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Protective role of *Trigonella foenum graecum* (fenugreek) seed extract on oxidative stress in salivary glands of aging accelerated mice

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ABSTRACT

The study was undertaken to evaluate anti-aging effect of fenugreek seed extract(FSE) on the protein content, amylase activity, fucose and sialic acid content of salivary glands of aging accelerated mice, because the protein content and amylase activity decreases while the sialic acid and fucose content increases with age. So to evaluate antioxidative property of fenugreek adult male albino mice (Mus musculus L.) were divided in to four groups viz, a) Control group received subcutaneous injection of 0.5 ml sterile water for 20 days. b) D-galactose group received subcutaneous injection of 0.5 ml 5% of D-galactose /day for 20 days. c) Protective group received subcutaneous injection of D-galactose + FSE (50 mg/kg BW) for 20 days. d) Curative group received subcutaneous injection of D-galactose for 20 days then after FSE (50 mg/kg BW) for 20 days. In D-galactose injected group the protein content and amylase activity was significantly decreased while sialic acid and fucose content were significantly increased as compared to control group, but after treatment of FSE protein content and amylase activity was significantly increased in protective and curative group, while sialic acid and fucose content were decreased. Thus above results elucidate the antioxidative property of fenugreek seeds.

Keywords: Salivary glands, Protein, amylase, sialic acid, fucose, aging, FSE.

INTRODUCTION

Aging is a natural and multifactoral process, which involves an inevitable decline in physiological functions [1]. The main cause is oxidative stress caused due to free radicals, which originate from different enzyme systems within the cell [2]. Free radicals are capable to cause oxidative tissue damage. Free radicals activate or inhibit expression of certain genes, functions of certain proteins and enzymes and damage membranes of cell organelles, leading to various disorders [3]. Normally, in adult age oxidative stress is prevented by cell's antioxidant system, including Superoxide dismutase, Catalase and Glutathione peroxidase. But this defense mechanism declines during aging process therefore organism suffer from oxidative stress. During aging the vital organs fail to play their normal functions. Salivary glands are also not the exception. Oxygen centered free radicals i.e. reactive oxygen species (ROS) are involved in alteration of the salivary gland functions. Several researchers have reported age related changes in morphology [4], ultrastructure [5-7], biochemistry [8, 9] and histology [10, 11] of salivary glands.

Salivary glands are plays important role in their secretions which is necessary in the development and maintenance of various organs in the body [12] and a number of biologically active polypeptides [13]. Their secretions contain various proteins, enzymes, glycoprotein as well as growth factors. Salivary proteins have important biological activities [14]. Salivary amylase is an important member of a complex of digestive enzymes that attack macromolecules. It play a major role in carbohydrate metabolism, organisms with a starch-rich diet depend on the effectiveness of their amylases for survival [15-22]. Salivary glands are rich in glycoproteins mainly sulfated hexoses, fucose and sialic acids [23]. Fucose is deoxyhexose sugar and is found in N-linked glycanes on the mammalian, insect and plant cell surface. It is required for optimal functions of cell to cell communication.

Fucosylated glycans play important role in variety of biological settings [24, 25]. It is a powerful immune modulator. Fucosylated glycoproteins play important role in salivary glands which are biomarkers of aging. It is essential to study the fucose content in salivary glands and prevention of its loss during aging and stress. Sialic acid is an acetylated derivative of neuraminic acid and it is attached to nonreducing residues of carbohydrate chains of glycoproteins and glycolipids. The monomeric sialic acid is a potent defense molecule against oxidative damage and cell death caused by H_2O_2 [26].

Salivary amylase activity, protein, sialic acid and fucose contents are considered to be good indicator of proper functioning of salivary gland as it shows alterations. So it is important to give attention on the effect of aging on the physiological processes involved in secretion of salivary glands.

To avoid the aging of salivary glands we used fenugreek seed extract (FSE) because fenugreek extract has reported a potent source of antioxidants [27]. It has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity [28, 29].

MATERIALS AND METHODS

Preparation of fenugreek seed extract:
Fenugreek seeds were collected from the local market of Kolhapur and subjected to various treatments for investigation of antioxidants potential. Extraction was carried out by the soxhelt method [30] (Lim Cheung, 2002). Dry fenugreek seeds were cleaned and ground into fine powder using a grinding machine. Ethanol was used for extraction. The extract was filtered and evaporated to dryness under reduced pressure 60ºC by a rotary evaporator. Extract was placed in dark bottle and stored at -8ºC until further analysis.

Animals:
Six month old Swiss albino mice (*Mus musculus*) weighing about 50-55 gm were used for the present study. Animals were housed in departmental animal house approved by the [CPCSEA/233]. Animals were kept under a 12:12 hr L: D cycle and fed Amrut mice feed [Pranav Agro Industries, Sangli, India] and water was *ad libitum*. The record of their age and body weight was maintained. Animals were divided into four groups.

1) Control group:
The six month male mice were given subcutaneous injection of 0.5 ml distilled water/ day/ animal for 20 days.

2) Aging accelerated group:
Male mice were given subcutaneously injection of 0.5 ml of 5% D-Galactose / day/Kg of the animal for 20 days to induce aging.

3) Protective group:
Male mice were subcutaneously injected with 0.5 ml of 5% D-Galactose/ day/ animal along with fenugreek seed extract 50 mg/kg body weight of animal/day for 20 days (very little volume of alcohol 0.01ml was used to dissolve fenugreek seed extract and volume raised to 100ml with 5% D-galactose).

4) Curative group:
Male mice were injected with 0.5 ml of 5% D-Galactose for 20 days and then for next 20 days they were injected subcutaneously fenugreek seed extract prepared as above.

After completion of the dose, the animals were killed by cervical dislocation. Submandibular (SM) and sublingual glands (SL) were dissected out, weighed and they were homogenized in distilled water and centrifuged at 3000 rpm for 10 minutes at 10ºC temperature to prepare sample and used for estimation of amylase activity and protein, fucose contents. For sialic acid content homogenate was prepared in 0.1N H2SO4.

1. Estimation of protein was carried out by using Bovine Serum Albumin as standard. [31].
2. Estimation of amylase activity was carried out using starch solution that yields maltose, which is estimated by using dinitrosalicylic acid [32].
3. Estimation of sialic acid was carried out using N- acetylneuraminic acid as standard [33].
4. Estimation of fucose was carried out using α- D (+) fucose as standard [34].

Statistical analysis:
The statistical analysis was performed using Student‘t’ Test.

RESULTS

The protein content and amylase activity was decreased significantly in aging accelerated group (Group II) as compared to control (Group I) and significance was (1:2, P<0.01), in both SM and SL glands, while in protective (Group III) and curative groups (Group IV) it was increased significantly as compared to aging accelerated group (Group II) (2:3, 2:4, P< 0.01) in both SM and SL glands. In curative group (Group IV) also both the protein content and amylase was still increased as compared to protective group (Group III) (3:4 P<0.01) (Table I and II).

The fucose content was decreased significantly in aging accelerated group (Group II) as compared to control (Group I) and significance was (1:2, P<0.01), in both SM and SL glands, while in protective (Group III) and curative groups (Group IV) it was increased significantly as compared to aging accelerated group (Group II) (2:3, 2:4, P< 0.01) in both SM and SL glands. In curative group (Group IV) also the fucose content was still increased as compared to protective group (Group III) (3:4 P<0.01) (Table III).

The sialic acid content was increased significantly in aging accelerated group (Group II) as compared to control (Group I) and significance was (1:2, P<0.01), in both SM and SL glands, while in protective (Group III) and curative groups (Group IV) it was decreased significantly as compared to aging accelerated group (Group II) (2:3, 2:4, P< 0.01) in both SM and SL glands. In curative group (Group IV) also the sialic acid content was still decreased as compared to protective group (Group III) (3:4 P<0.01) (Table IV).

Table I: Effect of fenugreek seed extract on protein content in Submandibular and Sublingual glands of aging accelerated male mice (µg protein/mg tissue)

Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

Sr. no.	Groups	Treatment	Protein content (SM)	Statistical significance	Protein content (SL)	Statistical significance
1	Group I	Control (5)	711±7.211	1:2 P<0.01	452±4.062	1:2 P<0.01
2	Group II	Aging accelerated group (5)	312.4±9.0167		150±7.9057	
3	GroupIII	Protective group (5)	353.4±5.4589	2:3 P<0.01	254.6±3.8471	2:3 P<0.01
4	Group IV	Curative group (5)	454.6±3.8471	2:4 P<0.01 3:4 P< 0.01	353.4±5.4589	2:4 P<0.01 3:4 P< 0.01

Table II: Effect of fenugreek seed extract on amylase activity in Submandibular and Sublingual glands of aging accelerated male mice (µg maltose/mg protein)

Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

Sr. no.	Groups	Treatment	Protein content (SM)	Statistical significance	Protein content (SL)	Statistical significance
1	Group I	Control (5)	38.5717±0.0157	1:2 P<0.01	26.5138±0.1221	1:2 P<0.01
2	Group II	Aging accelerated group (5)	25.2511±0.2011		20.6496±0.5096	
3	Group III	Protective group (5)	34.2756±0.032	2:3 P<0.01	24.2384±0.1814	2:3 P<0.01
4	Group IV	Curative group (5)	36.5825±0.0763	2:4 P<0.01 3:4 P< 0.01	25.6983±0.0467	2:4 P<0.01 3:4 P< 0.01

Table III: Effect of fenugreek seed extract on fucose content in Submandibular and Sublingual glands of aging accelerated male mice (µg fucose /mg tissue)

Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

Sr. no.	Groups	Treatment	Fucose content (SM)	Statistical significance	Fucose content (SL)	Statistical significance
1	Group I	Control (5)	0.007772±0.00005	1:2 P<0.01	0.007156±0.000687	1:2 P<0.01
2	Group II	Aging accelerated group (5)	0.003158±0.000033		0.003187±0.000053	
3	Group III	Protective group (5)	0.004802±0.000078	2:3 P<0.01	0.004556±0.000248	2:3 P<0.01
4	Group IV	Curative group (5)	0.006338±0.000146	2:4 P<0.01 3:4 P< 0.01	0.005153±0.000426	2:4 P<0.01 3:4 P< 0.01

Table IV: Effect of fenugreek seed extract on Sialic acid content in Submandibular and Sublingual glands of aging accelerated male mice (µg sialic acid /mg protein)

Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

Sr. no.	Groups	Treatment	Sialic acid content (SM)	Statistical significance	Sialic acid content (SL)	Statistical significance
1	Group I	Control (5)	5.5917±1.3527	1:2 P<0.01	9.6522±0.3234	1:2 P<0.01
2	Group II	Aging accelerated group (5)	21.7258±0.9743		28.2949±0.2088	
3	Group III	Protective group (5)	16.1405±0.1204	2:3 P<0.01	16.7891±0.1988	2:3 P<0.01
4	Group IV	Curative group (5)	14.0258±0.5335	2:4 P<0.01 3:4 P< 0.01	12.6278±0.4246	2:4 P<0.01 3:4 P< 0.01

DISCUSSION

A decline in the rate of total protein synthesis is one of the commonly observed age-associated biochemical change in the cells, tissues, organs and organisms. One of the consistent changes that occurred in many salivary glands of aged animals is decline in the rate of synthesis of protein and m-RNA [35]. Age related changes in protein synthesis are regulated both at the transcriptional and pretranscriptional levels in terms of the availability of individual mRNA species for translation, [36] and at the translation and post translation levels in terms of alteration in the components of the protein synthetic machinery. In the present study, protein content in SM and SL glands was decreased in aging accelerated group as compared to control group, indicating oxidative damage during aging. But after the treatment of FSE, the protein content was increased in both protective and curative groups indicating ameliorative effect of fenugreek.

Saliva is responsible for the initial digestion of food because of mainly by the presence of the digestive enzyme α-amylase (ptyalin) in its composition. Amylase is considered to be a good indicator of proper functioning of salivary glands, [37] contributing 40% to 50% of the total salivary protein produced by the glands. A salivary flow rate was decreases and diminishes saliva in aging process. [38-41]. In present study the amylase activity was reduced in aging accelerated mice because, the structural damage, reduction in protein synthesis and enzyme activity in salivary glands due to free radicals mediated oxidative stress [42]. But after the treatment of FSE, the amylase activity was increased in protective and curative group because FSE have capacity to neutralize the action of free radicals and to prevent the tissue from oxidative damage [43].

The SM and SL contain mainly glycoprotein complexes including sulphated hexoses, fucoses and sialic acid. Fucosylation of proteins takes place in luminal part of endoplasmic reticulum and Golgi apparatus. But this process may be impaired due to damage to these cell organelles during aging in rat salivary glands [44]. Because of this reason may be the fucose content was decreased in aging accelerated group as compared to control group. After the treatment of FSE fucose content was increased in protective and curative groups as compared to aging accelerated group.

Serum sialic acid has been reported as a marker of the acute phase response; increased sialic acid concentrations have been observed in several diseases, such as myocardial infarction and diabetes. Serum sialic acid is also increases during inflammatory processes as a consequence of elevated concentrations of richly sialylated acute phase glycoproteins [45]. Sialic acid levels were significantly increased in aging accelerated group as compared to control group because due to oxidative damage. After the treatment of FSE sialic acid content was decreased in both protective and curative groups.

The satisfactory result observed in curative group as compared to protective group indicates FSE administration after injury is more beneficial than co-treatment. Antioxidative enzymes are considered to be primary defense mechanism that protects biological macromolecule from oxidative damage. Thus the elevated level of antioxidative enzymes after fenugreek administration could be involved in increased protection of salivary glands against free radicals. All results of protein, sialic acid, fucose content and amylase activity suggests that FSE have significant antioxidative potential.

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