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Full Length Research Paper

Acute modulation of rat plasma glucose byan aqueous garlic extract

Meherzia Mokni[1], Sonia Hamlaoui[1]*, Ferid Limam[2], Mohamed Amri[1] and Ezzedine Aouani[2]

[1]Laboratoire de Neurophysiologie Fonctionnelle et Pathologies, Département des Sciences Biologiques, Faculté des Sciences de Tunis. Campus Universitaire El Manar II-2092 Tunis, Tunisie.
[2]Laboratoire des Substances Bioactives, Centre de Biotechnologie, Technopole Borj-Cedria, BP-901, 2050 Hammam-Lif, Tunis, Tunisie.

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In this study, the putative antidiabetic effect of garlic was re-investigated. Aqueous crude garlic solution was prepared at high concentration (2 g/ml) and extracts were obtained by ethanol precipitation followed by chromatography on C18 Sep-Pak cartridge. Garlic or extracts were administered by single intraperitoneal injection to euglycaemic rats. Plasma glucose, insulin and nitric oxide (NO) were determined after 30 min, 1 and 2 h, respectively. Garlic induced hypoglycemia and hyperinsulinemia which is mimicked by an ethanol soluble and non polar extract. This active principle appeared different from S-allyl-cystein sulfoxide based on physico-chemical properties and mode of action. Data of thin layer chromatography experiments indicated the presence of at least four molecular species, indicating a more non polar nature, with Rf values higher than S-allyl-cystein sulfoxide. The mechanism of action seemed to involve nitric oxide as its glucose induced lowering activity is abolished by diphenyleneiodonium which is a selective constitutive nitric oxide synthase inhibitor.

Key words: Garlic, Plasma Glucose, Insulinemia, Nitric Oxide, Thin Layer Chromatography.

INTRODUCTION

Garlic (*Allium sativum* L.), an indigenous dietary component, belongs to the Liliaceae family and is widely used as a condiment. Besides, it is also used widely in home remedies and pharmacotherapy against debilitated pathologies because of its antioxidant (Lieben et al., 2012), anticardiovascular (Ginter and Simko, 2010), and antihyperglycemic (Kumar et al., 2013) activities. The antidiabetic effect of garlic is still controversial. Although some investigators (Swanston-Flatt et al., 1990; Baluchnejadmojarad et al., 2003) were unable to detect any glucose lowering activity in garlic preparations, some others described plasma glucose lowering activity and insulin secretagogue effect on a sulfur derived amino acid identified as S-allyl-cysteine-sulfoxide (SACS) (Bordia et al., 1977; Sheela and Augusti, 1992; Kook et al., 2009). Moreover this insulin secreting activity was only demonstrated *in vitro*, using isolated cells from normal rat pancreas (Augusti and Sheela, 1996).

Although the mode of garlic's action or its derivatives is still uncertain, nitric oxide (NO) was suggested as a putative mediator (Mokni et al., 2006; Lieben et al., 2012) especially in antihypertensive effects (Pedraza-Chaverri et al., 1998). NO is synthesized from L-arginine by NO synthase (NOS) which exist in three isoforms: neuronal, endothelial constitutive and inducible form (Kerwin et al., 1995). NO, derived from constitutive NOS, is reported to modulate vasomotor tone, inhibition of platelet or leukocyte aggregation and adhesion to the endothelium

*Corresponding author. E-mail: sonia_hamlaoui@yahoo.fr. Tel: 216 98 968 113. Fax: 216 71 885 480.

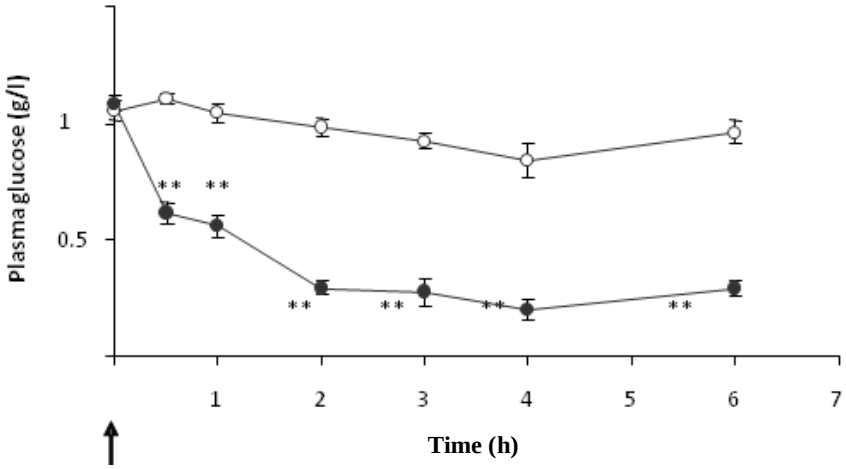

Figure 1. Effect of garlic on glycaemia (time course study). Rats were IP injected with aqueous crude garlic extract (80 mg / kg bw ●) or vehicle (○) and glycaemia determined at different times. Results are expressed by mean ± SEM (n = 10). **p < 0.01 vs control. The arrow indicates start of injection.

that suggests its anti-atherogenic properties (Moncada et al., 1991). In fact, a selective constitutive NOS (cNOS) inhibitor overcame the effect of aged garlic extract (AGE) (Morihara et al., 2006).

This research was aimed at studying the putative glucose lowering effect of aqueous extract of garlic on euglycaemic rats. In addition, attempts were made to identify the active component as well as its mechanism of action. We described a newly reported active principle, with a rapid onset of action and different from SACS based on physico-chemical properties and mode of action.

MATERIALS AND METHODS

Plant material and extraction

The raw garlic (*A. sativum* L.) cloves, purchased from local market, were peeled, weighted and blended with an electric mincer. The extraction was done using bi-distilled water at ambient temperature. The blended raw garlic was then dissolved in bi-distilled water at a concentration of 2 g/ml on the basis of the weight of the starting fresh material and centrifuged at 10,000 g for 15 min at 4°C (Beckman J20). Supernatant was sonicated with an ultraschall processor (UP 400S) and centrifuged again. Clear supernatant was then aliquoted and stored at -80°C until use. Aqueous solution (G) was subject to the extraction with ethanol as follows: briefly one volume of aqueous garlic was precipitated twice with seven volumes of ethanol and centrifuged at 10,000 g for 15 min at 4°C. Supernatant was dried using a rotavapor, dissolved in double distilled water and referred as ethanol-soluble extract (AS). After washing with ethanol/water (7v/1v) and drying, pellet was dissolved in double distilled water and referred as ethanol-insoluble extract (AP). AS was further subjected to chromatography on Sep-Pak C18 reverse phase cartridge. After extensive washing first with ethanol then with double distilled water loading of the cartridge with ethanol-soluble, extract gave two fractions: a polar fraction (Phile) eluted with double distilled water and a non polar fraction (Phobe) eluted with 10% ethanol.

Thin layer chromatography (TLC) analysis

30 µl corresponding to 1 mg dry product of Phobe extract was subject to TLC on silicagel plates 60 F_{254} (Merck, Germany) using butanol/acetic acid/water (12/3/5). Pure SACS (0.5 mg, Fluka, France) was run as control.

Animals and treatment

Male and female Wistar rats (Pasteur's institute, Tunis, Tunisia) weighting 180 to 220 g (6 to 7 weeks old) were maintained under standard laboratory conditions at 22 ± 2°C, on a light/dark cycle (12 h) supplied with standard pellet diet and tap water *ad libitum*. Procedures involving laboratory animals and their care were conducted in conformity with institutional guidelines of Tunis University and in accordance with the NIH guidelines. To determine the effects of aqueous extract of crude garlic on glycaemia, animals were divided in two groups: Group I was kept as control and received vehicle (H_2O) and Group II received aqueous crude garlic (G) (Figure 1). Each group contained 10 rats. To test the effect of the partially purified fraction of garlic, each group of rats received only one fraction. Rats were divided into 6 groups of 8 rats each (group I was kept as control, group II received aqueous crude garlic, group III received AP fraction, group IV received AS fraction, group V received Phobe fraction and group VI received Phile fraction) (Figure 2). Garlic or extracts were acutely administered by a single intraperitoneal injection (IP) at time = 0. Diphenyleneiodonium chloride (DPI, Fluka Aldrich, France) at 1 mg/kg body weight was dissolved in double-distilled water and IP injected 2 h prior to garlic or extract injection. Experimental duration never exceeded 3 h after which rats were anesthetized with urethane, sacrificed by decapitation and plasma used for glucose, insulin and NO determinations.

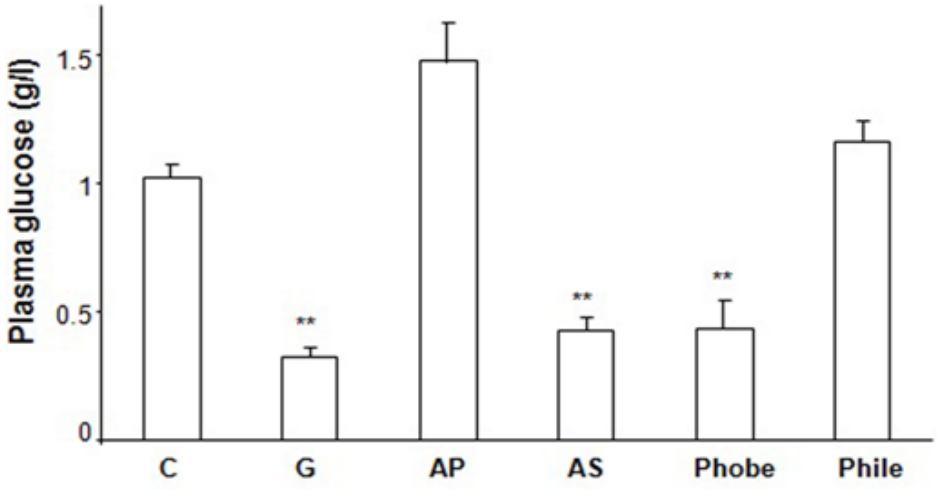

Figure 2. Effects of partially purified extracts from garlic on plasma glucose levels. C: control; G: aqueous crude garlic (80 mg / kg bw); AP: ethanol-insoluble extract; AS: ethanol-soluble extract; Phobe: non polar extract; Phile: polar extract. Extracts were IP administered to rats and glucose levels determined after 3 h of incubation. Results are expressed by mean ± SEM (n=8). **, $p < 0.01$ vs control.

Measurement of plasma glucose, insulin and NO levels

Glucose levels and plasma insulin were determined enzymatically using commercially available glucose oxidase (Sigma, France) and RIA kit (Immunotech, France), respectively. Plasma NO was measured by quantification of the NO metabolites nitrite and nitrate. These later were determined colorimetrically using a commercial kit (Roche diagnostics, France) according to Green et al. (1982).

Statistical analysis

Results are expressed by mean ± standard error of mean (SEM). Data were analyzed by unpaired Student's t-tests and expressed as means ± SEM, and $p < 0.05$ was considered significant.

RESULTS

Figure 1 shows the time related effects of aqueous extract of crude garlic on glycaemia. Data showed that garlic drastically induced hypoglycaemia from the first hour till several hours (6 h). The acute effects of crude garlic or partially purified extracts on plasma glucose levels were tested (Figure 2). All extracts were intraperitoneally injected (IP) at time 0 and glucose levels determined after 3 h. As expected, garlic exerted a glucose lowering effect, which is mimicked by the ethanol-soluble (AS) and the non polar extract (Phobe) but not by the ethanol-insoluble (AP) or the polar extract (Phile). Figure 3 showed that garlic as well as Phobe extract exerted their glucose lowering effect by increasing insulinemia (7-fold over control). Phobe extract was further subject to TLC on silicagel plates. Data from Figure 4 showed the presence of at least 4 spots in Phobe extract. However, none of them corresponded to SACS as assessed by Rf values.

The ability of Phobe extract to modulate plasma NO levels was also tested. Figure 5 showed the effect of Phobe extract either alone or in the presence of the specific constitutive NOS inhibitor DPI on plasma glucose (Figure 5A) and NO (Figure 5A) levels. Data clearly showed that Phobe extract lowered plasma glucose and simultaneously increased NO levels. It was clear that these effects are abolished by DPI.

DISCUSSION

The present work deals with a re-evaluation of the putative antidiabetic effect of garlic. We confirm that aqueous extracts exerts real glucose lowering effect *in vivo* (Sher et al., 2012), which is preceded by an increase in insulinemia (Sheela and Augusti, 1992). Some previous studies failed to show any antidiabetic effect probably because of the unappropriate use of streptozotocin-induced diabetic animals which no longer respond to any agonist (Baluchnejadmojarad et al., 2003). In this respect, it is generally recognized that an antidiabetic agent could exert a beneficial effect in the diabetic situation by enhancing insulin secretion and/or by mimicking insulin action (Gray and Flatt, 1999; Eidi et al., 2006). This lacking effect can also be the result of the use of too much low concentration of garlic, unable to elicit any detectable effect *in vivo*. In fact, neither garlic oil (100 mg/kg bw) nor DADS (40 or 80 mg/kg bw) significantly affected fasting blood glucose concentrations throughout the investigation period (Liu et al., 2006).

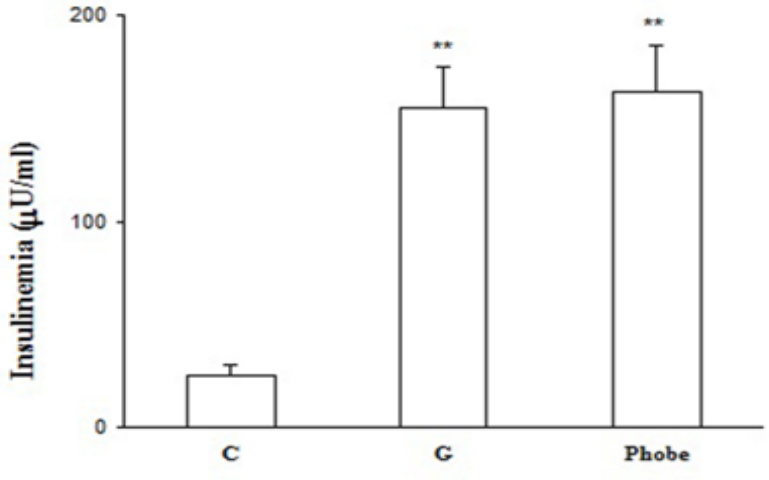

Figure 3. Effect of Phobe extract on insulinemia. Rats were IP injected with vehicle (10 % ethanol) or garlic (80 mg / kg bw) or Phobe extract (equivalent to garlic dose) and insulinemia determined by RIA after 1 h of incubation. Results are expressed by mean ± SEM (n = 8). **p < 0.01 vs control.

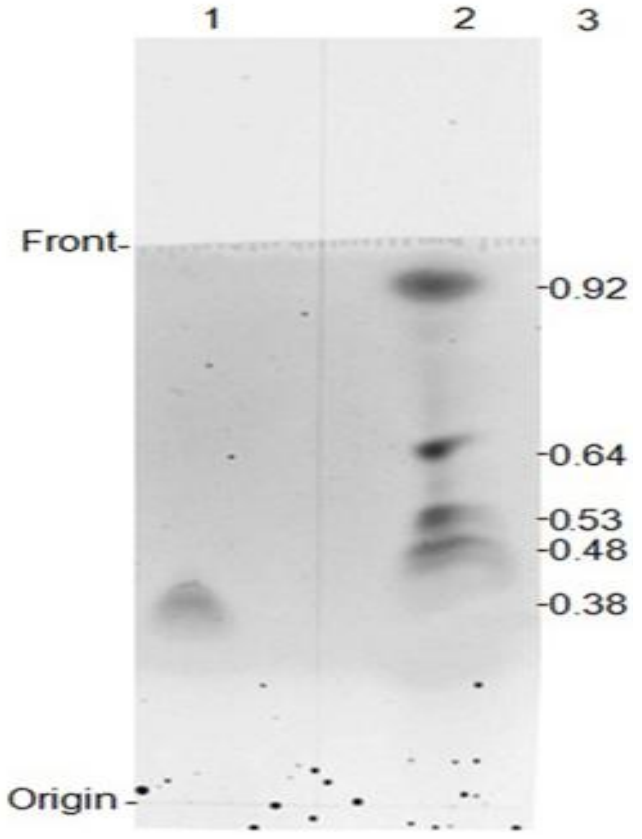

Figure 4. TLC analysis of Phobe extract. Phobe extract (line 2: 1 mg) or pure SACS (line 1: 0.5 mg) were subjected to reverse phase C18 silica gel plates using butanol/acetic acid/water (12/3/5). Line 3 indicate Rf values for pure SACS and 4 different spots of Phobe extract. Staining was performed with iodine.

In our hand garlic exerted dose related effects only at high concentrations. Indeed, on the basis of the weight of the starting material, our garlic preparation is approximately 1000 mg/kg/day which corresponds to 70 to 100 g crude garlic per day for a 70 kg adult, which is not safe (Alnaqeeb et al., 1996). These doses, which are much higher than previously reported in chronic (Ali and Thomson, 1995) or in acute experiments (Pantoja et al., 2000), outline the difficulty of comparing the two kinds of experiments in term of doses. In this respect, it is also well known that garlic activity depends closely on its mode of extraction or processing (Staba et al., 2001), doses (Banerjee et al., 2001) and ways of administration (Alnaqeeb et al., 1996; Sundaram and Milner, 1996). Our data rather support that garlic can no longer be used as a nutritional supplement (Ali and Thomson, 1995) but as a source of bioactive components and of potential new antidiabetic agents as yet to be isolated and identified (Saravanan and Ponmurugan, 2012).

Based on TLC experiments, SACS was identified as the major sulphur amino acid from aqueous extract of garlic implicated in insulin secretagogue effect (Augusti and Sheela, 1996). When submitted to TLC in the same conditions, Phobe extract exhibits at least 4 molecular species with Rf values higher than SACS, indicating a more hydrophobic nature (Rabinkov et al., 1998).

Phobe extract mode of action involved NO increase as found in kinetic as well as dose response experiments (data not shown). From pharmacological experiments on which we use selective constitutive NOS inhibitor as DPI, Phobe extract no longer induced glucose lowering and NO increasing activity. To our knowledge, our report is the first one that links garlic induced glucose lowering

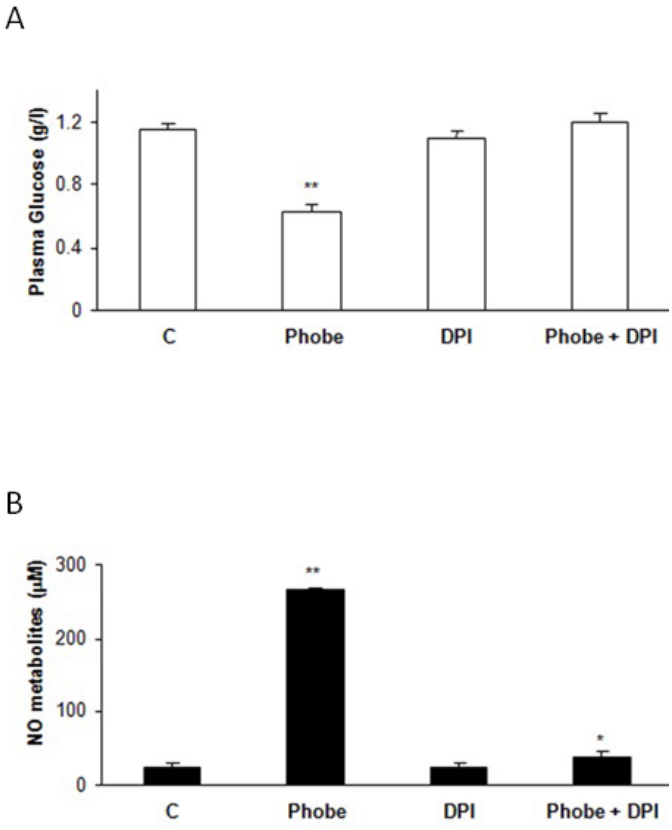

Figure 5. Effect of DPI on Phobe extract induced glucose (A) and NO (B) levels. Rats were pre-treated with DPI (1 mg / kg bw) during 2 h and IP injected with vehicle or Phobe extract. Plasma glucose levels were determined after 2 h (Figure 5A) and plasma NO after 30 min (Figure 5B). Results are expressed by mean ± SEM (n=8). *$p < 0.05$ vs control.

activity with NOS activation in euglycaemic rats. Our data also support that Phobe extract could not be alliin-derived products which have been previously shown to act by NO independent way (Morihara et al., 2002; Das et al., 1996). Further experiments using diabetic animals are underway to assess:

(i) The effectiveness of such new activity; (ii) the exact molecular nature of this active principle which might be a saponin (Matsuura, 2001); (iii) and the implication of constitutive NOS in glucose lowering and insulin secreting activity. Indeed NOS inhibition has been shown to reduce glucose uptake during exercise in individuals with type II diabetes more than in control subjects (Kingwell et al., 2002). In conclusion we described a new and not yet identified glucose lowering and insulin secreting activity from garlic exhibiting a rapid onset of action *in vivo*.

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