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Effect of *Trigonella foenum graecum* (fenugreek)-loaded PLGA nanoparticles on non-specific esterase enzyme activity in salivary glands of aging-accelerated male mice

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Summary

During oxidative stress, the reactive oxygen species (ROS) generally produce structural and biochemical alterations in cells and cell organelles. Impairment of lysosomal and mitochondrial function is the key for understanding the degenerative changes due to aging. Nonspecific esterase enzyme is particularly present in lysosomes and microsomes. In order to find the antioxidant property of fenugreek, non-specific esterase activity was measured to study the lysosomal membrane integrity in submandibular and sublingual salivary glands of D-galactose-induced aging male mice without and with supplementation of fenugreek. Adult male albino mice (*Mus musculus*) were divided in to six groups of five each and treated as follows: a) control for D-galactose (Dg) treatment; b) Dg-treated; c) Dg + fenugreek seed extract (FSE) treated concurrently; d) treated with Dg first, followed by treatment of FSE; e) Dg + FSE-loaded PLGA nanoparticle treated concurrently; and f) treated with Dg first followed by treatment of FSE-loaded PLGA nanoparticle. A significant decrease in non-specific esterase activity was observed in D-galactose treated mice, whereas FSE and FSE-PGLA groups of mice indicated protection against Dg-induced aging related oxidative stress in salivary glands. Thus, it is shown that fenugreek seed possesses antioxidant property, whereby non-specific esterase activity in salivary glands is increased but in a manner dependent on the treatment pattern, and the best result is obtained when FSE is administered post-treatment as loaded in PGLA nanoparticles.

Key words: Aging, fenugreek seed extract, fenugreek-loaded PLGA nanoparticles, non-specific esterase activity, ROS, salivary gland

Introduction

The study of mechanism of aging and possibility of development of diseases connected therewith is an important problem in modern medicine and biology. Aging is a natural process that is multifactoral. There is an inevitable decline in physiological functions during aging (Harman, 1992). Oxidative stress caused due to free radicals has a bearing on aging as well as tissue damage (Brown and Griendling, 2009). Free radicals activate or inhibit expression of certain genes, functions of certain proteins and enzymes and damage membranes of cell organelles, leading to various disease states such as cancer, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, etc. (Sorcha and Thomas, 2009). During the early age oxidative stress is prevented by due to the balance between formation of free radicals and cell's antioxidant system, including SOD, CAT and GPx. But this defense mechanism declines during aging and, therefore, the organism becomes incapable of managing the oxidative stress. During aging the vital organs deteriorate in their efficiency of carrying out their functions. This will apply to salivary glands as well.

Esterases are enzymes that hydrolyze carboxylic acid esters of alcohol and phenols (Gomori, 1952). The enzyme acting on simple esters such as methylbutyrate or napthyl acetate are defined as non-specific esterase or napthyl hydrolase. Biochemical and fractional studies indicate that esterase activity is chiefly present in microsomal compartment (Underhay et al., 1956; Markert *and Hunter,* 1959; Carruthers et al., 1960a). Histochemical reaction indicates its lysosomal localization also (Holt, 1963; Shibko and Tappel, 1964). Esterase acting on higher fatty acids esters is found to be concentrated in the lysosome (Mahadevan and Tappel, 1968).

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Esterases are found associated with membrane structures, primarily in the lysosomal and the microsomal fractions. Several mouse liver esterases show different degrees of attachment to membrane structure (Carruthers et al., 1960b). Histochemical methods have demonstrated the presence of esterase in almost every type of membrane structure and it is probable that esterase in general has membrane-associated functions specialized for the type of cell or tissue. There are reports on sex-dependent presence or absence of an esterase in the mouse kidney which is influenced by sex hormone (Shaw and Koen, 1963; Ruddle, 1966).

Nonspecific esterase enzyme is particularly present in lysosomes and microsomes. But during stress the balance between the cell's antioxidant system and ROS may be disturbed, leading to structural and biochemical alteration in the cells and cell organelles. Impairment of lysosomal and mitochondrial function is the key for understanding the degeneration changes due to aging (Vanneste and van den Bosch de Aguilar, 1981).

To avoid the damaging effect of free radicals in tissues we have to take antioxidant from dietary factors. Fenugreek seeds have antioxidant activity and have been shown to produce beneficial effects such as neutralization of free radicals and enhancement of antioxidant apparatus (Anuradha and Ravikumar, 1998; 2001). Furthermore, the polyphenolic fraction of fenugreek seed was found to inhibit peroxide-induced oxidative damage and prevent hemolysis of erythrocytes *in vitro* (Kaviarasan et al., 2004). Fenugreek, being hydrophobic, poses limitations such as low absorption by oral route, extensive metabolism and rapid elimination (Sharma, et al., 1990). Biodegradable polymeric nanoparticles are extensively used to improve the therapeutic properties of various drugs and bioactive compounds. The advantages in PLGA are its biodegradability, its biocompatibility, and drug products containing PLGA have been approved for extra-gastric route of drug administration by regulatory authorities around the world (Fredenberg et al., 2011).

In the present study esterase activity was measured to study the lysosomal membrane integrity in submandibular and sublingual glands of male mice induced to aging by treatment with D-galactose, with and without supplementation of fenugreek seed extract.

Material and Methods

Preparation of fenugreek seed extract (FSE)

Fenugreek seeds were collected from the Mahatma Phule Krushi Vidyapeeth, Rahuri, India, and dried, cleaned and ground into a fine powder using a grinding machine. Extraction in ethanol was carried out using a Soxhlet apparatus (Lim et al., 2002). The extract was filtered and evaporated to dryness under reduced pressure at 60º C using a rotary evaporator. The extract was placed in a dark bottle and stored at -8º C until further analysis.

Synthesis of fenugreek-loaded PLGA nanoparticles (FNP's)

Fenugreek loaded poly (lactic-co-glycolic acid) **(**PLGA)-based nanoparticle was prepared using oil in water single emulsion solvent evaporation process (Jaiswal et al., 2004).

Animals

Six month old male Swiss albino mice (*Mus musculus*) weighing 50-55 g were used for the present study. Mice were housed in an approved departmental animal house (1825/PO/EReBi/S/15/CPCSEA). They were kept under a 12:12 hr L: D cycle and fed Amrut mice feed [Pranav Agro Industries, Sangli, India] and water *ad libitum*. The record of their age and body weight was maintained. Mice were divided into six groups.

a) Control group

Mice were given subcutaneous (*sc*) injection of 0.5 ml distilled water/ day/ animal for 20 days.

b) D-galactose-treated group

Mice were given subcutaneous injection of 5% D-galactose 0.5 ml/ day/ animal for 20 days to induce aging.

c) D-galactose + FSE

Male mice were subcutaneously injected with 0.5 ml D-galactose/ day/ animal along with fenugreek seed extract dissolved in water at the dose of 50 mg/kg body weight of animal/day for 20 days (Deshmukh et al., 2014a,b).

d) D-galactose → FSE

Mice were injected with 0.5 ml of 5% D-galactose for 20 days and then for next 20 days they were subcutaneously administered fenugreek seed extract dissolved in water at a dose of 50 mg/kg body weight of animal/day.

e) D-galactose + FNP's

Mice received 0.5 ml of 5% D-galactose along with fenugreek extract loaded PLGA nanoparticles at the dose of 50 mg/Kg body weight of animals twice weekly, for 20 days.

f) D-galactose → FNP's:

Mice received 0.5 ml 5% D-galactose/ day for 20 days and then fenugreek extract-loaded PLGA nanoparticles 50 mg/Kg body weight of animal twice a week for 20 days.

After completion of these doses, mice were killed by cervical dislocation. Submandibular (SM) and sublingual (SL) glands were dissected out, weighed and used for biochemical study.

Determination of non- specific esterase activity

For measurement of lysosomal non-specific esterase activity, the weighed submandibular and sublingual glands were frozen and homogenized in 0.25M sucrose-1mM EDTA. The tissue homogenate (5mg/ml) was prepared in pre-chilled mortar using homogenization mixture. The homogenate was centrifuged at 1500 rpm for 10 min, the pellet was discarded and the supernatant was collected and again centrifuged at 10,000 rpm at 1-4^{0}C for 20 min. The supernatant was used for the estimation of post-lysosomal (i.e., microsomal fraction) non-specific esterase activity. The pellet was suspended in 0.66 M phosphate buffer pH 7 and refrigerated at 0-4^{0}C for 30 min, then used for estimation of non-specific esterase activity from lysosomal fraction (Bier, 1955).

Results

Table 1 illustrates the effect of fenugreek seed extract and fenugreek extract-loaded nanoparticles on activity of non-specific esterase (µ mols of p-nitrophenyl acetate/mg protein) in SMG and SLG of male mice induced to aging by treatment with D-galactose. The significant decrease in non-specific esterase activity in SMG and SLG of D-galactose-treated aging-induced mice was observed as compared to control group (1:2, $P<0.01$). In both SMG and SLG of Dg + FSE and Dg → FSE groups of mice, the enzyme activity was significantly increased as compared to D-galactose treated aging-induced group (2:3, $P<0.01$; 2:4, $P<0.01$). In SMG of Dg + FNP's and Dg → FNP's mice, the non-specific esterase activity was increased significantly as compared to D-galactose treated group (2:5, $P<0.01$; 2:6, $P<0.01$). Non-specific esterase activity in Dg + FNP's and Dg → FNP's groups of mice was increased as compared to Dg + FSE as well as Dg → FSE groups, and the difference was significant (3:5, $P<0.01$; 4:6, $P<0.01$).

Discussion

In the present study there was decrease in esterase activity of D-galactose treated mice in submandibular and sublingual glands. D-galactose is a reducing sugar which interacts with free amino group of proteins to produce advanced glycation end products (AGE's). These AGE's acts on membrane lipids causing increase in lipid peroxidation, which results in disintegrity of lysosomal membrane, due to which there may be possibility of decrease in non-specific esterase activity. Previously, Mote and Pillai (2009) observed in salivary glands of D-galactose stressed mice such

Table 1: Effect fenugreek-loaded PLGA nanoparticles on non-specific esterase activity (µ mols of p-nitrophenyl acetate/mg protein) of salivary glands during induced aging. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

S No.	Treatment (n=5)	Non-specific esterase activity (SM)	Statistical significance	Non-specific esterase activity (SL)	Statistical significance
1	Control	92.7592 ± 1.545	1:2 P < 0.01	74.2319 ± 0.9796	1:2 P < 0.01
2	Dg- treated	54.2395 ± 1.4352	2:3 P < 0.01	40.6513 ± 0.7063	2:3 P < 0.01
3	Dg + FSE	60.2812 ± 0.9006	2:4 P < 0.01	52.3549 ± 0.7016	2:4 P < 0.01
4	Dg → FSE	72.4802 ± 1.6417	2:5 P < 0.01 2:6 P < 0.01	63.0643 ± 1.103	2:5 P < 0.01 2:6 P < 0.01
5	Dg + FNP's	75.1601 ± 1.4962	3:5 P < 0.01	61.6929 ± 1.0327	3:5 P < 0.01
6	Dg→ FNP's	84.2637 ± 0.7888	4:6 P < 0.01	69.8429 ± 0.7783	4:6 P < 0.01

as reduction in total proteins and several other researchers observed structural damage (Brian et al., 1981; Kim and Allen, 1993; Azevedo et al., 2005; Mankapure, 2007; Sonavane, 2007). Similarly, the increase in lipid peroxidation in salivary glands (Deshmukh et al., 2014a,b), and alterations in lysosomal enzymes like acid phosphatase in brain (Pillai, et al., 2003; Vora, 2005) were observed in D-galactose treated mice.

Supplementation and treatment of antioxidants may help in prevention and removal of free radicals (Walvekar et al., 2013a, b; Deshmukh et al., 2015). In the present study the esterase activity was increased after supplementation of fenugreek extract and its nanoparticles, which indicated that fenugreek possesses free radical scavenging activity and thus prevents cellular damage thereby reducing lipid peroxidation. The good results seen in fenugreek nanoparticles-treated group, compared to fenugreek seed extract-treated groups, clearly indicate that the fenugreek nanoparticles are more efficacious than the very seed extract.

The increase in lysosomal enzyme activity produced by fenugreek may be due to increase in protein synthesis, inhibition of intramolecular linkage and protection of lysosomal stability as an anti-oxidant. This shows that fenugreek nanoparticles protect the cell from enzymatic degradation during oxidative stress.

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