THE ANTI-INFLAMMATORY EFFECTS OF AQUEOUS EXTRACT OF GINGER ROOT IN DIABETIC MICE

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ABSTRACT

On the basis of reports that ginger (Zingiber officinale, *Z. officinale*) extract has antiinfalammatory activity, the present study was undertaken to investigate whether the aqueous extract of *Z. officinale* has any significant beneficial effect on chronic inflammation in diabetic mice. Control mice received normal saline (0.1 ml, i.p.), and in the test group, diabetes was induced by injection of streptozotocin (STZ, 180 mg/kg, i.p.) which was confirmed by the measurement of blood glucose, 7 days after STZ injection. One week after saline or STZ injection, chronic inflammation was induced by implantation of cotton pellets (30 mg) on each side of the groin region subcutaneously. Then at the day of 3, the aqueous extract of *Z. officinale* was added to drinking water (100, 200 and 400 mg/100 ml) for 4 days. In another sets of experiments, L-NAME, a nitric oxide synthase inhibitor, (0.1 mg/kg, i.p.) and indomethacin, an inhibitor of the prostaglandin biosynthesis, (2 mg/kg, i.p.) were injected at the day of 5 of implantation. On the 8th day, the mice were killed and the pellets were removed, freed from extraneous tissue and dried at 60 °C for 24h. The increase in the weight of cotton pellets was higher in diabetic mice (control: 160 ± 13.6 mg, diabetic: 271 ± 11.8 mg, $P<0.001$). Pretreatment with the aqueous extract of *Z. officinale* caused a significant but not dose-dependent reduction in cotton pellet weight in diabetic animals (diabetic + *Z. officinale's* extract: 181.4 ± 21 mg, $P<0.05$ vs diabetic). The anti-inflammatory effect of extract was almost the same as L-NAME, but less than indomethacin. Results suggest that the anti-inflammatory effects of aqueous extract of *Z. officinale* are comparable to L-NAME.

Keywords: Diabetes, Streptozotocin, Inflammation, Nitric oxide, Zingiber officinale, Mice

INTRODUCTION

Ginger (Zingiber officinale, *Z. officinale*) has been used as a medicine since ancient time (1). In Asian medical practices, dried ginger has been used to treat stomachache, diarrhea and nausea (2-5). Recent studies have shown that *Z. officinale* has anti-inflammatory effects (6) and reduces pain and swelling associated with either rheumatoid or osteoarthritis (7). It has also antioxidant, analgesic and anti pyretic properties (8, 9, and 10). Cardiovascular actions of *Z. officinale* such as decrease in blood pressure, heart rate (8) and blood glucose (11) is well known. The major pharmacological activity of *Z. officinale* appears to be due to gingerol and shogaol (8, 12). These two active compounds are reported to be responsible for the analgesic, anti-emetic, anti-pyretic and prostaglandin suppression of *Z. officinale* (13).The amounts of pro-inflammatory cytokines are increased in diabetes (14, 15). For instance, it has been shown that hyperglycemia significantly increases the expression of endothelial adhesion molecules such as ICAM-1, E-selectin and P-selectin on human umbilical vein endothelial cells (16). The important role of inflammation on diabetes has also been reported (17). Although, *Z. officinale* has both hypoglycemic and anti-inflammatory effects, there is not enough scientific data on its anti-inflammatory activity during diabetes. Therefore, the present study was undertaken to investigate the anti-inflammatory effects of aqueous extract of *Z. officinale* in diabetic mice and also to compare these effects with those of NG-nitro-L-arginine methyl ester hydrochloride (L-NAME) and indomethacin.

MATERIALS AND METHODS

Albino adult mice of either sex (weighing between 30-35 g) from the animal house of Qhaem Hospital, Mashhad, Iran, were used and kept on the standard diet and water.

Preparation of the aqueous extract of Z. officinale rhizome

The ginger rhizome was purchased from a local store and then grounded in a coffee grinder. 10, 20, 40 mg of *Z. officinale* powder were dissolved in the boiled water (20 ml) and filtrates were added to the animal's drinking water.

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Induction of diabetes and inflammation

Diabetes was induced by injection of streptozotocin (STZ, 180 mg/kg, i.p.) and mice were kept on normal diet for seven days. Their plasma glucose levels were assessed spectrophotometrically with a quantitative enzymatic (glucose oxidase) commercial blood glucose measuring kit (Sigma). Chronic inflammation was induced by cotton pellet induced granuloma in mouse by the previously established method (18, 19). Briefly, under anesthesia (ketamine, 65 mg/kg and xylazine, 6.5 mg/kg) two sterilized cotton pellets (density cotton) weighing 30 mg were implanted subcutaneously in the groin region of mice, one on each side. These cotton pellets were sterilized in an air oven at 121°C for 20 minutes before implantation. The animals were scarified on the 8th day, and dry weight of granulation tissues with cotton pellets which were dried at 60˚C overnight were measured. The weight of the cotton pellets before implantation was subtracted from the weight of the dried, dissected pellets.

Experimental groups

Mice were divided into different groups: control (received saline, 0.1 ml, i.p., n=6); diabetes (received 180 mg/kg of STZ in 0.1 ml of saline, i.p., n=6); control + *Z. Officinale* (received saline, 0.1 ml, i.p. + 100, 200, 400 mg/100 ml of *Z. officinale*, which was added to drinking water for 4 days from the 3rd day of implantation, n=18); diabetes + *Z. Officinale* (received 180 mg/kg of STZ, i.p. + 100, 200, 400 mg/100ml of *Z. officinale*, which was added to the drinking water for 4 days, n=18); control + L-NAME (received saline, 0.1 ml, i.p. + L-NAME 0.1 mg/kg, i.p. at 5th day of implantation, n=6); diabetes + L-NAME (received 180 mg/kg of STZ, i.p. + L-NAME 0.1 mg / kg, i.p. at 5th day of implantation, n=6); control + indomethacin (received saline, 0.1 ml, i.p. + indomethacin 2 mg /kg, i.p. at 5th day of implantation, n=5); diabetes + indomethacin (received 180 mg/kg of STZ, i.p. + indomethacin 2 mg / kg, i.p. at 5th day of implantation, n=5); control + *Z. officinale* + L-NAME (received saline, 0.1 ml, i.p. + 200 mg/100 ml of *Z. officinale*, + L-NAME 0.1 mg/kg, i.p., n=5) and diabetes + *Z. officinale* + L-NAME (received 180 mg/kg of STZ, i.p. + 200 mg/100 ml of *Z. officinale* + L-NAME 0.1 mg/kg, i.p., n=6).

Drugs

The following drugs were used: streptozotocin (Pharmacia & Upjohn Company, Kalamazoo, USA), ketamine (Rotexmedica, Germany), N^G-nitro-L-arginine methyl ester hydrochloride, indomethacin and xylazine (Sigma Laboratories).

Statistical Analyses of Data

Results are expressed throughout as means ± S.E.M and were analyzed by one way ANOVA followed by a Tukey-Kramer multiple comparison test (for comparison of the increase in the weight of the cotton pellets). A *P* value of less than 0.05 was considered to be significant. Statistical analysis was performed using Minitab for Windows.

RESULTS

One week after induction of diabetes (by injection of streptozotocin), blood glucose was significantly higher in streptozotocin-induced diabetic than control mice (control; 94.6 ± 8, diabetes; 158 ± 2 mg/dl, P<0.001). The increase in the weight of cotton pellets granuloma (a marker of chronic inflammation) was significantly higher in the diabetic mice in comparison to the control (Fig. 1). *Z. officinale* showed an anti-inflammatory effect in both control and diabetic mice which was not dose-related (Fig. 1).

Table 1. Anti-inflammatory effects (increase in the weight of cotton pellets, mg) of Indomethacin and L-NAME in control and diabetic mice. Values are mean ± S.E.M; n= 4 - 6 animals in each group.

Drug/Group	Control	Diabetes
Normal Saline	160 ± 13.66	270 ± 13.90 ***
Indomethacin	70 ± 5.77 *	113.34 ± 17.63 ###
L-NAME	78 ± 8 *	166 ± 23.5 ##

(* P < 0.05, *** P < 0.001 vs Control; ## P < 0.01 and ### P < 0.001 vs Diabetes)

The anti-inflammatory effect of *Z. officinale* (Table 1) was comparable to those of L-NAME (0.1 mg/kg, i.p.,) and less than indomethacin (2 mg/kg, i.p.). However, injection of L-NAME to the *Z. officinale*-treated diabetic mice did not significantly modify the anti-inflammatory effects of ginger (increase in the weight of cotton pellets (mg), *Z. officinale*+ diabetes: 181.4 ± 21, *Z. officinale* + diabetes + L-NAME: 188 ± 34, data are not shown).

DISCUSSION

Ginger is widely grown in China, India, and Japan (3, 5) and it has anti-inflammatory activity. The exact anti-inflammatory mechanism of ginger is not well known and possible mechanism of the ginger action may involves a change in the synthesis of prostaglandins and leukotrines that mediate inflammation (7, 20). In the present study, it was shown that the addition of ginger (Z. Officinale's) to drinking water reduces inflammation in diabetic mice. In agreement with

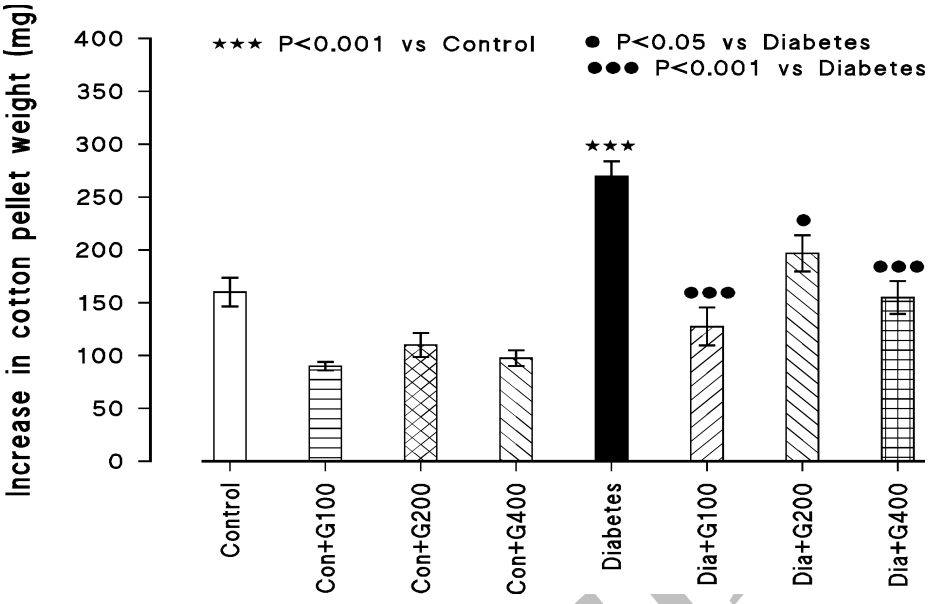

Figure 1. Mean ± S.E.M of the increase in cotton pellets weight (mg) in the different groups of control and diabetic mice which have been treated by different dose of *Z. officinale* (100, 200 and 400 mg/100 ml). One way ANOVA followed by a Tukey-Kramer multiple comparison test was applied to compare the data (n = 5-6), which showed a significant increase in the weight of cotton pellets in diabetic mice (*** P<0.001 vs control) and a significant reduction by ginger treatment (• P<0.05 and ••• P<0.001vs diabetes) in diabetic mice, but no significant reduction in control mice.

pervious studies, it was found that anti-inflammatory effect of *Z. officinale* was not dose-related (7), and there was a non-significant increase in the weight of cotton pellets using 200 mg/100 ml of *Z. officinale* in comparison with other doses. The reason of this increase is not known. It was also shown that oral consumption of ginger (200 mg/100 ml) is as effective as L-NAME in reducing inflammation but less effective than indomethacin.

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