

Effect of Garlic Extract on Esterases Activity in Tissues and Serum of Male Mice

Jamaan S. Ajarem and Mohammad Ahmad

Zoology Department, College of Science, King Saud University,
P.O. Box 2455, Riyadh 11451, Saudi Arabia

(Received 26/6/1996; accepted for publication 18/3/1997)

Abstract. Male Swiss-Webster mice were injected (injectable volume 0.1 ml) subcutaneously with fresh garlic extract at doses of 1, 5 and 10 mg/kg body weight. The injections were given daily for 5 days, and on the last day the mice were subjected to biochemical investigations. Acid (AcPase) and alkaline phosphatase (AlPase) activities were stimulated in the serum and in liver tissue. Brain acetylcholinesterase (AChE) activity was inhibited in the treated males only at the highest dose (10 mg/kg). It is suggested that the enzymes AcPase, AlPase and AChE can be used as convenient markers in teratological studies of garlic and its isolated active fractions.

Introduction

Garlic (*Allium sativum* L.) in the family Alliaceae is a vegetable food item, widely used both in the West and the East. It has traditionally been reported to have expectorant, diaphoretic, disinfectant and diuretic properties [1]. Recently, garlic has also been reported to have lipid lowering properties in blood [2,3].

Garlic has long been recognized as a herbal medicament with antifungal and antibacterial properties, used to treat the infections of the skin [4], but recent studies have indicated that it is also a contact dermal sensitizer due to the presence of active substances like diallyldisulphide [5] and alliin [3]. Garlic can be prepared in various forms such as paste, ointment, juice or oil, to be used alone or in combination with other medicaments for the treatment of vaginal and scalp infections [6, pp. 110-115].

Biochemical investigations of the effect of garlic in rats are limited to lipid metabolic studies only. It has been shown that garlic extract has preventive effects on atherosclerosis [7,8,9], inhibits lipogenesis [10,11], inhibits hepatic triglyceride and

lowers lipid contents in injured liver [12]. A case of urticaria was observed in horses due to allergic reaction from garlic feeding [13].

The purpose of this study was to investigate the effect of fresh garlic extract on the level of esterase (AcPase, AlPase and AChE) activities in liver, brain and blood serum of male mice.

Materials and Methods

Animals

Adult male Swiss-Webster albino mice (*Mus musculus*) were used in the present study. The animals, of approximately the same age and weight, were housed (six per cage) in plastic cages measuring 30 × 12 × 11 cm, in environmentally controlled conditions with an ambient temperature of 20-22°C and a 12-hour light/dark cycle. Unless otherwise stated, Pilsbury's mouse food and water were provided *ad libitum*.

Garlic extract

Fresh garlic bulbs were purchased from the local markets in Riyadh. A known weight of peeled garlic (1, 5 and 10 g) was minced finely, squashed and mixed separately with 100 ml of normal saline (0.9% NaCl) and electromagnetically stirred at low speed overnight at room temperature. Subsequently, the mixture was filtered under a low vacuum and the filtrate was used in a constant injectable volume (0.1 ml) containing 1, 5 and 10 mg garlic extract per kg mouse body weight. Each dose was injected subcutaneously for five consecutive days. The control animals received saline (0.9%) injections only. On the last day, the animals were subjected to biochemical investigations.

Blood collection

The blood sample from each animal was collected from their retro-orbital plexuses with the help of capillary tubes. The blood was allowed to clot and the clear serum was isolated and stored at 0°C until required for enzyme assays.

Tissue homogenate preparation

After collecting blood sample, the experimental mice were killed by decapitation. The brain and liver were immediately removed, rinsed gently in physiological saline (0.9%), blotted on a Whatman filter paper, weighed and frozen. A 10% (w/v) homogenate of each tissue was prepared in teflon-glass homogenizer at $4 \pm 1^\circ\text{C}$, centrifuged at $1000 \times g$ for 10 min to remove cell debris and the supernatant was used for enzyme assays.

Enzyme estimations

The brain homogenate was prepared in ice-cold phosphate buffer, 0.067 M, pH 7.2,

and its acetylcholinesterase (AChE) activity was estimated using acetylcholine chloride as the substrate [14] and expressed as μ moles acetylcholine chloride hydrolysed per gram wet tissue weight per hour at $37 \pm 1^\circ\text{C}$.

The liver was homogenized in chilled 0.25 M sucrose solution and the protein content was estimated according to the method of Lowry *et al.* [15]. The levels of acid phosphatase (AcPase) and alkaline phosphatase (AlPase) were estimated in liver homogenate and blood serum, using sodium p-nitrophenol phosphate as the substrate [16]. The specific activity of these phosphomonoesterases was expressed in liver as 'n' moles p-nitrophenol liberated per mg protein per minute at $37 \pm 1^\circ\text{C}$, and in serum, as I.U. per litre.

Statistical analysis

The data of enzyme estimations were analysed by ANOVA using the Minitab programme. Subsequently, the data were subjected to student's t-test [17].

Results

The effect of garlic extract on the level of AcPase and AlPase activity in liver is shown in Fig. 1. The levels of these phosphomonoesterases are significantly ($P < 0.05$) higher in the treated groups.

In serum (Fig. 2), AcPase and AlPase are significantly stimulated ($P < 0.05$ and $P < 0.02$) at higher doses of garlic (5 and 10 mg/kg respectively).

AChE activity is significantly ($P < 0.05$) inhibited only at the highest dose (10 mg/kg) of garlic; the lower dose (5 mg/kg) appear to inhibit the AChE activity but the inhibition is statistically insignificant (Fig. 3). The lowest dose (1 mg/kg) remains ineffective.

Discussion

The enzymes AcPase and AlPase are known indicators of the well-being of eukaryotic cell membranes [18,19,20], and are frequently associated with the transport of nutrients across the cell membranes [21,22]. Although, the levels of these phosphomonoesterases are elevated significantly in liver and serum of the male mice, the garlic effect appears to be different at these two sites. Since the garlic extract is injected subcutaneously, the active substance present in garlic, possibly, acts directly on the serum phosphatases and the stimulatory effect produced therein is important from the pharmacokinetic point of view. Whereas absorption of garlic extract in the liver is probably delayed which ultimately shows a steady level of stimulation of the phosphatase activity. In both cases, however, an increase in the levels of these phosphomonoesterases might have led to variations in the phosphate pool of the animal

which might lead to disturbed energy sources available to the animal with the consequent disturbances in its metabolism [23]. The other enzyme tested in the brain tissue is AChE, an enzyme required for hydrolyzing acetylcholine that has been implicated as an important neurotransmitter in aggressive behavior [24]. The AChE is inhibited in the present study by the garlic extract at the highest dose only.

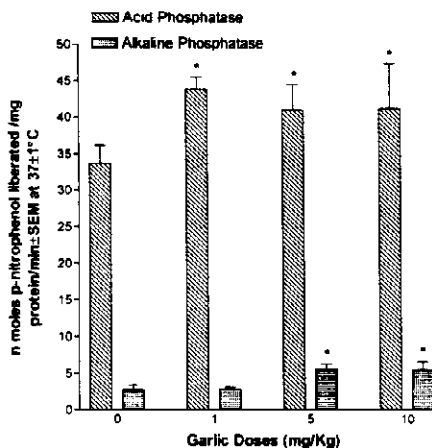


Fig. 1. Effect of garlic extract on the level of activity of phosphomonoesterases in the liver of male mice.

*Indicates ($P < 0.05$) as compared to controls.
Number of mice in each group was ten.

The pharmacological effects of garlic are primarily of interest in humans because of its principal usage as a foodstuff, with a secondary use as a topical treatment. Whilst statistically significant biochemical changes have taken place in the present study following subcutaneous injection, this mode of entry into the body is a highly abnormal for garlic. Since garlic is known not to be toxic to humans by oral administration, changes in serum enzyme activities could be investigated on human volunteers to understand its relevance from pharmacological point of view.

However, the enzymes AcPase, AlPase and AChE are affected substantially by the garlic extract and could be used as convenient markers in teratological studies as suggested earlier by Ajarem and Ahmad [25]. It would be worth investigating the effect of the sensitive active substances present in garlic [3, 5] in experimental models to understand the interactions between biochemical changes and the usage of garlic as a traditional medicament.

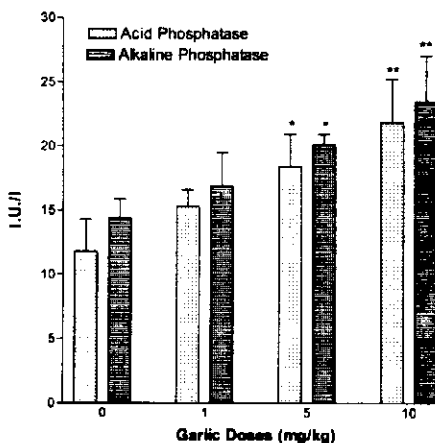


Fig. 2. Effect of garlic extract on the level of activity of phosphomonoesterases in the serum of male mice.

*Indicates ($P < 0.05$) as compared to control.

**Indicates ($P < 0.02$) as compared to control.

Number of mice in each group was ten.

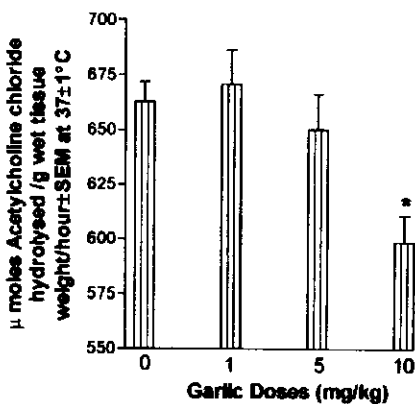


Fig. 3. Effect of garlic extract on the level of acetylcholinesterase activity in the brain of male mice.

*Indicates ($P < 0.05$) as compared to control.

Number of mice in each group was ten.

References

- [1] Lee, T.Y. and Lam. "Contact Dermatitis Due to Topical Treatment with Garlic in Hong Kong." *Contact dermatitis*, 24, No. 3 (1991), 193-196.
- [2] Reynolds, J.E.F. *Martindale. The Extra Pharmacopoeia*. 29th ed. London: The Pharmaceutical Press, 1989.
- [3] Mader, F.H. "Treatment of Hyperlipidaemia with Garlic-powder Tablets." *Arzneim. Forsch./Drug Res.*, 40, No. 10 (1990), 1111-1116.
- [4] Elnima, E.I., Ahmed, S.A., Mekkawi, A.G. and Moosa, J.S. "The Antimicrobial Activity of Garlic and Onion Extracts." *Pharmazie*, 38 (1983), 747.
- [5] Lembo, G., Balato, N., Patruno, C., Auricchio, I. and Ayala, F. "Allergic Contact Dermatitis due to Garlic (*Allium sativum*)." *Contact Dermatitis*, 25, No. 5 (1991), 330-331.
- [6] Jiangsu New Medical College. *Dictionary of Chinese Medicine*. Shanghai: Shanghai Science and Technology Press, 1977.
- [7] Jain, R.C. "Effects of Alcoholic Extract of Garlic in Atherosclerosis." *Am. J. Clin. Nutr.*, 31 (1978), 1982-1983.
- [8] Sainani, G.S., Desai, D.B., Natu, M.N., Katrodia, K.M., Valame, V.P. and Sainani, P.G. "Onion, Garlic and Experimental Atherosclerosis." *Japan. Heart J.*, 20 (1979), 351-357.
- [9] Kamanna, V.S. and Chandrasekhara, N. "Effect of Garlic (*Allium sativum* Linn) on Serum Lipoproteins and Lipoprotein Cholesterol levels in Albino Rats Rendered Hypercholesteremic by Feeding Cholesterol." *Lipids*, 17 (1982), 483-488.
- [10] Chi, M.S. "Effects of Garlic Products on Lipid Metabolism in Cholesterol-fed Rats." *Proc. Soc. Exp. Biol. Med.*, 171 (1982), 174-178.
- [11] Chang, M.L.W. and Johnson, M.A. "Effect of Garlic on Carbohydrate Metabolism and Lipid Synthesis in Rats." *J. Nutr.*, 110 (1980), 931-936.
- [12] Kagawa, K., Matsutaka, H., Yamaguchi, Y. and Fukuhama, C. "Garlic Extract Inhibits the Enhanced Peroxidation and Production of Lipids in Carbon Tetrachloride-induced Liver Injury." *Japan. J. Pharmacol.*, 42 (1986), 19-26.
- [13] Miyazawa, K., Ito, M. and Ohsaki, K. "An Equine Case of Urticaria Associated with Dry Garlic Feeding." *J. Vet. Med. Sci.*, 53, No. 4 (1991), 747-748.
- [14] Hestrin, S. "The Reaction of Acetylcholine and Other Carboxylic Acid Derivatives with Hydroxylamine and Its Analytical Application." *J. Biol. Chem.*, 180 (1949), 249-261.
- [15] Lowry, O.H. Rosebrough, N.J., Farr, A.L. and Randall, R.J. "Protein Measurement with Folin Phenol Reagent." *J. Biol. Chem.*, 193 (1951), 266-275.
- [16] Bergmeyer, H.U., Gawhn, K., and Grassl, M. "Enzymes as Biochemical Reagents." In: Bergmeyer, H.U., ed. *Methods of Enzymatic Analysis*. 2nd ed. Vol. 1., New York: Academic Press, 1974.
- [17] Yamane, T. *Statistics an Introductory Analysis*. London: Harper and Row, 1973.
- [18] Hopfer, U. "Transport in Isolated Plasma Membranes." *Am. J. Physiol.*, 234 (1978), 89-96.
- [19] Oaks, J.A., Knowles, J. and Cain, G.D. "A Simple Method of Obtaining an Enriched Fraction of Tegumental brush Border from *Hymenolepis diminuta*." *J. Parasitol.*, 63 (1977), 476-485.
- [20] Rahman, M.S., Mettrick, D.F. and Podesta, R.B. "Use of Saponin in the Preparation of Brush Border from Parasitic Flatworm." *Can. J. Zool.*, 59 (1981), 911-917.
- [21] Starling, J.A. "Tegumental Carbohydrate Transport in Intestinal Helminth: Correlation between mechanisms of membrane Transport and the Biochemical Environment of Absorptive Surfaces." *Trans. Am. Microsc. Soc.*, 94 (1975), 508-523.

- [22] Ussing, H.H., Ery, D. and Lassen, U. "Transport Pathways in Biological Membranes." *Annu. Rev. Physiol.*, 36 (1974), 17-49.
- [23] Wilson, J.G. "Mechanism of Teratogenesis." *Am. J. Psychiatry.*, 136 (1973), 129-132.
- [24] Allikmets, L.H. "Cholinergic mechanisms in Aggressive Behavior." *Med. Biol.*, 52 (1974), 1930.
- [25] Ajarem, J.S. and Ahmed, M. "Behavioural and Biochemical Consequences of perinatal Exposure of mice to instant Coffee: a Correlative Evaluation." *Pharmacol. Biochem. Behav.*, 40, No. 4 (1991), 847-852.

دراسة تأثير مستخلص الثوم على مستوى الاستريز في الأنسجة ومصل الدم لذكور الفئران المختبرية

جمعان سعيد عجارم و محمد أحمد

قسم علم الحيوان، كلية العلوم، جامعة الملك سعود، ص.ب. ٢٤٥٥،

الرياض ١١٤٥١، المملكة العربية السعودية

(استلم في ١٠/٢/١٤١٦هـ، وقبل للنشر في ٩/١١/١٤١٧هـ)

ملخص البحث. درس تأثير مستخلص الثوم الطازج على نشاط بعض الأنزيمات الموجودة في المخ، مصلى الدم والكبد لذكور الفئران المختبرية، وذلك بحقنها تحت الجلد يوميا ولمدة خمسة أيام وبتركيزات مختلفة من مستخلص الثوم هي ١، ٥ و ١٠ كجم/ وزن الجسم، بالإضافة إلى الكنترول. درس نشاط أنزيم الفسفاتيز الحامضي والقاعدي في كل من الكبد ومصلى الدم، وأنزيم أستيايل كولين أستريز في المخ بعد الانتهاء من التجربة مباشرة.

تشير النتائج إلى أن المعالجة بمستخلص الثوم أحدثت زيادة في نشاط أنزيم الفسفاتيز الحامضي والقاعدي في الكبد ومصلى الدم، بينما قلّ نشاط أنزيم الأستيايل كولين أستريز في المخ عند الجرعة العالية.

وتشير النتائج أيضاً إلى أن أنزيم الفسفاتيز الحامض والقاعدي وأنزيم الأستيايل كولين يمكن أن تكون مؤشرات يستدل بها لمعرفة التغييرات المختلفة التي يحدثها الثوم.