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Mechanism of Action of the Egyptian Garlic "*Allium sativum*" on Excitable Tissues

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Abstract: Garlic has been used for millennia in the traditional medicine practice of many cultures to treat cardiovascular and others disorders. In this study, mechanism of action of garlic on several excitable membranes have been discussed. three groups of isolated toads' hearts were used to investigate the effect as well as the mechanism of garlic solution (4 μg ml^{-1}) on the physiological properties of the myocardium through the electrocardiogram (ECG) recordings. A significant decrease of the heart rate (negative chronotropism) accompanied by significant increase in the conduction time (positive dromotropism) were resulted following the garlic application and sustained 20 min. Several cases of heart block were recorded after 10 min from garlic perfusion. Application of atropine didn't abolish the effect on the myocardium. Moreover, an increase of the ventricular voltage has been noticed after 10 min from the garlic application. The calcium-channel blocker verapamil apparently abolished the positive inotropic effect of the garlic. Smooth-muscle activity was studied before and after garlic application (4μg ml^{-1}). An increase of the spontaneous contraction of rabbit-duodenum was established by garlic solution. Blockade the M_3 muscarinic receptors of the smooth muscle by atropine sustained normal contraction. Perfusion of toad gastrocnemius-muscle/sciatic-nerve preparation with (4μg ml^{-1}) garlic solution weakened the mechanical contraction of the muscle without recovery. Pretreatment with the nicotinic receptor antagonist gallamine sustained normal contraction of the skeletal muscle.

Key words: Garlic (*Allium sativum*), ECG, muscles, T-type calcium channels, nicotinic and muscarinic receptors

Introduction

Garlic belongs to the oldest traditional medicinal plants. It has been mentioned 1500 A.D. in an ancient Egyptian medical script, the Papyrus Ebers (Weiss and Fintelmann, 2000). It can be assumed that the Egyptians workers needed the garlic to combat the then widespread problem of amebic dysentery. In modern times, they used the garlic for both prevention and cure (Weiss and Fintelmann, 2000). Recently, Antitumoral and antiarteriosclerotic effects are attributed to garlic-derived drugs (Gao *et al.,* 1999). The beneficial effects claimed for the use of garlic as a nutritional-supplement include detoxification, antioxidation, antifungal, antibacterial activity and tumor suppression have discussed by several investigators (Svendsen *et al.,* 1994; Ledezma and Castro 1998). The effect of standardized garlic power on arterial oxygenation and dyspnea in subjects with hepato-pulmonary syndrome (HPS) was investigated by (Abrams and Fallon, 1998), their results claimed that garlic improved arterial oxygenation in younger subjects or those with lower macro-aggregated albumin shunt fractions.

Despite the long tradition of use of garlic as a medicinal herb, pharmacists only started to investigate its constituents in the 20th century. Agarwal (1996) reported a description of the chemical constituents of *Allium sativum* exhibiting antithrombotic actions which include: alliin, ajoene, amino acid glycosides, adenosine, adenosine deaminase inhibitor and cyclic AMP phosphodiesterase inhibitor. He described also the lipid-lowering and cardiovascular effects and antitumour actions of garlic. On the other hand, sulphur compounds from Allium species were pharmacologically investigated to have activity against infection and in the prevention of cardiovascular disorders and cancer (Vannereau and Mellouki, 1996; Gao *et al.,* 1999).

Aged garlic is suggested to have an effective antioxidant character in preventing or treating disorders related to endothelial cell injury associated with free radicals (Wei and Lau, 1998). Kim *et al.* (2000) determined the characteristics of garlic extract-induced relaxation in rat-isolated pulmonary arteries and its protective effect against hypoxic pulmonary vaso-constriction. Recently, a review of possible therapeutic applications of nutritional supplements in cardiovascular diseases has been introduced by (Gaytan and Prisant, 2001), these supplements included a mixture of vitamins, selenium, garlic, flavenoids, phytoestrogens and ubiquinone.

Although numerous studies point to the fact that garlic could normalize the plasma lipids, enhances fibrinolytic activity, inhibits plasma aggregation and reduces blood pressure and glucose (Kleijnen *et al.,* 1989; Rahman, 2001) as well as its beneficial effect on several organs and

Table 1: Effect of direct perfusion of garlic solution (4μg ml^{-1}) on the heart rate, conduction time (P-R interval), depolarization voltage (R-wave amplitude) and repolarization voltage (T-wave amplitude) of isolated toad' hearts

Time recording	Heart rate (beat/min)	P-R interval ms	R-wave mV	T-wavemV
Zero (pre-perfusion)	62.7±2.07	260±8.7	0.82±0.1	0.32±0.20
5 min	49**±5.4	370**±20	0.93±0.06	0.28±0.04
10 min	38.8**±8.2	520**±48.9	1.3*±0.2	0.35±0.15
15 min	52.5**±2.5	450**±28.9	1.2*±0.2	0.47±0.20

A Values represents means ± SE (n=9/group)
B (*,** p< 0.01, 0.001) significantly different form zero values (ANOVA single factor)

tissues. There is no details data about its physiological effect on the excitable muscles contraction. This study was to determine the mechanism of action of the Egyptian fresh garlic on the myocardium, skeletal and smooth muscles contraction.

Materials and Methods

Chemicals

Garlic (*Allium sativum*): Highly concentrated Egyptian garlic powder prepared from organically grown garlic without using chemical fertilizers or pesticides was produced and obtained from Sekem Lab. For biological products, Cairo, Egypt. Garlic powder was dissolved in frog Ringer solution or Tyrode solution to a concentration of (4μg ml^{-1}) for the *in vitro* ECG recording of isolated toads' hearts and Gastrocnemius-sciatic/nerve preparation.

Animals: In this *in vitro* study, adults male toads '*Bufo regularis*' 34-40 g body weight, male and female rabbit weighting about 0.5 kg were used.

Experimental design and doses

Cardiac muscle: Experiments on cardiac muscle were carried out on isolated toad' heart preparations. Three groups each of 9 animals were used. They were directly perfused with (4μg ml^{-1}) garlic solution. Electrocardiogram (ECG) data were recorded directly from the surface of the heart before the garlic application which serve as zero point and self control (pre-perfusion). Then, after the garlic perfusion, signals were recorded every 5 min for 20 minutes. ECG was recorded by a multi-pen rectilinear recorder (DBE, UK) with paper speeds of 10 mm/sec.

Skeletal muscle: Toad gastrocnemius-muscle/sciatic-nerve preparations (n=6) were used to study the effect of garlic on the mechanical contraction of skeletal muscle. Muscles were directly perfused with (4μg ml^{-1}) of garlic solution. Activity of the skeletal muscle was recorded by the Universal kymograph (Harvard Apparatus Ltd, TN8 6HE, England) of paper speed of 1.4 mm/sec. The sciatic nerve was electrically stimulated with 1V, 0.1 ms Width, 1.25 mm/sec speed and 5 Hz square-pulse waves.

Smooth muscle: Duodenums of rabbit was used to study the effect of garlic (4μg ml^{-1}) on the smooth muscle contraction (n=3). The gut preparation was placed in a bowl of tyrode solution and was mounted in a mono-organ bath with an inner vessel 40 mm. Spontaneous activity of smooth muscle was recorded by an ink Kymograph (model 10500, Bioscience, UK) with a paper speed of 1 mm/sec.

Antagonists: Atropine sulphate was purchased from (Memphis Co, Dorpharm and Chem, Ind. Cairo). A dose of 4μg ml^{-1}-Ringer was used on heart preparations and a dose of 5X10^{-6} M in saline was added into smooth-muscle preparations. Verapamil hydrochloride, the calcium channel antagonist was obtained from (ADWIC Pharmaceutical Division, El Nasr Pharm. Chem. Co., Egypt). It was applied directly to the perfused toad hearts at a dose (5μg ml^{-1}). Gallamine triethiodide, a nicotinic antagonist for skeletal muscle was used in a dose (3 μg ml^{-1}) in Ringer solution, it was purchased by Alexandria CO. Pharmaceuticals, Alexandria, Egypt. Propranolol (non-selective adrenergic receptor antagonist) was used at a dose of (2 x 10^{-5} M) in the smooth muscle experiments. It was purchased from (Memphis Co, Dorpharm and Chem, Ind. Cairo).

Statistical analysis: Changes of the heart rate (HR) and the other electrocardiographic parameters (P-R interval, R-wave amplitude and T-wave-amplitude) were expressed as means ± standard error. The data were analysed by ANOVA at a statistical probability of $P<0.05$ and $P<0.001$.

Results

Effects of the Egyptian garlic on the myocardium activity: The effect of direct perfusion of the Egyptian garlic solution (4μg ml^{-1}) on the heart rate, conduction time, depolarization voltage and repolarization voltage of the isolated toads hearts (group 1) are shown in Table 1. It is clear from these data that garlic induced highly significant bradycardia accompanied with highly significant increase of the atrioventricular conduction time (P-R interval) after 5 min from garlic perfusion and sustained 15 min. On the other hand, depolarization voltage has been increased significantly at 10 and 15 min recording (Table 1). Although there is an increase in the repolarization voltage

Plate 1

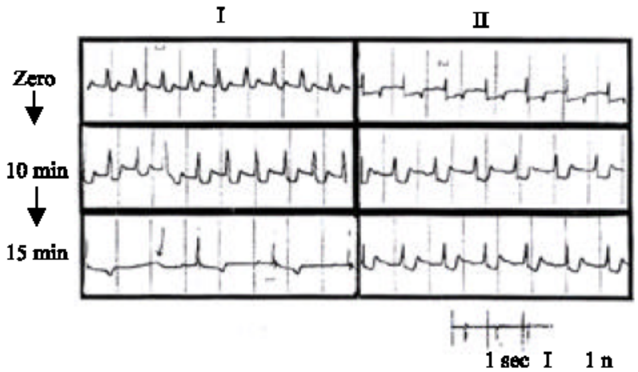

Plate 1: Two examples (I, II) of recorded electrocardiogram showing the effect of direct perfusion of isolated toads' hearts with the Egyptian garlic solution (4 μg ml^{-1}). The arrow shows second degree heart block

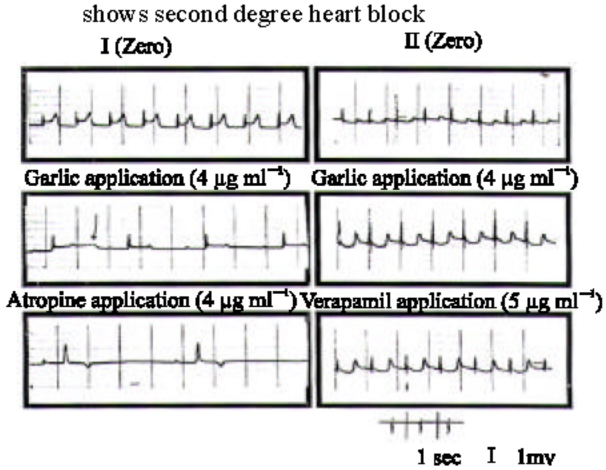

Plate 2: Effect of atropine (4 μg ml^{-1}) and verapamil (5μg ml^{-1}) direct application on isolated toads' hearts perfused with the Egyptian garlic solution (4 μg ml^{-1}). The arrow shows second degree heart block

of the toad' heart after 15 min from the garlic perfusion, but it isn't statistically significant. Examples of *in vitro* electrocardiographic representation of garlic effect is demonstrated in Plate 1, they showed the different changes induced by the garlic application on the myocardium. Cases of second degree heart block has been obtained after 15 min from the garlic perfusion. A noticeable ST depression was also noticed after 10 min.

Mechanism of action of the Egyptian garlic on the myocardium: In the second group of isolated toads hearts, atropine was used as a muscarinic-cholinergic antagonist following the changes of the ECGs parameters as a result of garlic application. Fig. 1 represented the

Plate 3

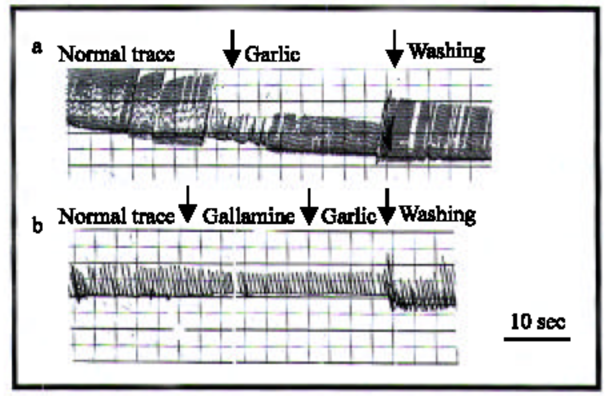

Plate 3: Effect of direct perfusion with (4μg ml^{-1}) of the Egyptian garlic solution on the mechanical activity of the toads gastrocnemius muscle
a Effect of garlic
b Pretreatment with the blocker (gallamine)

significant percentage of changes of the heart rate of the isolated hearts with the application of atropine following 15 min from the garlic perfusion ($P<0.05$). As clear from the figure, atropine didn't abolish the negative decrease of the heart rate induced by the garlic (-27%). Moreover, cases of bradycardia and A-V block were sustained even after atropine application as clear in Plate 2.

The increase of conduction time of the isolated hearts as a result of garlic perfusion was significant and sustained after the atropine application. Fig. 2 shows the percentage of changes of P-R interval (73 % & 70%) after garlic and atropine respectively ($P<0.005$). Plate (2, I) represents examples of the P-R interval changes of the ECG after the perfusion of garlic and their stability after the application of atropine.

The percentage of changes of the ventricular voltage of the isolated hearts (group 3) perfused with garlic followed by the application with verapamil have been demonstrated in Fig. 3. As shown from the figure, the significant increase of R-wave amplitude ($P<0.05$) as a result of garlic (+42%) was absolutely abolished by verapamil. Plate (2, II) shows the effect of verapamil on the increase of the ventricular depolarization on the ECG recordings of the isolated hearts perfused with garlic.

Effect of the Egyptian garlic on the activity of skeletal muscle: Plate 3 demonstrates the activity of toad skeletal muscle (gastrocnemius muscle) before and after the application of garlic (4 μg ml^{-1}). A decrease in muscle contraction was noticed following garlic perfusion (Plate 3, a). Blocking the nicotinic receptors with gallamine before garlic application almost sustained normal contraction of the muscle (Plate 3, b).

Plate 4

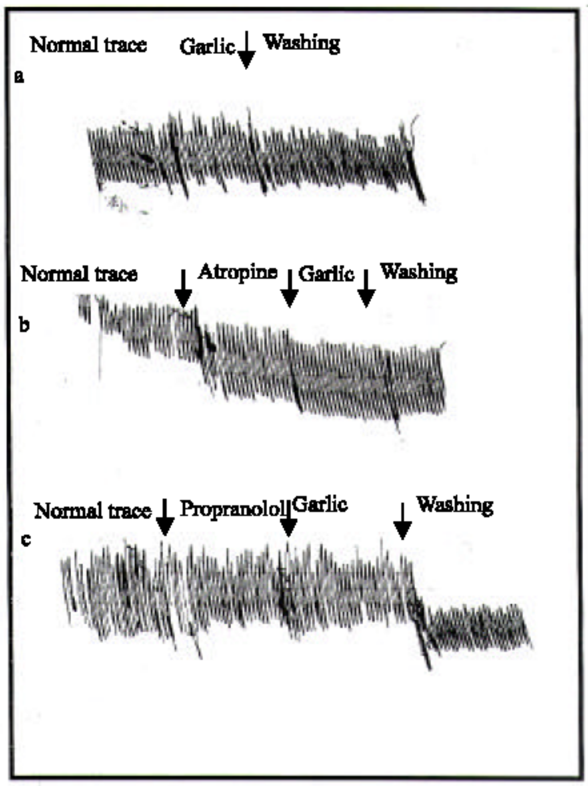

Plate 4: Effect of direct perfusion with ($4 \mu g\ ml^{-1}$) of the Egyptian garlic solution on the spontaneous activity of the smooth muscle of rabbit duodenum
a Effect of garlic
b Pretreatment with the blocker (atropine)
c Pretreatment with the blocker (propranolol)

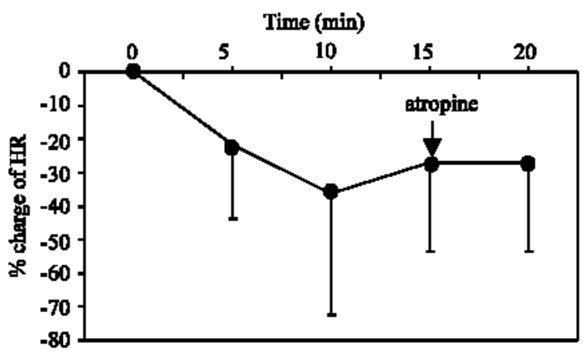

Fig. 1: Effect of atropine on the percentage of change of the heart rate (HR) of the isolated toads' hearts after the perfusion with the Egyptian garlic solution ($4\ \mu g\ ml^{-1}$). Values represents means ± SE (n= 9/group)

NB: All the values of the percentage are significantly different from the zero values (ANOVA, P< 0.05)

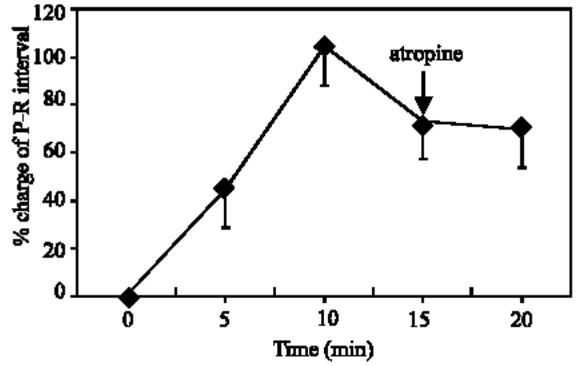

Fig. 2: Effect of atropine on the percentage of change of the conduction time (P-R interval) of the isolated toads' hearts after the perfusion with the Egyptian garlic solution ($4\ \mu g\ ml^{-1}$). More details are shown in Fig. 1

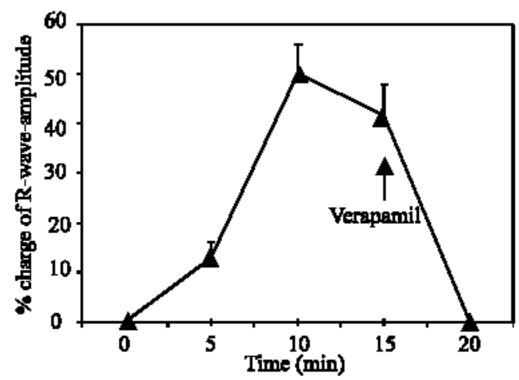

Fig. 3: Effect of verapamil on the percentage of change of the R-wave amplitude of the isolated toads' hearts after the perfusion with the Egyptian garlic solution ($4\ \mu g\ ml^{-1}$). More details are shown in Fig.1

Effect of the Egyptian garlic on the activity of smooth muscle: Plate 4 demonstrates the activity of the smooth muscle (duodenum of rabbits) before and after the garlic application ($4\ \mu g\ ml^{-1}$). As clear from the plate, garlic increased the contraction of the duodenum (Plate 4, a). Blocking the muscarinic receptors with atropine sustained normal contraction of the smooth muscle (Plate 4, b). Moreover, blocking the Ü adrenergic receptors with the non-selective antagonist propranolol sustained the increased of the muscle contraction (Plate 4, c).

Discussion

Interest in herbal medicine is enjoying a renaissance at present. Garlic (*Allium sativum*) is an intriguing herb with a long history of medicinal use for a variety of diseases including ringworm infections (Taha and Tosson, 1980).

Although the huge results deals with the protective and therapeutic usage of garlic in different cells and organs, its precise effect on the excitable tissues needs more investigations.

The present work deals with the effect as well as the mechanism of action of the Egyptian garlic on the myocardium, skeletal and smooth muscle contraction. As clear from the present data, garlic induced negative chronotropism and negative dromotropism at the isolated hearts. The muscarinic cholinergic antagonist atropine didn't abolish the noticed effect which stimulates an argument of garlic-direct action on the myocardium. Moreover, the positive inotropic effect of garlic has been abolished by verapamil the calcium-channel antagonist which in the same manner postulates the direct action of garlic on the heart function. A direct action of garlic on rat pulmonary isolated artery was reported by (Kim *et al,* 2000). They concluded its direct action due to nitric oxide-induce protection against hypoxic pulmonary vasoconstriction. On the other hand, an antioxidant effect of oil-garlic against nicotine–induced lipid peroxidation was established (Helen *et al.,* 1999). Others researchers noticed that the administration of nitric oxide synthase inhibitor inhibited the vasodilatory effect of garlic and they concluded that garlic blocks hypoxic pulmonary hypertension *in vivo* and there is a combination of endothelium-dependent and independent mechanisms for its effect in pulmonary arterial rings (Fallon *et al.,* 1998).

The above reports of the direct action of garlic support our finding on the myocardium since, the bradycardia and prelongation of the conductance distance were sustained and increased after atropine application. Thence, a postulation that garlic can exert a local auto-regulation mechanism on the myocardium can be profound. This local mechanism can be guessed from the rich garlic constituents. Garlic contains minerals like calcium, iron, potassium, germanium, selenium and zinc. All of these add up to its mechanism, for example, the action of potassium ions is complicated and could manifest the bradycardia and the heart block, since a moderate increase in extracellular potassium K^+ concentration causes the membrane potassium conductance to increase; this brings the potential closes to the potassium equilibrium potential so that the membrane hyperpolarizes and relaxation follows (Levick, 1991). On the other hand , the increased of the repolarization voltage of the isolated hearts in the present data (although it isn't statistically significant) is a sign for hyperkalemia in the myocardium (Heger *et al.*, 1994).

Our data confirms that the positive inotropic action of garlic on the heart is due to a direct effect on the T-calcium channels, since verapamil the T-type calcium channels antagonist could abolish the increase of ventricular voltage followed the garlic application. Moreover, the negative dromotropic effect of garlic could be ascribed to its pronounced level of calcium, which lead to prolongation of the P-R interval (Heger *et al.,* 1994).

We postulated the direct positive inotropic effect of garlic on the myocardium to a factor derived from its constituents. Previous garlic reports identified a potent enzyme which could inhibit the activities of adenosine deaminase and cyclic AMP phosphodiesterase (Agarwal, 1996). It is well known that the synthetically phosphodiesterase (PDE) inhibitors are used for cardiac failure treatment (Brown and Kozlowski, 1997) through the increase of intracellular cyclic adenosine monophosphate (cAMP) which in turn opens calcium channels enhancing the inward calcium current and produce a positive inotropic effect which is established in the present investigation. On the other hand, these inhibitors proved to have a significant role in the pharmacological actions of garlic in the body and explained the antithrombic, vasodilatory and anticancer actions (Agarwal, 1996). Also, adenosine is another important component of garlic, it has a delator effect on the vascular smooth muscle (VSM) which is endothelium-independent and it inhibits neurotransmitter release by a pre-junction effect (Levick, 1991).

It is worth-noting that atropine antagonizes several subclasses of muscarinic receptors as M_1 and M_3 type which can be found on neurons, gastric parietal cells and smooth muscle. Our data showed that garlic increased the smooth muscle contraction of the rabbit duodenum which was blocked by atropine pretreatment. This data postulates that garlic could induce a post-cholinergic outflow through the M_3 receptors activation which may enhance the GIT motility. On the other hand, M_2 receptors which are found in cardiac cells didn't stimulate by garlic as shown from the present data. Actually, this data confirms a specific mechanism of garlic on acetylcholine receptors even they are on the same subclasses. Recently, it is known that a number of different molecular mechanisms are transmitting the signal generated by acetylcholine occupation of the receptor (Mycek *et al.,*2000). For example, when M_1 or M_3 receptors are activated, the receptor undergoes a conformational change and interacts with a G protein, which in turn activates phospholipase C. This leads to the hydrolysis of phosphatidylinositol-(4,5)-biphosphate (PIP_2) to yield diacyl-glycerol (DAG) and inositol (1,4,5)-triphosphate (IP_3), which cause an increase in intracellular Ca^{2+}. This hypothesis is coincide with the increase of contraction of smooth muscle as a result of garlic application through its stimulation to M_3 subtype of muscarinic receptors.

The antimicrobial and anitfungal effect of garlic have been discussed early by many investigators (Naganawa *et al.,* 1996; Yoshida *et al.,* 1998). Other animals studies have found that garlic can increase the intestinal activity levels of enzymes used by the body to eliminate toxins (Sigounas, 1997). From the present data, we can postulate that garlic by its increasing to the smooth muscle contraction of the GIT can increase the toxins elimination and the liver function. On the other hand, sulphur compounds from Allium species were pharmacologically investigated to have activity against infection and in the prevention of cardiovascular disorders and cancer (Vannereau and Mellouki, 1996; Gao *et al.,* 1999).

The present finding showed the relaxant effect of garlic on the skeletal muscle contraction following the application. The blockade of nicotinic receptors at the neuromuscular junction before garlic application sustained normal contraction and ensured the direct action of garlic on the nicotinic receptors. The decrease of contraction was supposed due to non-depolarizing action of garlic that mimic the non-depolarizing blockers. This data encourages further investigations towards the relaxant property of garlic on the neuromuscular-junction, perhaps it could used for local medication during surgery, since increased muscle tone is a disadvantage during surgery and increases the metabolic demands and in many cases interferes with the procedures (Davies *et al.,* 2001).

In conclusion, the present data on the traditional medicinal plant garlic '*Allium sativum*' provides new documents about its direct action on the myocardium, its increase to the smooth-muscle motility and its relaxant to skeletal muscle contraction. The biologically active chemical constituents as well as the rich-minerals contents help in the manifestation of its mode of action on the excitable tissues. They could affect directly the cell membrane probably through the receptors coupling to G-proteins which regulate the ion channels physiology as in the myocardium and indirectly through activation of the cholinergic muscarinic receptors of the smooth muscle and/or inhibition of the somatic nicotinic receptors.

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