investigated various types of garlic preparations, only some of them analyzed oily garlic preparations, despite their wide availability and easy absorption from gastrointestinal tract [31]. It is for this reason that we focused on an oily garlic preparation to confirm its hypolipemic activity. Hypolipemic properties of allicin and its derivatives are thought to be mainly linked to their inhibitory effect on cholesterol synthesis and to decreased intestinal absorption of cholesterol [2, 3] as other hypolipemic drugs [23].

In a study similar to ours, healthy individuals received oily garlic preparations and had their lipid parameters and BP analyzed. Four months of such treatment did not confirm hypolipemic, hypotensive or antioxidant action of garlic [28] based on BP values, cholesterol level or serum vitamin E concentration. It must be stressed, however, that the authors investigated young and healthy subjects aged 17 to 45 years who received a total daily dose of garlic preparation that differed from the one given in our study.

Several reports focused on antioxidant properties of some active compounds found in garlic, e.g. allicin. Our study analyzed and confirmed antioxidant potential of garlic supplementation in hypertensive patients and the results confirm the opinions of several authors [3, 9] that allicin and its metabolites are scavengers of free radicals as shown through significantly decreased levels of lipid peroxidation products, expressed as TBARS levels, in the study subjects.

Earlier clinical and experimental studies have suggested that garlic and its allicin containing preparations can lower the levels of oxidative stress biomarkers, such as TBARS and malonyldialdehyde (MDA) [10–12] and increase the activity of superoxide dismutase, catalase and glutathione peroxidase [3]. This has been documented by Dhawan and Jain [10] study who administered powdered garlic to hypertensive patients for two months with resulting significant decrease in lipid peroxidation products and augmentation of total antioxidant plasma potential. Huang et al. [18] have demonstrated *in vitro* that dithioallyl compounds of garlic, including allicin metabolites, i.e. diallyl sulfides and diallyl disulfide, reduce oxidation of LDL and inhibit decrease in catalase and glutathione peroxidase activity, as well as plasma vitamin E level in diabetic patients. Durak et al. [11] have shown that garlic extract taken by patients with arteriosclerosis for six months can decrease serum MDA levels, but does not change the activity of superoxide dismutase or glutathione peroxidase. In numerous reports garlic and its preparations have been found to elevate tissue glutathione (GSH) levels [3, 4, 19]. Our study has confirmed these observations to a certain extent, particularly in female subjects, as the effect of garlic phytotherapy on total GSH level in blood differed between male and female subjects. The inter-gender difference is most probably linked to metabolic differences. Nevertheless, it must be stressed that our re-

sults may have been affected by numerous endo- and exogenous factors beyond the focus of this study.

There are few published data on the effects of garlic preparations on the levels of antioxidant vitamins. Slight increase in vitamin C and E concentrations observed in our hypertensive subjects following supplementation with garlic preparation (Tab. 1) might have reflected protective effects of garlic based antioxidants on serum levels of these vitamins, especially vitamin E, which has been projected to be the most effective antioxidant for reducing lipid peroxidation [7].

It should also be stressed that the investigated preparation contained oil – itself a rich natural source of vitamin E – so its significant serum level increase might have reflected this extra intake of the vitamin delivered with the oil fraction of the capsules. It must be also taken into account that rape seed oil in the capsules might have augmented bioavailability of other lipid-soluble dietary vitamins, such as vitamin A and β-carotene.

In conclusion, the garlic phytotherapy used in our study appears to lower oxidative stress related to hypertension and to decrease lipid oxidation, thus, it can be assumed that it decreases the risk of atherosclerosis in these patients. Minor degree of BP normalization observed among subjects receiving oily garlic supplementation for one month suggests the need for further, long-term studies and confirms that comparative analysis should involve the same type of garlic formulations, as garlic preparations can significantly differ with regard to the contents of biologically active substances. Our results seem to confirm hypolipemic potential of the analyzed garlic preparation which appeared particularly pronounced among subjects with hypertriglyceridemia. Therefore, it may be hypothesized that it is not only alliin and allicin, but also some of their sulfide derivatives, e.g. vinyl dithiins, that can exhibit such properties.

Based on the positive effects of the investigated oily garlic preparation, even though these could not be observed for all the analyzed parameters, it seems justified to attempt to recommend its use as an adjunct agent in the treatment of hypertension.

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