EFFECT OF SOME FOODSTUFFS ON THE DEVELOPMENT IN DIABETES

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ABSTRACT

From many reports it is clear that diabetes will be one of the major diseases in the coming years. Diabetes shows a wide range of variation in prevalence around the world and it is expected to affect 300 million by the year 2025. In a prevention framework where banning policies and educational strategies lead the interventions, functional foods (FFs) with their specific health effects could, in the future, indicate a new mode of thinking about the relationships between food and health in everyday life. Functional ingredients, such as cinnamon, garlic and onion have been addressed for their specific actions towards different reactions involved in diabetes development. This study was undertaken to assess the effect of garlic (*Allium sativum L.*) and onion (*Allium cepa L.*) consumptions on metabolic parameters related to diabetes. The animals were divided into six groups: one control and five experimental groups. All injections (onion and garlic extracts 200 mg/kg b.w./daily for 15 days) were oral administered (per os = p.o.) at the volume of 100 µl. Thirty minutes after the last injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery. The results indicate that the application of onion and garlic extracts were reduced glucose, cholesterol and triglyceride concentrations in blood serum of the mice with induced experimental diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a serious problem in developing as well as developed countries **(Wild *et al.*, 2004).** Hyperglycemia, a typical symptom in non-insulin dependent diabetes mellitus (NIDDM, type 2 diabetes) patients, is a condition characterized by a rapid rise in blood glucose levels and is due to hydrolysis of starch by pancreatic α-amylase and absorption of glucose in the small intestine by α-glucosidases. The intestinal absorption of dietary carbohydrates such as maltose, sucrose, and starch is carried out by a group of α-glucosidases. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard digestion of glucose by the inhibition of these carbohydrate hydrolyzing enzymes, α-glucosidases, in the digestive tract **(Deshpande *et al.*, 2009)**. Diet has been recognized as a corner stone in the management of diabetes mellitus. Spices are the common dietary adjuncts that contribute to the taste and flavour of foods. Besides, spices are also known to exert several beneficial physiological effects including the antidiabetic influence. In a prevention framework where banning policies and educational strategies lead the interventions, functional foods (FFs) with their specific health effects could, in the future, indicate a new mode of thinking about the relationships between food and health in everyday life. Functional ingredients, such as stevioside, cinnamon, bitter melon, garlic and onion, ginseng, Gymnema sylvestre and fenugreek, have been addressed for their specific actions towards different reactions involved in diabetes development **(Ballali and Lanciai, 2012)**. Among these plant foods *Allium species*, onions and garlic have long been used for a large range of purposes including medicine, nutrition, flavoring, condiment, foodstuff, and the treatment of common ailments as folk medicine **(Kim *et al.*, 2006)**.

Allium cepa L. (onion) and *Allium sativum L.* (garlic) are bulbous herbs belonging to family Alliaceae and are commercially cultivated worldwide **(World Health Organization, 1999)**. Onion (*Allium cepa L.*) and garlic (*Allium sativum L.*), among the oldest cultivated plants, are used both as a food and for medicinal applications. Onion bulbs have been recognized as the richest source of dietary flavonoids. At least 25 different flavonoids have been characterized and quercetin and its glycosides are the most important ones **(Slimestad *et al.*, 2007)**. Especially higher concentrations of quercetin occur in the outer dry layers of onion bulb **(Park *et al.*, 2006; Amagase, 2006; Smith *et al.*, 2003; Kris-Etherton *et al.*, 2002;**

Cook and Samman, 1996; Goldman *et* al., 1996; Patil and Pike, 1995) anthocyanin pigments are reported from red onions. In *A. sativum* a small amount of non-volatile sulphur-containing compounds such as S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SAMC) have significant antioxidant activity. Allixin and organo-selenium compounds are other characteristic major chemical constituents of garlic which exhibit antioxidant effect synergistically with organo-sulphur compounds **(Eidi *et al.*, 2006; Miron *et al.*. 2000)**. Diabetes is characterized as an imbalance in carbohydrate, fat, and protein metabolism, primarily due to increased insulin resistance and relatively impaired insulin secretion .

This study examined the anti-diabetic effect of onion and garlic extracts in the streptozotocin (STZ)-induced diabetic mice.

MATERIAL AND METHODS

Preparation of garlic and onion extracts

Fresh garlic and onion bulbs (*A. sativum L.; A. cepa L.*) were purchased from a commercial source. Sliced, dried and ground bulbs (100g) were submitted to extraction with 300ml ethanol (80%) in a Soxhlet apparatus for 72h. After extraction, the solvent was filtered and then evaporated by Rotavapor. The obtained garlic extract was stored at-20°C until being used.

Experimental animals

The experiments were carried out on male mice, average body weight 25–26g, bred in the constant light conditions LD 12:12 and fed with standard diet with unlimited access to water.

Animals were divided into four groups of ten mice each. Group I – control (C), Group II – diabetic untreated (DM), Group III –was administered ethanol extract of *Allium cepa L.* (in dose 200mg.kg^{-1} body weight/day for 15 days) (AC); Group IV - was administered ethanol extract of *Allium sativum L.* (in dose 200mg.kg^{-1} body weight/day for 15 days) (AS); Group V - diabetic treated with onion (DM+AC); Group VI - diabetic treated with garlic (DM+AS). All injections (onion and garlic extracts 200mg/kg b.w./daily for 15 days) were oral administered (per os = p.o.) at the volume of 100μl. Diabetes mellitus (DM) was induced in groups II, V, VI and received a single i.p. injection of streptozotocin - STZ, 60mgKg^{-1} body

weight, freshly dissolved in 0.1M citrate buffer (pH 4.5). DM was verified by measuring blood glucose in tail nick blood samples. Mice with non fasting blood glucose levels of ≥20mmol/L after 48h of STZ injection or greater and symptoms of polyuria, polyphagia, and polydipsia were considered diabetic. Thirty minutes after the fourth injection animals were anaesthetized and decapitated. The blood samples were collected from the carotid artery.

Biochemical analysis

The concentrations of glucose, cholesterol and triglycerides were estimated in the blood serum with STAMAR kits.

Glucose estimation was based on the enzymatic reaction of glucose with oxygen catalyzed by glucose oxidase. The reaction leads to the formation of D-gluconate and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with phenol and 4-aminoantipyrine producing chinone dye and water. We measured the intensity of the colour spectrophotometrically at the wavelength 500 nm.

The estimation of cholesterol was based on the reaction of cholesteryl ester with water. The reaction was catalyzed by cholesterol esterase. As a result of this reaction cholesterol and fatty acids are produced. At the second stage, cholesterol is oxygenised by cholesterol oxydase producing 4-cholestenon-3 and hydrogen peroxide. Hydrogen peroxide reacts with the participation of peroxidase with phenol and 4-aminoantipyrine. As a result, chinone dye and water are produced. The intensity of the color was estimated spectrophotometrically at the wavelength 500 nm.

The enzymatic estimation of triglycerides involves three stages. First, lipase hydrolyses triglycerides to glycerol and fatty acids. Then, glycerol, at the presence of glycerol kinase and ATP, is phophorylised to 3-P-glycerol. 3-P-glycerol oxidase (GPO) catalyses the formation of hydrogen peroxide which reacts with p-chlorophenol and 4-aminoantipyrine (4-AA) producing a coloured complex. The intensity of the colour is directly proportional to the concentration of triglycerides. All the spectrophotometric measurements were performed using Marcel 330 spectrophotometer.

Statistical analysis

The results were expressed as means ± standard deviation. The statistical analysis of the results was carried out with Statistica program version 9.0.

The distribution was tested using Shapiro-Wilk test. Differences between consecutive groups were analysed using one-way ANOVA followed by post hoc analysis with Tukey test. Statistical significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Diabetes mellitus, a common metabolic disease characterized by elevated blood glucose, has been linked to diabetic complications such as hyperlipidemia. Currently available drugs for diabetes have a number of limitations, such as adverse effects and high rates of secondary failure **(Ajjan and Grant, 2006; Shapiro and Gong, 2002)**. This situation has led to the search for alternative therapies from natural products that have low or no side effects and multi-target actions **(Greń and Formicki, 2012; Greń *et al*., 2012; Ozsoy-Sacan *et al*., 2004)**. More than 1000 plants have been described as efficacious in the treatment of diabetes mellitus. However, many of these descriptions are anecdotal accounts of traditional usage, and fewer than half of these plants or plant extracts have received a thorough medical or scientific evaluation of their purported benefits.

In Poland's indigenous system of medicine, *Allium cepa L.* (onion, AC) and *Allium sativum L.* (garlic, AS), commonly used as a food ingredient, is claimed to be useful for various ailments. To establish its utility in diabetes mellitus, the present study evaluated the antidiabetic effects of AC and AS in streptozotocin-induced diabetic mice.

The antihyperglycemic, antihyperlipidemic and antioxidative properties of hydroethanolic extract of AC and AS were investigated in streptozotocin-induced diabetic mice. STZ administration (group II) resulted in higher blood glucose level as compared to normal animals. Besides, serum lipid profile parameters such as total cholesterol (TC) and triglyceride (TG) were also found to be significantly elevated in diabetic animals. The pharmacological intervention with onion and garlic (in dose 200 mg.kg^{-1} body weight/day for 15 days) significantly relieved hyperglycemia and hyperlipidemia in STZ-induced diabetic mice (p < 0.05). The results are shown in Fig. 1, 2 and 3.
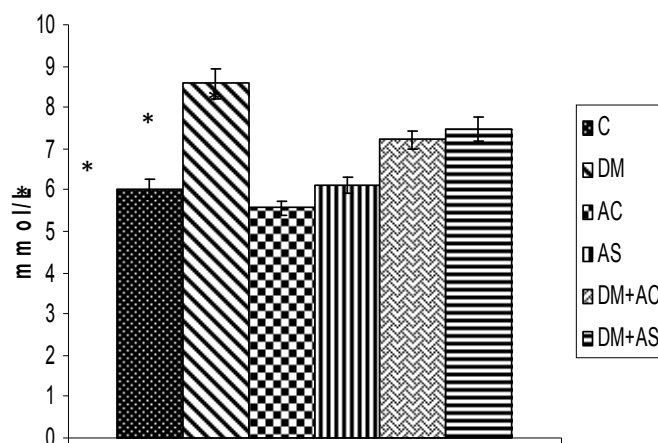
Figure 1 Effect of *Allium sativum L.* (AS) *and Allium cepa L*. (AC) treatment on serum glucose levels in diabetic mice (DM). The values are means±SE of eight rats per group. *$p<0.05$ vs. the diabetic group (Group II)
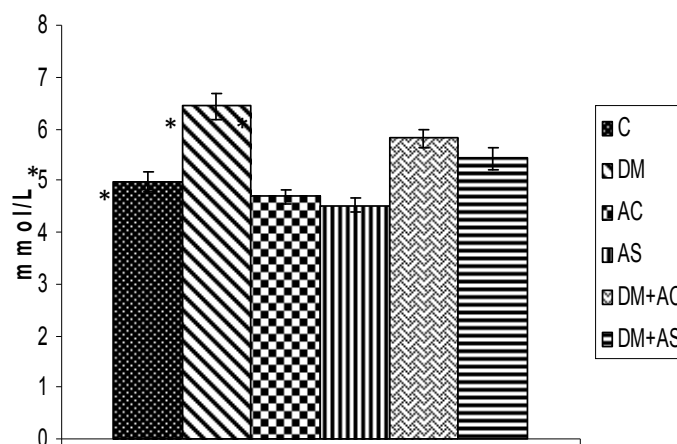

Figure 2 Effect of *Allium sativum L.* (AS) *and Allium cepa L*. (AC) treatment on serum cholesterol levels in diabetic mice (DM). The values are means±SE of eight rats per group. *$p<0.05$ vs. the diabetic group (Group II)
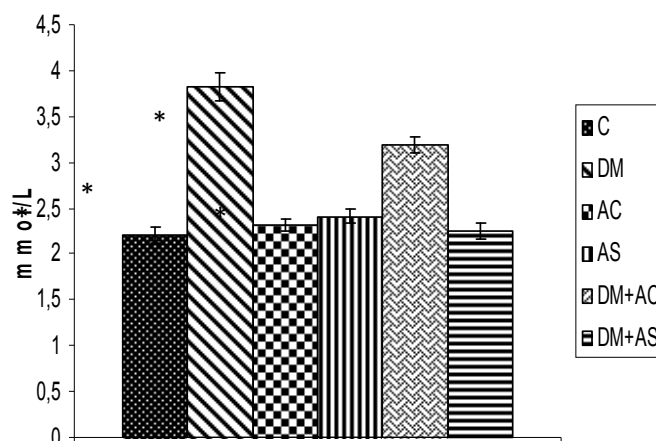
Figure 3 Effect of *Allium sativum L.* (AS) *and Allium cepa L.* (AC) treatment on serum triglycerides levels in diabetic mice (DM). The values are means±SE of eight rats per group. *$p<0.05$ vs. the diabetic group (Group II)

Streptozotocin - induced diabetes is characterized by a severe loss in body weight **(Soria *et al.*, 2000)**, which might be the result of protein wasting due to the unavailability of carbohydrate as an energy source. Hyperglycemia, a typical symptom in non-insulin dependent diabetes mellitus (NIDDM, type 2 diabetes) patients, is a condition characterized by a rapid rise in blood glucose levels and is due to hydrolysis of starch by pancreatic α-amylase and absorption of glucose in the small intestine by α-glucosidases **(Deshpande *et al.*, 2009)**. The intestinal absorption of dietary carbohydrates such as maltose, sucrose, and starch is carried out by a group of α-glucosidases. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard digestion of glucose by the inhibition of these carbohydrate hydrolyzing enzymes, α-glucosidases, in the digestive tract **(Deshpande *et al.*, 2009)**. Therefore, inhibition of these carbohydrate-hydrolyzing enzymes can significantly decrease the postprandial hyperglycemia after a mixed carbohydrate diet and can be a key strategy in the control of diabetes mellitus **(Hirsh *et al.*, 1997)**. Quercetin was considered the most bioactive compound of onion skin. This research suggested that although quercetin does have blood glucose lowering potential via α-glucosidase inhibition, there are other bioactive compounds present in onion skin that contribute towards the observed bioactivities for type 2 diabetes management. Although, in this study, we provided evidence for onion extract as a α-glucosidase inhibitor and its properties to decrease blood glucose, such detailed relationship between onion is not yet clear. Therefore, to provide the precise mechanism based on a single compound, further pharmacological and genetic studies are needed. In our study, oral administration of onion and garlic reduced the levels of TG and cholesterol in diabetic mice.

It is likely that the hypolipidemic effect of *Allium sativum L.* and *Allium cepa L.* results from the inhibition of cellular cholesterol and triglyceride synthesis, thus suggesting that onion and garlic may be beneficial to diabetic individuals with hyperlipidemia.

CONCLUSION

The results of this study show that *Allium sativum L.* and *Allium cepa L.* supplementation had a favorable effect on plasma glucose and lipid profile concentrations. The use of plants especially vegetables by the population as antidiabetic remedies have added interest of joining two basic diabetes mellitus control factors: food and medication. *Allium species* such as onion has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health, warding off illnesses and providing vigor.

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