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ORIGINAL ARTICLE

EFFECTS OF GARLIC EXTRACT TREATMENT IN NORMAL AND STREPTOZOTOCIN DIABETIC RATS INFECTED WITH *CANDIDA ALBICANS*

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ABSTRACT

The anti-candidial effect of garlic extract (Allium sativum L.) was investigated in normal and streptozotocin-induced diabetic rats. Diabetes was induced after a single intraperitoneal injection of streptozotocin (60 mg/kg). Rats were divided into six groups with fifteen rats in each group: (1) Normal control rats (2) Control rats + C. albicans (3) Control rats + garlic extract + C. albicans (4) Diabetic control rats (5) Diabetic rats + C. albicans (6) Diabetic rats + garlic extract + C. albicans. The concerned groups were inoculated with C.albicans on the 15 th day. At the end of one month experiment, fasted rats were killed by cervical decapitation. Blood was collected for estimation of glucose and C. albicans concentrations were estimated in liver and kidneys homogenates. A significant increase was observed in serum glucose levels in diabetic rats. A loss of bodyweight, polydipsia and polyphagia were observed in diabetic rats. Administration of alcoholic extract of garlic (0.25 g/kg body weight) reduced the hyperglycemia, polydipsia, polyphagia and associated weight loss of streptozotocin-treated rats. Administration of garlic extract significantly reduced C. albicans concentrations in liver and kidneys homogenates in infected control and diabetic rats. It is concluded that garlic extract improves candidia infection in diabetic rats.

KEY WORDS

Garlic (Allium sativum L.), Streptozotocin induced-diabetic rats, Candida albicans.

INTRODUCTION

Diabetes mellitus comprises a group of chronic diseases characterized by hyperglycemia or diminished insulin secretion, or both (1). In the year 2004, according to the World Health Organization reports, more than 150 million people throughout the world suffered from diabetes (2) while the mankind has been unable to solve this problem. Although present therapy for diabetes mellitus relies heavily on an arsenal of drugs developed since the introduction of insulin (3), prior to 1922 diabetes therapy revolved around dietary measures including the use of traditional anti-hyperglycemic plants(4). The World Health Organization has recommended that traditional plant treatments for diabetes warrant further evaluation (5). Many herbal medicines have been recommended for the treatment of diabetes (6, 7). Plant drugs are frequently considered to be less toxic and free from side effects more than synthetic ones (8). Some studies confirmed anti-hyperglycemic (9, 10) and anti-microbial (11-15) effects of garlic.

In the current literature, there is a paucity of information regarding the in-vivo anti-candidiasis efficacy of garlic extract. Thus the purpose of the present study was to compare the efficacy of garlic extract in treatment of established systemic candidiasis in normal and diabetic rats.

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MATERIALS AND METHODS

***Chemicals*:** Streptozotocin was obtained from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of the highest purity available.

Animals: Ninety male Wistar rats, weighting 200-250 g, were used in all experiments. Groups of rats were housed in an air conditioned room at 22±2°C with a lighting schedule of 12 h lighting and 12 h darkness. Animals had free access to a standard pellet diet (Rat breeding diet, Pillsbury Ltd., Birmingham, UK) and tap water. The overall nutrient composition of the diet was 36.2% carbohydrates, 20.9% protein, 4.4% fat and 38.5% fiber with added vitamins and minerals. Four rats were housed per cage. Permission for the study was obtained from the government of Iran.

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

Drug administration: Garlic extract was suspended in distilled water and administered orally through orogastric tube at dose 0.25 g/kg body weight of rats (groups 3 and 6) 5 day before and after intraperitoneal administration of streptozotocin. The volume of administrated extract was 1 ml for each animal.

Microorganism: *C. albicans (ATCC 10731*) was cultured for 24 hours at 37°C on Sabouraud dextrose agar (SDA) then stored at 4°C. The morphology and biochemical characteristics of yeast cells were verified by microscopic observations, colonial morphology on SDA, formation of germ tubes in serum, formation of chlamydospores on chlamydospore agar, and sugar fermentation reactions. For experiments, a single stock was prepared with organisms that were grown on SDA for 24 hours at 37°C, washed from the slants in 2 ml of sterile saline at a concentration of 1×10^6 colony forming units (CFU) ml^{-1}. Rats in groups 2, 3, 5 and 6 were inoculated (day 15) intraperitoneally with 0.2 ml of 10^5 CFU ml^{-1} suspension of organism (16). Verification of the concentration was done using serial Sabouraud dextrose pour plates of 10-fold dilutions of the original suspension.

Experimental induction of diabetes: Rats in groups 4 to 6 were injected (day 5) with a freshly prepared solution of streptozotocin (60 mg/kg body weight) in 0.1 mol/L sodium citrate buffer (pH 4.5) (17). Control rats (groups 1 to 3) were treated with the same amount of citrate buffer without streptozotocin. The dosing volume was 1 ml /kg. Successful induction of diabetes was confirmed by measuring the fasting blood glucose concentration in rats 48 h after injection of streptozotocin. Rats with fasting blood glucose levels over 180 mg/dl were defined as diabetic and included in the study. The food and water were removed from cages 12 h before testing.

Experimental design: In the present experiment, 90 rats (45 diabetic and 45 normal rats) were used. The rats were divided into six groups, with fifteen rats in each group, as follows: (1) Normal control rats were administrated with 1 ml of distilled water (2) control rats were administrated with 1 ml of distilled water and injected with *C. albicans* (3) control rats were administrated with garlic extract (0.25 g/kg body weight) and injected with *C. albicans* (4) diabetic control rats were administrated with 1 ml of distilled water (5) diabetic rats were administrated with 1 ml of distilled water and injected with *C. albicans* (6) diabetic rats were administrated with garlic extract (0.25 g/kg body weight) and injected with *C. albicans.* Daily measures of body weight, food intake and water intake were done. Postprandial blood samples obtained from the cut tail tip of conscious rats were collected on days 15 and 30 for serum glucose analysis.

***Measurement of glucose in rats' serum*:** One ml of blood was taken from rats in order to measure glucose. The samples were collected in sterilized tubes and kept at 4°C and after separating the clot, the serum was separated by centrifuging. Serum glucose was estimated by glucose oxidase method (18).

Quantification of Candida in organs: After 30 days of treatment, overnight-fasted rats were killed by cervical decapitation. To quantitate *Candida* organism in organs, both kidneys and liver were removed, rinsed of any adhering blood and homogenized in 5 ml PBS (pH 7.2) in a motor-driven Teflon-glass homogenizer (Glas-Col). Serial 200-fold dilutions of the homogenates were plated (at 0.1 ml) on Petri dishes containing Sabouraud agar, inverted and incubated for 48 h at 37°C. The CFU per gram of tissue for each kidney and the liver from each rat was converted to log 10 values for statistical manipulation.

***Statistical analysis*:** All data are expressed as the mean ±SEM for fifteen rats in each experimental group. Statistical analysis was performed using SPSS 10.0 statistical software. One-way analysis of variance (ANOVA) followed by the Tukey

Table 1: Effects of garlic extract administration on body weight, water intake, food intake and serum glucose concentrations of normal and streptozotocin-treated rats

	Normal control rats	Control rats +*C. albicans*	Control rats +garlic extract +*C. albicans*	Diabetic control rats	Diabetic rats +*C. albicans*	Diabetic rats +garlic extract +*C. albicans*	Between groups sig.
Body weight (g)							
Study Day 0	208.3±4.3	204.6±4.9	209.5±4.1	205.9±4.5	206.3±4.1	207.6±4.8	0.354
Study Day 30	330.7±4.4	321.4±6.3	323.2±5.5	178.2±4.6[a]	173.5±4.2	238.7±5.2[c]	0.042
Water intake (mL/day)							
Study Day 0	34.5±3.4	32.6±3.8	33.4±3.3	32.2±2.8[b]	31.3±2.9	33.8±2.3	0.490
Study Day 30	49.8±1.8	47.5±2.2	48.3±2.5	301.2±4.1	281.5±5.1	170.7±6.6 [d]	0.009
Food intake (g/day)							
Study Day 0	12.8±0.9	12.6±0.7	13.1±1.1	12.7±0.7	12.6±0.8	12.9±0.9	0.381
Study Day 30	22.3±0.5	21.3±0.9	22.9±0.7	60.2±1.6 [a]	57.1±1.3	38.4±2.2 [d]	0.008
Serum glucose (mg/dL)							
Study Day 30	110.3±3.1	112.3±2.8	111.9±2.6	296.3±4.8 [a]	287.9±4.6	218.6±4.3[c]	0.045

The values represent the mean± SEM for fifteen animals in each group. Comparisons were made by one-way ANOVA test. [a] P<0.05 compared to normal control rats. [b] P<0.01 compared to normal control rats. [c] P<0.05 compared to diabetic control rats. [d] P<0.01 compared to diabetic control rats.

post hoc test was used to compare mean values of quantitative variables among groups. The criterion for statistical significance was *P* <0.05.

RESULTS

Bodyweight ,water and food intake, and serum glucose: Tables 1 and 2 show significant differences in body weight, water intake, food intake and serum glucose in the different experimental groups.

Normal level of glucose in healthy adult rats was measured as 110.3±3.1 mg dl^{-1}. Daily consumptions of water and food in healthy adult rats were measured as 49.8±1.8 ml and 22.3±0.5g respectively but in diabetic rats the level of glucose was measured as 296.3±4.8 mg dl^{-1} and daily consumptions of water and food were measured as 301.2±4.1 ml and 60.2±1.6g respectively. Compared with normal rats, rats administered with streptozotocin showed a significant weight loss (*P*=0.042: %13.4 decrease in percentage), polydipsia (*P*=0.009: %835.4 increase in percentage) and hyperphagia (*P*=0.008: %374.0 increase in percentage) by the end of the study period. Administration of garlic extract reduced the weight loss (*P*=0.04: %14.9 increase in percentage), polydipsia (*P*=0.009: %405.0 increase in percentage) and hyperphagia (*P*=0.008: %197.6 increase in percentage) associated with streptozotocin treatment.

The results showed that serum glucose of diabetic rats increased when compared with normal rats (*P*=0.045: %168.6 increase in percentage). Oral administration of the garlic extract at dose of 0.25 g/kg body weight tended to bring serum

Table 2: Percentage* changes in body weight, water intake, food intake and serum glucose concentrations in different experimental groups

	Normal control rats	Control rats +*C. albicans*	Control rats +garlic extract +*C. albicans*	Diabetic control rats	Diabetic rats +*C. albicans*	Diabetic rats +garlic extract +*C. albicans*	Between groups sig.
Body weight (g)	+%58.7	+%57.0	+%54.2	-%13.4	+%15.8	+%14.9	0.042
Water intake (mL/day)	+%44.3	+%45.7	+%44.6	+%835.4	+%799.3	+%405.0	0.009
Food intake (g/day)	+%74.2	+%69.0	+%74.8	+%374.0	+%353.1	+%197.6	0.008
Serum glucose (mg/dL)	%0.0	+%1.8	+%1.4	+%168.6	+%161.0	+%98.1	0.045

-Negative numbers indicate decrease in percentage; +Positive numbers indicate increase in percentage; *Percentages indicate the differences between first and thirtieth days in each group

glucose significantly (*P*=0.047: %98.1 increase in percentage) toward normal values, while normal rats did not exhibit any significant alteration in serum glucose level during the experiment.

C. albicans in liver and kidneys homogenates: Table 3 shows the *C. albicans* content in liver and kidneys homogenates for rats in different experimental groups. There was a significant increase (P<0.01) in the concentration of *C. albicans* in the liver and kidneys homogenates in diabetic rats compared with control rats. Administration of garlic extract significantly reduced Candida titers in the liver (*P*=0.009) and kidneys (*P*=0.008) in both normal and diabetic rats compared with the titer in the controls.

DISCUSSION

Diabetes mellitus is a highly prevalent chronic illness and a major socio-economical burden with serious health consequences. A study conducted by the World Health Organization reported that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million by 2030 (19).

We used streptozotocin for diabetes induction in the rats. Streptozotocin is a commonly used compound for inducing type1 diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic β-cells (20). Researchers around the world have used streptozotocin to create experimental diabetes because it is a simple, inexpensive and available method (21, 22). Intra-venous or intra-peritoneal injection of 60 mg/kg dose of streptozotocin in adult wistar rats, makes pancreas swell and at last causes degeneration in Langerhans islet beta cells and induces experimental diabetes mellitus in 2-4 days (16, 23).

In the present study, consumptions of water and food increased in diabetic animals in comparision with normal rats but body weight decreased. These data are consistent with the findings of Dhuley (16), Holemans *et al* (22) and Swanston –Flatt *et al* (24) who found water and food increase and body weight decrease in streptozotocin induced diabetes.

The present data indicated that the garlic extract significantly decreased serum glucose levels in treated diabetic rats as compared with control diabetic rats. In agreement with the present results, several studies have shown the hypoglycemic effect of garlic (9, 10, 25). The hypoglycemic potency of garlic has been attributed mainly to allicin and its derived sulphur compounds (10, 25).

In the present study, administration of garlic extract reduced the weight loss, polydipsia and hyperphagia associated with streptozotocin treatment. In consistent with present data, other researchers have reported that administration of garlic extracts prevented loss of body weight, polydipsia and hyperglycemia of streptozotocin diabetic rats (9, 26). The dosage of garlic extract in our study was 0.25 g/kg body weight. It has been reported that the anti-diabetic beneficial effects of garlic extract are prominent with a dosage of 0.25 g/kg body weight in comparison with other dosages (26).

We used *C. albicans* for inducing systemic candidiasis in rats. The model of candidiasis in rats used in the present experiment is one of a sub-acute systemic infection that is usually well tolerated by the animals for several weeks but is not cleared spontaneously (16). This model, with treatment delayed for several days, mimics human infection. This is unlike mouse lethality studies, in which therapy is initiated concomitant with injection of an otherwise lethal inoculum (16). In the present study, there was an increase in the concentration of *C. albicans* in the kidneys homogenates compared with the liver homogenates in normal rats.

In the majority of experimentally induced systemic infections, the kidneys of animals were the organ that bore the heaviest foci of infections through out the experiments (27, 28). It is unclear why kidneys are more susceptible to Candida

Table 3: *C. albicans* concentration in liver and kidneys homogenates for rats in different experimental groups

	Normal control rats	Control rats +*C. albicans*	Control rats +garlic extract +*C. albicans*	Diabetic control rats	Diabetic rats +*C. albicans*	Diabetic rats +garlic extract +*C. albicans*	Between groups sig.
`Mean log 10 CFU g^{-1} of tissue± SEM							
Liver	0.00±0.00	3.13±0.11	2.21±0.08 [b]	0.00±0.00	5.32±0.14 [a]	4.38±0.18 [b]	0.009
Kidney [*]	0.00±0.00	4.655±0.13	3.35±0.12 [b]	0.00±0.00	5.41±0.15 [a]	4.43±0.20[b]	0.008

[*]Mean for right and left kidneys. The values represent the mean± SEM for fifteen animals in each group.Comparisions were made by one-way ANOVA test. [a] P<0.05 compared to control rats. [b] P<0.01 compared to control rats.

infections and it has been suggested that the physiological conditions (28), anatomical architecture (29) and the poor phagocytic system of kidneys (30, 31) may be contributing factors for predisposition of kidneys to the long-term systemic Candida infections.

Tsao *et al* (32) reported that methicillin-resistant *Staphylococcus aureus* was markedly present in kidneys of diabetic mice and Raffel *et al* (33) reported that the inflammatory reaction in kidneys infected with *C. albicans* was greater in diabetic rats and fungus balls associated with ureteral obstruction and gross multiple renal abscesses occurred in diabetic, but not in non-diabetic rats infected with Candida. In the latter study, it has been reported that the renal microbial populations of *C. albicans* were found to be greater than 10^5 colony-forming units per g in diabetic rats, whereas the kidneys tended to become sterile in non-diabetic rats (33).

In the present study, the concentrations of *C. albicans* in both liver and kidneys homogenates of diabetic rats were high. It has been shown in the normal individuals, a formidable barrier to *C. albicans* is provided by a multitude of effectors and control mechanisms (28, 29) but in diabetic patients, *C. albicans* remains one of the organisms most frequently producing infection (34).

Patients with diabetes mellitus have an increased susceptibility to infections. In other studies, diabetic animals were more susceptible to microbial infections than normal ones (16, 32, 33). Hepatic predisposition of experimental animals to Candida infection reported previously (27, 28) is an effect which is observed in the long-term infections and it has been suggested that this predisposition could be due to recruitment of premature monocytes and PMNs from bone marrow (35). These cells contain greater numbers of abnormal giant lysosomes which are defective in phagosome-lysosome fusion process (35). This situation provides an opportunity for the yeast to grow and produce infectious foci in liver. Yamashiro *et al* (36) reported that in diabetic mice, the levels of IL-12 and IFN-gamma in liver were lower than those in control animals on day 14 post-infection, while the opposite was true for IL-4 levels. Treatment of normal and diabetic rats with garlic extract after Candida inoculation caused a considerable inhibitory effect on the growth of the organism in both liver and kidneys.

Tsao *et al* (15, 32) reported that garlic extract and its two diallyl sulphides (DAS and DADS) could effectively decrease methicillin-resistant *Staphylococcus aureus* viability in blood and organs in diabetic and non-diabetic mice. Bhagyalakshmi *et al* (37) reported that oral administration of DAS or DADS could effectively reduce methicillin-resistant *Staphylococcus aureus* viability in kidney from 10^7 to 10^4 CFU g^{-1} in diabetic mice.

Some in-vitro studies confirmed anti-bacterial (11, 15) and anti-Candidal (12- 14) effects of garlic extract but there is a paucity of information regarding the in-vivo anti-candidiasis efficacy of garlic extract. Our findings indicate that garlic extract exhibits inhibitory effects against candidiasis in both control and diabetic rats. The anti-candidial effects of garlic attributed mainly to allicin (38). Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of anti-bacterial (against a wide range of gram-negative and gram-positive bacteria), antifungal (particularly against *C. albicans*), anti-parasitic and anti-viral activities (38).

Some approaches suggest that garlic extract exerts its effect by the oxidation of thiol groups present in the essential proteins, causing inactivation of enzymes and subsequent microbial growth inhibition (13). It has been also reported that blockage of lipid synthesis (39), enhancement of phagocytosis and increase in natural killer cell activity (40) may be important components of the anti-candidal activity of garlic.

We used fresh garlic extract in our investigation. It has been reported that the use of fresh garlic is more effective for anti-microbial activity than that from old garlic (11, 14).

These results indicate that garlic extract exhibits inhibitory effects against candidiasis and therefore validates the traditional use of the plant in fungal infections in diabetic patients. In addition, further comprehensive pharmacological investigations, including experimental chronic studies, should be carried out.

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