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MODULATORY EFFECT OF FENUGREEK (*TRIGONELLA FOENUM GRAECUM)* SEED EXTRACT ON SALIVARY GLANDS LIPOFUSCHINOGENESIS IN AGING ACCELERATED MALE MICE.

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ABSTRACT

The present investigation was undertaken to evaluate antioxidative property of fenugreek seed extract (FSE) on lipid peroxidation and fluorescence product in salivary glands of mice. The aging was induced by D-galactose (Dg). Adult albino male mice (*Mus musculus* L) were divided into four groups viz. 1)Control group received subcutaneous injection of 0.5 ml sterile water/day for 20 days 2) Dg- treated group received subcutaneous injection of 0.5 ml of 5% Dg/ day for 20 days 3)Protective group received Dg+ FSE (50mg/kg BW) by subcutaneous injection for 20 days 4)Curative group received Dg then after FSE (50mg/kg BW) subcutaneously for next 20days.After the completion of treatment submandibular and sublingual glands were removed and used for the estimations of lipid peroxidation in the form of malondialdehyde (MDA) and fluorescence product. The MDA level and fluorescence product in both glands were significantly raised in Dg treated group as compared to control group but after the treatment of FSE significant decrease in both parameters in protective and curative group was observed. The results of curative group were more satisfactory as compared to the protective group indicating that FSE give protection against the aging.

KEY WORDS: Lipofuschinogenesis, *Trigonella foenum graecum*, Salivary gland, Aging

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INTRODUCTION

Salivary glands are not only the organ of digestion and maintenance of oral health, but they synthesize and secrete enzymes, growth factors and glycoproteins. These are essential for normal development and maintenance of the body. Several researchers have reported age related changes in morphology[1], histology[2], biochemistry[3] in the salivary glands. The free radical theory of aging states that free radicals derived from oxygen are responsible for damage associated with aging[4]. ROS such as superoxide radical anion, singlet oxygen, hydrogen peroxide and hydroxyl radical are products of oxidative metabolism[5]. Reactive free radicals that are formed within cells can oxidize biomolecules, leading tissue injury and cell death. Free radicals can attack almost any component of the cell, particularly lipids, proteins, and nucleic acids are important targets. Lipids of cell membranes and organelles are frequently damaged, due to lipid peroxidation resulting loss of enzymes and proteins ultimately organelle gets damaged[6]. To nullify the damaging effect of free radicals antioxidant system is present in the body. Antioxidant system in the cells scavenges free radicals. Cellular antioxidant system consists of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidatase (GPx). The non-enzymatic antioxidants include Tocopherol (Vit. E), glutathione, carotenoids, ascorbic acid etc. Organisms have evolved a wide array of enzymatic antioxidant defenses to balance the physiological generation of free radicals. But in case of aging this system diminish function, so the reinforcement of enzymatic antioxidant with dietary antioxidants is important. A number of dietary antioxidants substances exist such as plant derived substances; termed as “phytonutrients” or “phytochemicals”. The antioxidative properties of plants are being investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, without side effects and economic viability[7-8]. Thus antioxidant compounds, which are able to scavenge free radicals or modulate oxidative stress, are beneficial due to their high therapeutic value.

Trigonella foenum graecum (fenugreek) is one of the most popular plant which is widely used in ayurvedic medicine and also applications in food and beverages. Fenugreek seeds are known to have diuretic, hypoglycemic and hypolipidemic effects[9-12]. Fenugreek extract has also reported to exhibit antioxidant activity and could be used as a potent source of antioxidants[13]. Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, and antioxidant activity[14-15]. Seeds are reported to have restorative and nutritive properties[16]. The main chemical constituents of fenugreek are fiber, tannic acid, steroidal saponins, flavonoids, polysaccharides, alkaloids, trigonelline, trigocoumarin, trigomethyl coumarin, mucilage and vitamins A, C, B1, B2 and B3[17-19]. The above review indicates several properties of fenugreek but the only purpose of this study was to evaluate the role of fenugreek as an antilipofuschinogenetic agent in salivary glands because the glands secrete several enzymes, growth factors which are important to maintain cellular activity described above.

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 their antioxidants potential. Extraction was carried out by the soxhelt method[20]. Dry fenugreek seeds were cleaned and ground into fine powder using a grinding machine. Ethanol was used for extraction for six hrs. The extract was filtered and evaporated to dryness under reduced pressure 60ºC by a rotary evaporator. Extract was placed in dark bottle and stored -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 given *ad libitum*. The record of their age and body weight was maintained. Animals were divided into four groups.

1) Control group

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 these doses, the mice were killed by cervical dislocation. Submandibular (SM) and sublingual glands (SL) were dissected out, weighed and one side gland was used for estimations homogenized in 75mM phosphate buffer (pH 7.0) and another side gland used for histochemical study of lipofuschin granules.

A) DETERMINATION OF LIPID PEROXIDATION

Tissue homogenate were prepared in chilled mortar and pestle using 75mM potassium phosphate buffer pH 7.0.[21] The lipid peroxidation was estimated by measurement of malondialdehyde (MDA) an end product of fatty acid peroxidation and reacts with TBA to form coloured complex that has maximum absorbance at 532 nm by using calorimeter.

B) MEASUREMENT OF FLUORESCENCE PRODUCT

The lipofuscin granules from SM and SL glands were extracted using chloroform: Methanol mixture (2:1 v/v). The fluorescence was measured by using quinine sulphate as a standard by using photoflurometer [22].

C) HISTOCHEMICAL DEMONSTRATION OF LIPOFUSCIN GRANULES BY CARBOL FUSCHIN METHOD[23]

After dissection SM and SL glands fixed in 10% neutral buffered formalin for 24 hours at 4^{o}C. The formalin fixed halves of brain were washed under running tap water for 24 hours, dehydrated through alcohol grades, cleared in xylene and embedded in paraffin. 5µ thick sections were cut on the rotary microtome and used for the histochemical demonstration of lipofuschin granules.

STATISTICAL ANALYSIS

The statistical analysis was performed using Student't' Test.

Table 1

Effect of fenugreek seed extract on total lipid peroxidation in Submandibular and Sublingual glands of aging accelerated male mice (Lipid peroxidation in n mol MDA /mg wet tissue). Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

Sr. no.	Groups	Treatment	Total lipid peroxidation (SM)	Statistical significance	Total Lipid peroxidation (SL)	Statistical significance
1	Group I	Control (5)	11.9630±4.422		7.8553±1.678	
2	Group II	Aging accelerated group (5)	46.4394±4.126	1:2 P<0.001	23.7554±3.272	1:2 P<0.001
3	Group III	Protective group (5)	27.9396±4.548	2:3 P<0.001	16.1898±1.239	2:3 P<0.001
4	Group IV	Curative group (5)	19.7295±3.618	2:4 P<0.001 3:4 P<0.01	10.8959±1.259	2:4 P<0.001 3:4 P<0.01

Table 2

Effect of fenugreek seed extract on fluorescence product in Submandibular and Sublingual glands of aging accelerated male mice (fluorescence product in µg/mg tissue). Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

Sr. no.	Groups	Treatment	Total fluorescence product (SM)	Statistical significance	Total fluorescence product (SL)	Statistical significance
1	Group I	Control (5)	0.001817±0.0001767	1:2 P<0.001	0.00181±0.0001951	1:2 P<0.001
2	Group II	Aging accelerated group (5)	0.009772±0.0001316		0.005562±0.0003212	
3	Group III	Protective group (5)	0.005632±0.0002306	2:3 P<0.001	0.003709±0.0002230	2:3 P<0.001
4	Group IV	Curative group (5)	0.003773±0.0001759	2:4 P<0.001 3:4 P<0.01	0.001937±0.0001218	2:4 P<0.001 3:4 P<0.01

RESULTS

A) The total lipid peroxidation in SM as well as SL was increased in aging accelerated group (Group II) as compared to control (Group I) and increase was highly significance (1:2, P<0.001), while it was decreased significantly in protective (Group III) and curative (Group IV) (2:3, P<0.001; 2:4, P<0.001) as compared to aging accelerated group (Group II), (Table 1).

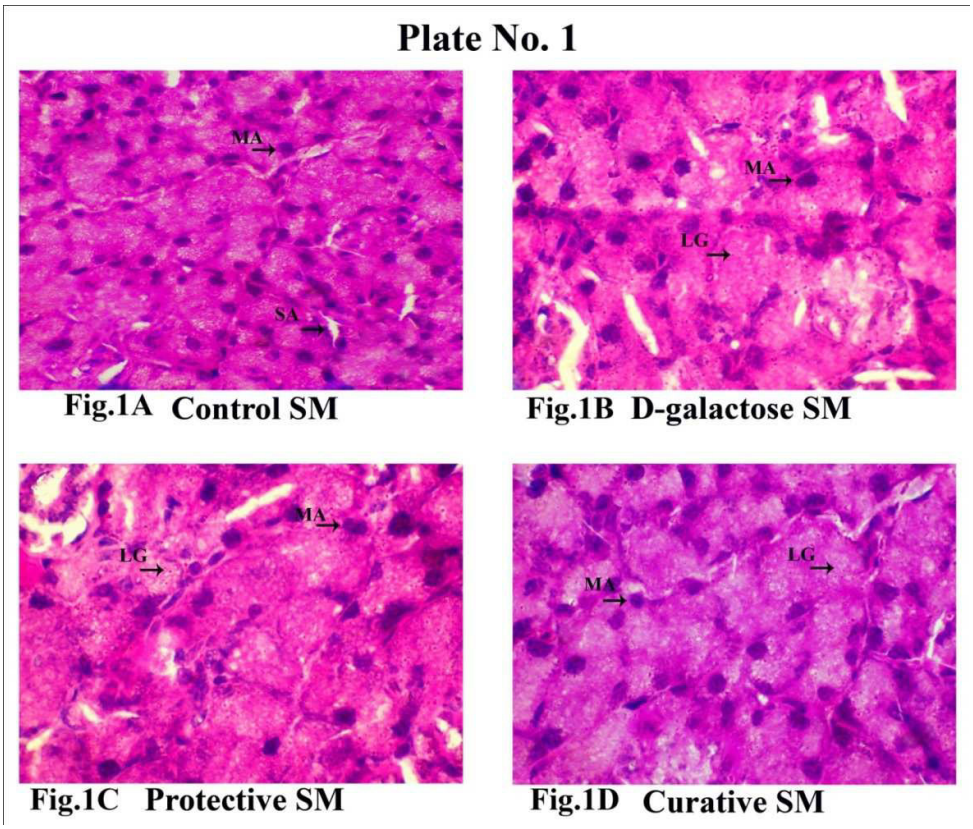
B) The fluorescence product in SM as well as SL gland was increased in aging accelerated group (Group II) as compared to control (Group I) (1:2, P<0.001), while it was decreased significantly in protective group and curative group as compared to aging accelerated group (2:3,P<0.001; 2:4, P<0.001) (Table 2). The MDA and fluorescence product was significantly decreased in curative group (Group IV) as compared to protective group (Group III) (3:4, P<0.01).

C) The light microscopic observation of lipofuschin granules in SMG (Plate No. 1) and SLG (Plate No. 2) was displayed in figure. The increased accumulation of lipofuschin granules were observed in SMG of aging accelerated group as compared to aging control group (Plate no. 1, Fig A and B). While in FES co-treated group, (protective) there was decrease in accumulation of lipofuschin granules as compared to aging accelerated group (Plate no. 1, Fig. B and C). In case of curative group negligible number of lipofuschin granules was observed as compared to protective group (Plate no. 1, Fig. C and D). Similar type of changes was observed in SLG (Plate no. 2, Fig. A, B, C, D).

DISCUSSION

D-galactose is aging inducing agent[24]. The given dose of D-galactose caused a significant oxidative damage to the SM and SL glands within very short time of 20 days. D-galactose interacts with free amino group of proteins to produce advance glycation end products (AGEs) [25-27] and increase in fluorescence product indicates the formation of lipofuscin granules during aging because membrane damage. Fluorescence product lipofuscin granules are autofluorescence. The significant increase in lipid peroxidation formation of MDA and fluorescence product in D-galactose injected male mice was observed in SM and SL glands while significant decrease in both parameters in protective and curative groups as compared to aging accelerated mice this indicates protective property of fenugreek. The satisfactory result observed in curative group as compared to protective group indicates fenugreek 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. The accumulation of lipofuschin pigment as a function of age, oxidative stress and antioxidant deficiency has been known for many years and these pigments results from lipid peroxidation[28] as well as these increase fluorescence product also. The elevated level of antioxidative enzymes after fenugreek administration could be involved in increased protection of salivary glands against free radicals. The antioxidant mechanism of

fenugreek has recently been a focus of interest of free radicals chemist and biologist. Fenugreek seeds have been documented for their multiple pharmacological activities including antioxidation[29] as well as polyphenols prevented oxidative hemolysis and lipid peroxidation induced by H_2O_2 in vitro in human erythrocyte[30]. Moreover, it was demonstrated that the supplementation of fenugreek seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxan-diabetic rats[31]. The reduction in the lipid peroxidation and fluorescence level in salivary glands of mice after receiving fenugreek concludes that, fenugreek seeds having antioxidant and anti-peroxidative properties.



MA- Mucous acinar cells
LG- Lipofuschin granules
SA- Serous acinar cells
Figure 1A: Cross section of Submandibular Gland of Control Group Mice.
Figure 1B: Cross section of Submandibular Gland of D-galactose treated Group Mice.
Figure 1C: Cross section of Submandibular Gland of Protective Group Mice.
Figure 1D: Cross section of Submandibular Gland of Curative Group Mice.

Plate No. 2

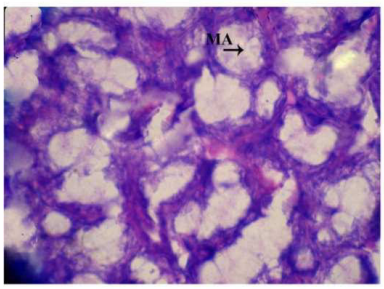


Fig. 2A Control SL

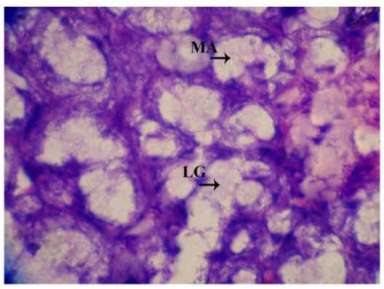


Fig. 2B D-galactos SL

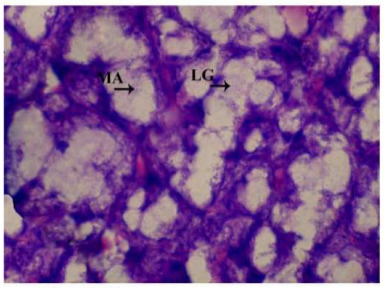


Fig. 2C Protective SL

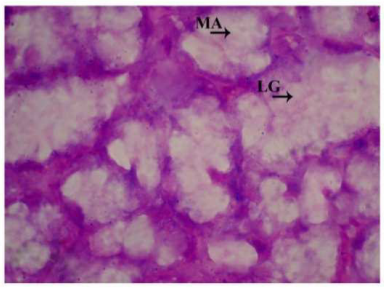


Fig. 2D Curative SL

MA- Mucous acinar cells
LG- Lipofuschin granules
Figure 2A: Cross section of Sublingual Gland of Control Group Mice.
Figure 2B: Cross section of Sublingual Gland of D-galactose treated Group Mice.
Figure 2C: Cross section of Sublingual Gland of Protective Group Mice.
Figure 2D: Cross section of Sublingual Gland of Curative Group Mice.

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