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Does Lycopene Tomato Paste Consumption Effectively Decrease Clastogenicity in Streptozotocin-Induced Diabetic Rats?

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ABSTRACT

The present study was designed to determine the possible therapeutic effects of oral tomato paste lycopene supplementation on clastogenicity induced by hyperglycemia in diabetic rats. Diabetes was induced by streptozotocin (STZ) injection. Twenty eight male Wistar rats were divided into 4 groups (7 rats per group) as follow: 1-The first group served as control. 2-The second was a diabetic (STZ induced) group, 3-The third was a diabetic+lycopene treated group and 4-The fourth was a lycopene treated group. The treatment period was 2 months and the animals were subjected to micronucleus assay. The results of the present study revealed that a significant increase in micronucleus frequency in the diabetic group (STZ-induced) compared to control. However, supplementation with lycopene in STZ-induced diabetic group decreased significantly the incidence of MN frequency and restores the MN range to the normal control. In conclusion, oral tomato paste lycopene supplementation effectively decreases clastogenicity in Streptozotocin-induced diabetic rats through its antioxidant activity.

Keywords: Lycopene, Anticlastogenic, Streptozotocin, Diabetes, Rats

INTRODUCTION

Diabetes mellitus is a long term metabolic disorder and its incidence has been increasing markedly in parallel with obesity. There are many mechanisms involved in the pathogenesis of diabetic complications. Oxidative stress is the most commonly accepted cause of diabetes which caused by free radicals [1,2].

Free radicals (reactive chemical species) are involved in many diseases, including diabetes [3]. When the number of reactive chemical species increases more than the antioxidants, they can attack the immune system and somatic cells [4]. Free radicals effects could be neutralize by antioxidants molecules which donating an electron to pair with the free radical's unpaired electrons. A balance between free radicals and antioxidants is required in healthy people. However, it has been shown that diabetic people have higher levels of free radicals, which can cause diabetic disorder complications [5].

Many studies showed an increasing use of medicinal plants or their extracts to ameliorate diseases [6,7]. Lycopene is a carotenoid and has antioxidant effect and abundantly present in tomatoes but in smaller amounts in other fruits such as guava, grapefruit, papaya and watermelon [8]. A significant increase in antioxidant activity of lycopene with reducing oxidative stress has been reported [9-11].

Lycopene administration to streptozotocin-induced hyperglycemic rats was found to cause a decrease in glucose, in H_2O_2 and thiobarbituric acid reactive substances levels and an increase in insulin concentration, as well increased the total antioxidant status, and antioxidant enzyme activities (i.e., catalase, superoxide dismutase, glutathione peroxidase) with improvement in serum lipid profile [12]. In addition, lycopene at doses of 1, 2, and 4 mg/kg has significant, dose-dependent anti-diabetic effect in streptozotocin-induced diabetic rats [13]. Investigating the role of lycopene in a clinical study in diabetic patients (N=133), showed that the risk of diabetic retinopathy was reduced by lycopene treatment [14].

Lycopene reduced diabetes-induced learning and memory impairment in some animal models by reducing oxidative stress and inflammation [13]. Lycopene also may attenuate diabetic neuropathic pain by inhibiting action of nitric oxide and tumor necrosis factor-alpha [15]. Lycopene may be useful in patients with type 2 diabetes by suppressing oxidative stress and enhancing innate immunity or serum levels of immunoglobulin M [16].

Streptozotocin (Streptozocin, STZ, (U-9889)) is a small molecule, N-nitroso-containing antibiotic and a potent DNA methylating agent and antineoplastic properties. It has been used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic-cells. STZ is a potent alkylating agent and is highly genotoxic, producing chromosomal aberrations, DNA strand breaks, DNA adducts, alkali-labile sites, unscheduled DNA synthesis, sister chromatid exchanges, micronuclei and cell death by mechanisms which involve free radicals generated during STZ metabolization [17].

Streptozotocin mutagenicity was first reported in mice and rats [18]. A few years later, STZ was found to induce mutations inV79 chinese hamster and also induced chromosomal aberrations, SCEs and micronuclei in mouse [19,20]. Therefore, the present study was designed to determine the possible therapeutic effects of oral tomato paste lycopene supplementation on clastogenicity in streptozotocin- induced diabetic rat.

MATERIALS AND METHODS

Chemicals

Streptozotocin or Streptozocin (STZ) is a synthetic antineoplastic agent. Each vial of sterilized Streptozotocin powder contains 1 gr. of Streptozotocin active ingredient with the chemical name, 2-Deoxy-2-[[(methylnitrosoamino)-carbonyl]amino]-D-glucopyranose and 200 mg citric acid. Streptozotocin was supplied by Pharmacia Company. Pure Streptozotocin has alkaline pH. When it is dissolved inside the vial in distilled water as instructed, the pH in the solution inside the vial will be 3.5-4.5 because of the presence of citric acid. This material is prepared in 1-gr vials and kept in cold store and refrigerator temperature (2-8°C) away from light.

Egyptian Tomato paste bottle (360 g bottle) of three different brands (Heinz®, Wady Foods® and Faragello®, Cairo, Egypt) was purchased from a local market for lycopene extract to the experimental trail. The containers had glass bottled content and undamaged when purchased, and were in the same desirable state (not expired) during the time of experimentation. Ten samples of each brand were from the same lot (same manufacture date or batch number) to maintain experimental consistency, to minimize chances of variation and to maintain experimental homogeneity in sample selection. The purchased bottles were mixed to form a composite representative sample.

Lycopene was extracted without saponification using ethyl acetate (100%), with subsequent removal of the solvent according to the procedure described [21,22]. The extract was evaporated in the rotary evaporation (at temperature <35°C), with avoiding to reach temperatures above 40°C, to avoid degradation. The extract was concentrated just to dryness, were stored at –18°C until used in the same week, complete dryness was avoided. For the experimental trail use, the concentrated sample was complete dried under N2 immediately before reconstituted in oil for injection by stomach tube.

Animals

Twenty eight male Wistar rats weighing 120-150 g were kept under standard laboratory conditions such as temperature 23 ± 2°C, 12 light/dark cycles and was provided with water and food ad libitum.

Diet composition

Rats were fed commercial diet and tap water ad libitum for 1 week before the experiment. After 1 week of acclimation, the dietary treatment consisted of AIN-93M based diet as stated by Reeves et al. [23].

Induction of diabetes in rats

The animals were injected by STZ at the dose of 45 mg/kg of the body weight in 0.1 M citrate buffer, pH 4.5 to induce diabetes. Streptozotocin induces diabetes within 3 days by destroying the beta cells [24]. Those rats with blood glucose 270 mg/dl and above were regarded as diabetic, and were included in the study.

The groups were as follows: Control group (C): In seven male rats weighing 120-150 g, with blood sugar levels measured before the experiment with Roche, ACCU-CHEK Active® and strips, a 45 mg/kg single dose of sterile solution of saline (NaCl, 0.09%) was injected intraperitoneally.

Group with diabetes but lycopene was not given (D): In seven male rats weighing 120-150 g, with blood sugar levels measured before the experiment with Roche, ACCU-CHEK Active® and strips, a 45-mg/kg single dose of STZ was given in a cold citrate tampon (pH 4.5) intraperitoneally. After 72 h, glucose levels in the blood samples taken from the eye vein were determined through the Roche, ACCU-CHEK Active® glucose measuring device and strips. Rats with a blood level >250 mg/dl were diagnosed as having diabetes.

Group with diabetes and given lycopene (DL): In seven male rats weighing 120-150 g, with blood sugar levels measured before the experiment with Roche, ACCU-CHEK Active® and strips, a 45-mg/kg single dose of STZ was given in a cold citrate tampon (pH 4.5). After 72 h, glucose levels in the blood samples taken from the eye vein were determined with the Roche, ACCU-CHEK Active® glucose measuring device and strips. Rats with a blood level >250 mg/dl were diagnosed as having diabetes and given lycopene dissolved in corn oil, 10 mg/kg/day for 5 weeks by stomach tube orally.

Group to which lycopene was given (L) In seven male rats weighing 120-150 g, with blood sugar levels measured before the experiment with Roche, ACCU-CHEK Active® and strips, lycopene dissolved in corn oil was given, 10 mg/kg/day for 5 weeks by stomach tube orally.

Determination of tomato paste lycopene

Stock standard solution of lycopene for calculating lycopene content were prepared by dissolving 1 mg lycopene standard (vial contain 1 mg) in 25 mL volumetric flask with tetrahydrofuran (THF) containing 250 ppm tert-butyl-4-hydroxytoluene (BHT) and 0.05% triethylamine (TEA), to yield concentrations of 40 µg/mL.

The obtained solution was stored at -80°C and before use, the concentration of the lycopene was spectrophotometrically determined after dilution in hexane up to solution giving an absorbance value of below 0.7 units. The UV/VIS spectrophotometer and 1-cm cell was used, the absorbance of this solution at 472 nm was determined, using hexane as a blank. The lycopene concentration (CST mg/l) in standard solution was calculated using the A1% of 3450 in hexane at 472 nm [25].

HPLC analysis of tomato paste lycopene

Lycopene concentration of the tomato paste extract was measured using a reversed phase high-performance liquid chromatography (RP-HPLC) described by Shi et al. [21]. An Agilent 1100 series liquid chromatogram instrument was used, consisting of a solvent delivery pump, controller, and UV/visible detector at 472 nm for the determination of lycopene. Separation was achieved by using an analytical polymeric C30 column (4.6 mm × 25 cm), column temperature was 35˚C. A guard column packed with C 30 stationary phase was used in-line for all separations. The mobile phase consisted of MeOH/isopropyl alcohol/THF (30:30:35) containing 250 ppm BHT and 0.05% TEA as mobile phase. A 20 µl sample was injected into the detector via a micro syringe. The flow rate was adjusted to 1 ml/min, and peak areas were measured at 450 nm. The peaks of lycopene were identified by comparing their retention times and peak area with that of a pure standard. Detector signals were acquired and integrated on a chart printer. Finally, the extracted tomato paste lycopene were dissolved in sunflower and injected daily by stomach tube to rats with concentration at 10 mg/kg body weight, daily for 5 weeks.

EXPERIMENTAL DESIGN AND TREATMENT

Animals were divided into 4 groups (seven animals per group) as follow: 1) First group served as control. 2) The second was a diabetic (STZ induced) group (n=7), and 3) The third was a lycopene treated group (n=7) and the fourth was a diabetic+lycopene group (n=7). The treatment period was 2 months and the animals were subjected to micronucleus assay [26]. The bone marrow from the femur was flushed in the form of a fine cell suspension into a centrifuge tube containing fetal calf serum. The cell suspension was centrifuged at 500 × g for 10 min and the supernatant was discarded. The pellet was resuspended in a drop of serum and used for preparing slides. The air-dried slides were stained with May-Grünwald and Giemsa. A total of 1000 polychromatic erythrocytes (PCEs) were scored per animal from a single slide to determine the frequency of MnPCEs.

Statistical analysis

Data were expressed as the mean ± standard error (SE) of the means. The results were statistically analyzed using one way analysis of variance (ANOVA) with least significant difference (LSD). Results were considered significantly different if the *p*-value was <0.05.

RESULTS AND DISCUSSION

Effect of STZ induced-diabetic and/or lycopene treatment on the frequency of bone marrow micronucleus in Wistar male rats

The results of the present study (Table 1) revealed that the STZ treatment increased significantly ($p<0.001$) the incidences of MN compared to control animals (9.04 ± 0.16^{a} *vs*. 5.5 ± 0.14^{b}). However, supplementation with lycopene to STZ-induced diabetic group decreased significantly the incidence of MN frequency compared to diabetic group (5.10 ± 0.10 *vs*. 9.04 ± 0.16^{a} for diabetic+lycopene and diabetic groups, respectively) and restore the MN range to the normal control (48-54 *vs*. 50-58 for diabetic+lycopene and control group, respectively). The percentage inhibition was found to be about 50% for MN compared to the diabetic group. As well the treatment with lycopene for diabetic rats lowered the frequency of Mn near to lycopene treated group, where, no significant difference between diabetic+lycopene and lycopene treated group (5.10 ± 0.10 *vs*. 4.98 ± 0.11 for diabetic+lycopene and lycopene treated groups, respectively).

Table 1: Effect of STZ induced-diabetic and/or lycopene treatment on micronucleus frequency in wistar male rats

Treatment	No. of animals	MN frequency	MN range
Control	7	5.5 ± 0.14^{b}	50-58
Diabetic	7	9.04 ± 0.16^{a}	60-68
Diabetic+Lycopene	7	5.10 ± 0.10^{c}	48-54
Lycopene	7	4.98 ± 0.11^{c}	47-53

N.B.: diabetic (STZ induced – diabetic rats)
Small different superscript letters are significantly different.

These findings are consistent with the studies of Bolzán and Bianchi [17] who found that STZ induced–diabetic is highly genotoxic, producing DNA strand breaks, DNA adducts, unscheduled DNA synthesis, micronuclei, chromosomal aberrations and sister chromatid exchanges. Similarly, Liegibel et al., and Chinnasamy et al., [19,20] found that this antibiotic was a good inducer of micronuclei in mouse bone marrow cells.

Free radicals generated during STZ metabolization may be the mechanism involved in clastogenicity in diabetes via oxidative stress [4,27]. However, antioxidants such as lycopene donate an electron to pair with the free radical's unpaired electrons, which neutralize the effects of free radicals and reduce such oxidative stress [5].

As well with [9-11] who concluded that lycopene intake increases the plasma levels of this compound. Lycopene and other tomato derivatives were found to had a beneficiary effect on the oxidative stress in diabetic disease.

CONCLUSION

It could be recommended that the treatment with lycopene should be considered in the medications of diabetic and hyperglycemia. Lycopene supplementation can be useful for diabetic patients to reduce the complications such as clastogenicity and we can conclude that lycopene compound may prevent the genetic disorders generated by reactive oxygen species, which are generally found in type II diabetic patients. The results indicated that lycopene treatment inhibit the free radical generation in hyperglycemic patients as an antioxidant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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