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Research Article

Structural and Cytochemical Study of Salivary Glands after Fenugreek Seed Extract Administration in Oxidatively Stressed Mice.

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ABSTRACT

The study was undertaken to evaluate protective effect of fenugreek seed extract (FSE) on the salivary glands of aging induced mice. For that we have studied the histology as well as histochemistry of the salivary glands. So to evaluate protective effect of fenugreek against accelerated aging, male albino mice (*Mus musculus* L.) were divided into four groups viz, a) Control group- received subcutaneous injection of 0.5 ml sterile water for 20 days. b) Aging accelerated group- received injection of 0.5 ml of 5% of D-galactose/ day for 20 days. c) Protective group- received injection of D-galactose + FSE (50 mg/kg body weight of animal) for 20 day. d) Curative group- received D- galactose for 20 days then after FSE (50 mg/kg body weight) for 20 days. The histological study by HE showed loss of their normal architecture and cellular integrity .Histochemical study by PAS, AB-1, AB-2.5 showed decreased staining intensity of salivary glands in D-galactose injected aged mice as compared to control. While protective and curative groups showed recovery in architecture of salivary acini and increase in acini number. Increased staining intensity indicates increase in glycoprotein content. Better results are seen in curative group as compared to protective group. Thus above results elucidate the protective action of fenugreek seeds against aging in salivary glands of mice.

Keywords: Salivary glands, Histology, Histochemistry, *Trigonella foenum graecum* seed extract

INTRODUCTION

Free radicals or reactive oxygen species (ROS) are formed in our body as a result of biological oxidation. The over production of free radicals such as hydroxyl radical, super oxide anion radical, hydrogen can cause damage to the body and contributes peroxide to oxidative stress[1, 2]. Oxidative damage caused due to free radicals to proteins, DNA and lipid is associated with chronic degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc[3]. Lipids of cells membranes and organelles are frequently damaged, resulting in lipid peroxidation[4]. DNA and RNA are susceptible to oxidative damage and it has been reported that especially DNA is a major target in aging and cancer[5].

During aging the vital organs fail to play their normal functions. ROS are involved in alterations of the salivary gland functions. Several researchers have reported age related changes in morphology, ultrastructure, biochemistry and histology of salivary glands.[6-13] Age-related structural changes observed in salivary glands of humans and other animals involve both the glandular (parenchymal) and supportive (stromal) tissues. The stromal changes in the human salivary glands include an increase in the amount of fibrous and fatty tissues, as well as lymphocytic infiltration[14-18].

Most of the reactive oxygen species are scavenged by endogenous defense systems such as catalase, superoxide dismutase and peroxides-glutathione system[19]. The researchers have focused on natural anti-oxidants. Numerous crude extracts and pure natural compounds have been recognized to have beneficial effects against free radicals in biological systems as anti-oxidants[20-22]. Compounds that can scavenge free radicals have great potential in ameliorating various disease processes[23-25].

Fenugreek (*Trigonella foenum-graecum*) being rich in phytochemicals has traditionally been used as a food, and medicinal plant. Bioactive compounds isolated from fenugreek seeds include saponins (i.e. fenugreekine, diosgenin), alkaloids (i.e. trigonelline, gentianine, and carpaine), amino acids, flavonoids, 4-hydroxyisoleucine, arginine, coumarin, mucilaginous fibers (galactomannan), nicotinic acid and other vitamins and minerals[26, 27].

The objective of the present study was to study recovery of salivary glands structure and glycoprotein content after the administration of *T. foenum graceum* seeds extract.

MATERIALS AND METHODS

Preparation of fenugreek seed extract

Fenugreek seeds were collected from the local market of Kolhapur and dried fenugreek seeds were cleaned and ground into fine powder using a grinding machine. Extraction was carried out by the soxhelt method[28]. Ethanol was used for extraction for six hrs. The extract was filtered and evaporated to dryness under reduced pressure at 60ºC by a rotary evaporator. Extract was placed in dark bottle and stored at -8ºC until further analysis.

**Author for Correspondence*

Animals

Six month old Swiss albino mice (*Mus musculus*) weighing about 50-55 gm were used for the present study. Animals were housed in an approved departmental animal house .Animals 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 four groups.

Control group

Male mice were given subcutaneous injection of 0.5 ml distilled water/ day/ animal for 20 days.

Aging accelerated group

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

Protective group

Male mice were subcutaneously injected with 0.5 ml of 5% D-Galactose/ day/ Kg 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 in 5% D-galactose).

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 used for histological and histochemical study. Both glands were fixed in 10% Neutral Buffer Formalin (NBF) for 24 hours. After hydration, SM and SL were properly dehydrated through the alcohol grades, cleared in xylene and then embedded in paraffin wax. With the help of microtome the sections of thickness 5µ were obtained. The sections were used for Histological study of SM and SL glands by HE technique[29] and Histochemical study of SM and SL glands by PAS[30], AB pH 1.0 and AB pH 2.5[31].

RESULTS

The submandibular (SM) and sublingual gland (SL) of D-

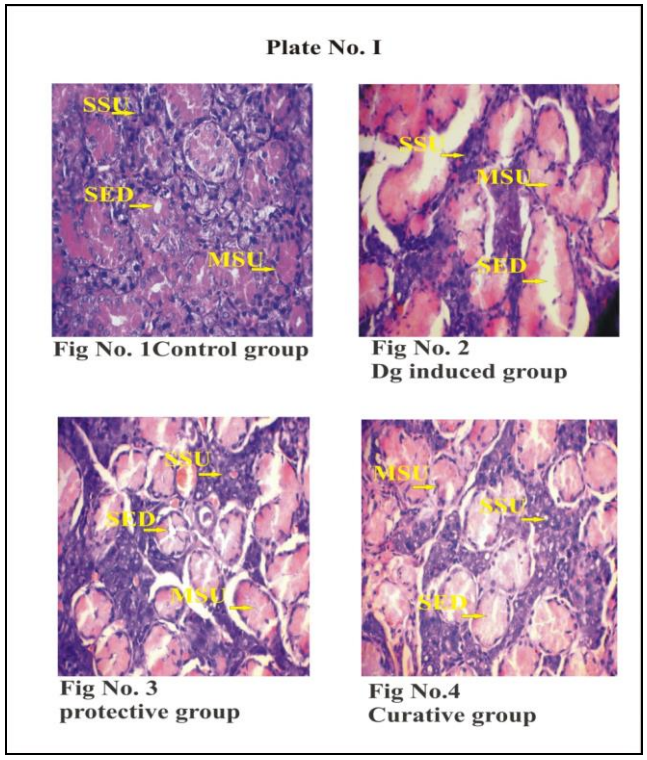

PLATE NO – I

Effect of Fenugreek seed extract on submandibular gland structure of D-galactose induced aged male mice stained with hematoxylene –eosin (HE). Figure no. 1: T.S. of Submandibular gland of control group mice stained with HE (400X) Figure no. 2: T.S. of Submandibular gland of Aging accelerated mice stained with HE (400X) Figure no.3: T.S. of Submandibular gland of D-galactose treated protective group mice stained with HE (400X) Figure no. 4: T.S. of Submandibular gland of D- galactose treated curative group mice stained with HE (400X)

MSU-Mucous Secretory Unit ; SSU-Serous Secretory Unit; SED- Striated Excretory Duct

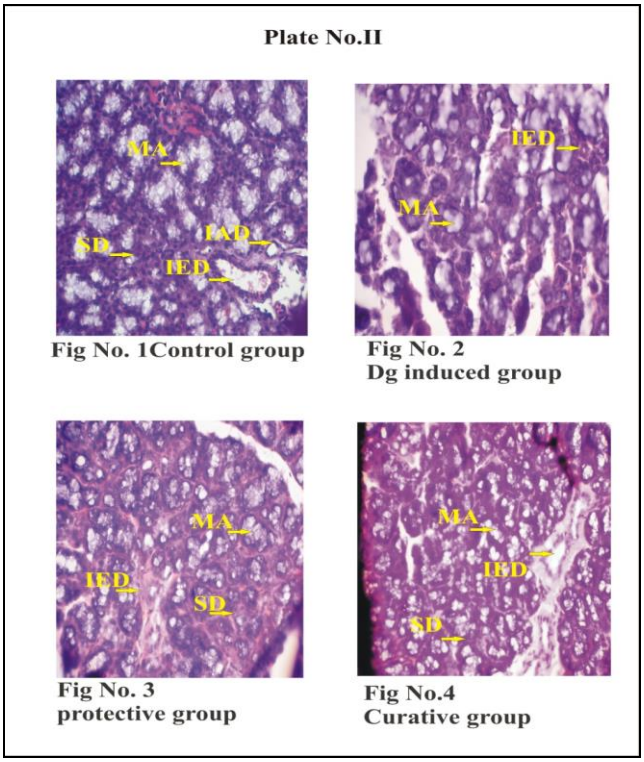

PLATE NO – II

Effect of Fenugreek seed extract on sublingual gland structure of D-galactose induced aged male mice stained with hematoxylene –eosin (HE). Figure no. 1: T.S. of sublingual gland of control group mice stained with HE (400X) Figure no. 2: T.S. of sublingual gland of aging accelerated mice stained with HE (400X) Figure no. 3: T.S. of sublingual gland of D-galactose treated protective group mice stained with HE (400X) Figure no. 4: T.S. of sublingual gland of D-galactose treated curative group mice stained with HE (400X)

MA- Mucous acini; SD- Serous demilunes; IAD- Intralobular duct

IED- Interlobular duct

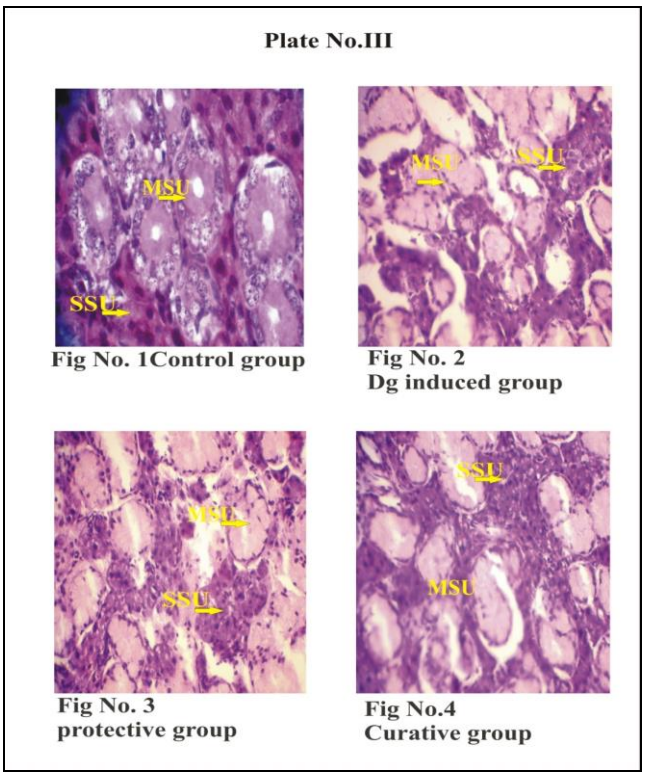

PLATE NO –III

Effect of Fenugreek seed extract on neutral glycoproteins from submandibular gland of D-galactose induced aged male mice stained with PAS-HE. Figure no. 1: T.S. of Submandibular gland of control group mice stained with PAS- HE (400X) Figure no. 2: T.S. of Submandibular gland of aging accelerated mice stained with PAS- HE (400X) Figure no. 3: T.S. of Submandibular gland of D-galactose treated protective group mice stained with PAS-HE (400X) Figure no. 4: T.S. of Submandibular gland of D-galactose treated curative group mice stained with PAS-HE (400X)

MSU-Mucous Secretory Unit ; SSU-Serous Secretory Unit; SED- Striated Excretory Duct

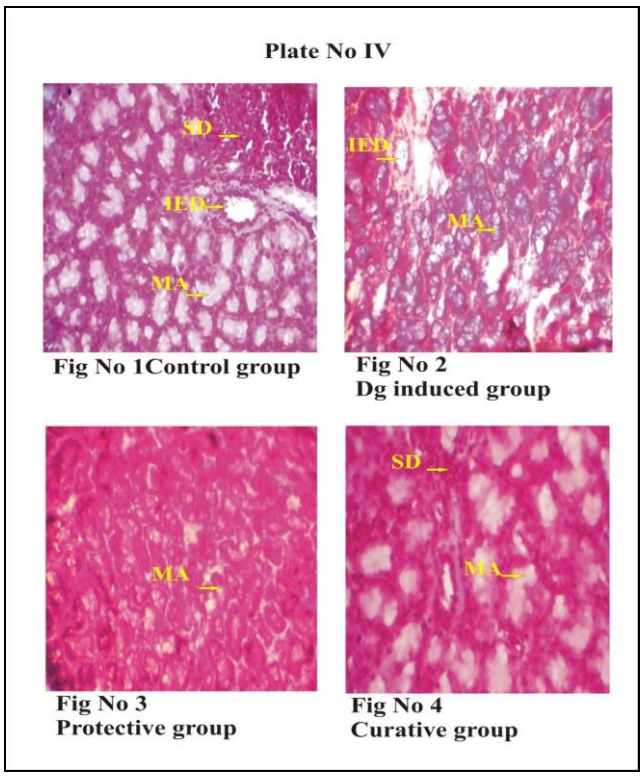

PLATE NO –IV

Effect of Fenugreek seed extract on neutral glycoproteins from sublingual gland of D-galactose induced aged male mice stained with PAS-HE. Figure no. 1: T.S. of sublingual gland of control group mice stained with PAS- HE (400X) Figure no. 2: T.S. of sublingual gland of aging accelerated mice stained with PAS- HE (400X) Figure no. 3: T.S. of sublingual gland of D-galactose treated protective group mice stained with PAS- HE (400X) Figure no. 4: T.S. of sublingual gland of D-galactose treated curative group mice stained with PAS-HE (400X)

MA- Mucous acini; SD- Serous demilunes; IAD- Intralobular duct; IED- Interlobular duct

galactose treated (Plate No. I and II, fig. no. 2) mice showed loss of their normal architecture, cellular integrity as well as number of secretory units as compared to control group (Plate No. I and II, fig. no.1). The loss of glandular structural integrity was due to increase in free radicals in D-galactose induced aged mice. After the simultaneous treatment of FSE and D-galactose in protective group (Plate No. I and II, fig. no. 3) the most of acinar cells of SM and SL have slightly restored their normal histological appearance, increase in number as well as cellular integrity as compare to D- galactose treated group. Better results were observed in case of curative group as compared to protective group. The histological structure of curative group (Plate No. I and II, fig no. 4) was more markedly restored and retained in normal condition. The sections of SM and SL glands when stained with PAS-HE stain, the staining intensity of PAS positive neutral glycoproteins was decreased in D-galactose treated (Plate No. III and IV, fig no. 2) as compared to control group (Plate No. III and IV, fig no.1). While in Dg-treated protective (Plate No. III and IV, fig no. 3) and curative groups (Plate No. III and IV, fig no. 4) after fenugreek treatment the staining intensity was again increased up to control levels. When the SM and SL glands were stained with AB pH-1 (Plate no. VII and VIII) for sulphated glycoproteins and with AB pH 2.5 (Plate no. V and VI) for acidic glycoproteins, the staining intensity was decreased in both the D-galactose treated mice as compared to control group, and was again increased after fenugreek treatment in protective and curative groups.

DISCUSSION

The SM and SL glands of D-galactose stressed mice showed loss of their normal architecture, cellular integrity as well as number of secretory units. The loss of glandular structural integrity was due to increase in free radicals in D-galactose induced aged mice. The age related changes in SM and SL involving the parenchymal tissues include reductions in the amount of rough

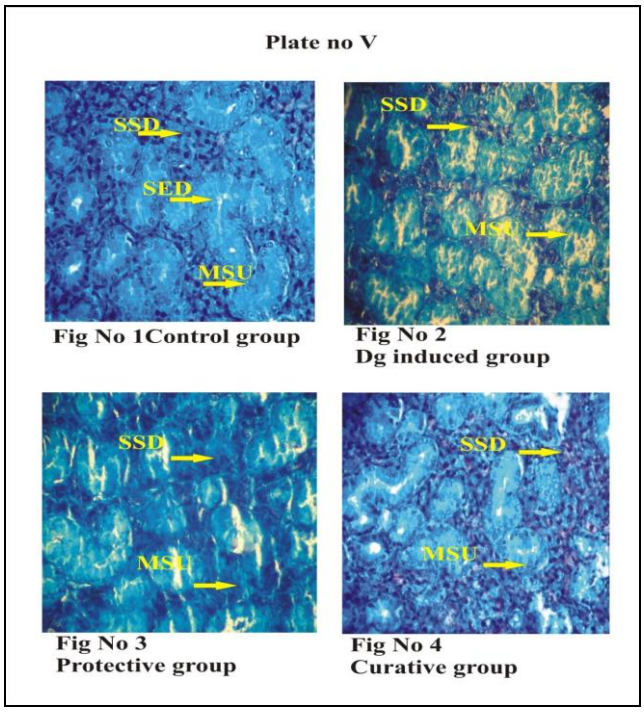

PLATE NO –V

Effect of Fenugreek seed extract on acidic glycoproteins from submandibular gland of D-galactose induced aged male mice stained with AB pH-2.5. Figure no. 1: T.S. of Submandibular gland of control group mice stained with AB- pH 2.5 (400X) Figure no. 2: T.S. of Submandibular gland of aging accelerated mice stained with AB- pH 2.5 (400X) Figure no. 3: T.S. of Submandibular gland of D-galactose treated protective group mice stained with AB- pH 2.5 (400X) Figure no. 4: T.S. of Submandibular gland of D-galactose treated curative group mice stained with AB- pH 2.5 (400X)

MSU-Mucous Secretory Unit; SSU-Serous Secretory Unit; SED- Striated Excretory Duct; ILED- Inter Lobular Striated Duct

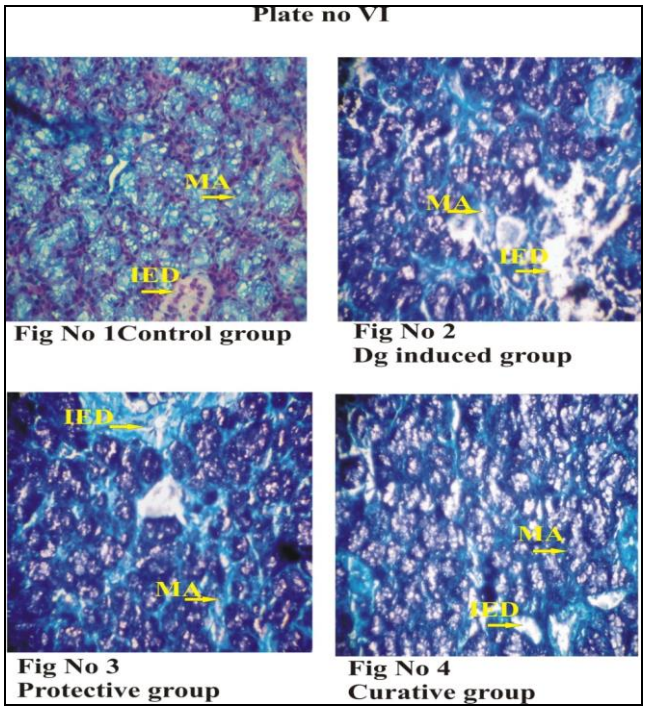

PLATE NO –VI

Effect of Fenugreek seed extract on acidic glycoproteins from sublingual gland of D-galactose induced aged male mice stained with AB pH-2.5. Figure no. 1: T.S. of sublingual gland of control group mice stained with AB- pH 2.5 (400X) Figure no. 2: T.S. of sublingual gland of aging accelerated mice stained with AB- pH 2.5 (400X) Figure no. 3: T.S. of sublingual gland of D-galactose treated protective group mice stained with AB- pH 2.5 (400X) Figure no. 4: T.S. of sublingual gland of D-galactose treated curative group mice stained with AB- pH 2.5 (400X) MA- Mucous acini; SD- Serous demilunes; IAD- Intralobular duct; IED- Interlobular duct

endoplasmic reticulum and the number of secretory granules in acinar cells as well as in cells of the granular convoluted tubules[32, 33, 34, 35]. That means given dose of D-galactose caused a significant oxidative damage to the SM and SL glands within very short time. Thus D-galactose is aging inducing agent[36].

Protective and curative groups after the treatment of FSE revealed that most of acinar cells of SM and SL have restored their normal histological structural appearance and increased staining intensity. This study indicates that the fenugreek seeds were active and scavenges free radicals Phytochemicals analysis has shown that the fenugreek seeds contain a variety of alkaloids, saponins, flavonoids and carbohydrates[37]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen.

Flavonoids are wide spread in all natural compounds and posses a broad spectrum of biological activities. The chemical composition of *Trigonella foenum graecum* has the presence of phenolic compounds including tannins and flavonoids these may be responsible for its free radical scavenging activity[38]. These findings are in accordance with the results of other investigators who reported that fenugreek is a hypoglycemic, hypolipidemic and antioxidant agent[39, 40, 41, 42, 43].

In conclusion, recovery in the structure of SM and SL after fenugreek treatment in aged mice proves that fenugreek seed have free radical scavenging activity.

ACKNOWLEDGEMENT

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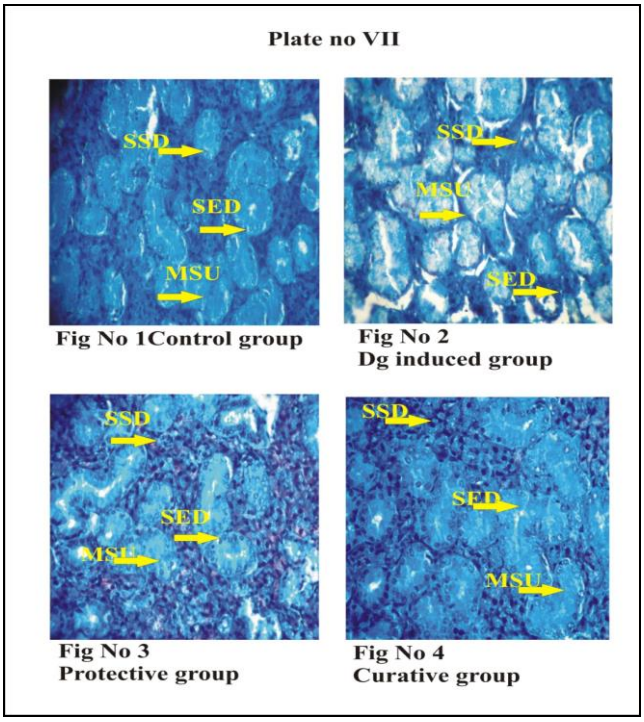

PLATE NO –VII

Effect of Fenugreek seed extract on sulphated glycoproteins from submandibular gland of D-galactose induced aged male mice stained with AB pH-1. Figure no. 1: T.S. of Submandibular gland of control group mice stained with AB- pH 1 (400X) Figure no. 2: T.S. of Submandibular gland of aging accelerated mice stained with AB- pH 1 (400X) Figure no. 3: T.S. of Submandibular gland of D-galactose treated protective group mice stained with AB- pH 1 (400X) Figure no. 4: T.S. of Submandibular gland of D-galactose treated curative group mice stained with AB- pH 1 (400X)

MSU-Mucous Secretory Unit; SSU-Serous Secretory Unit; SED- Striated Excretory Duct

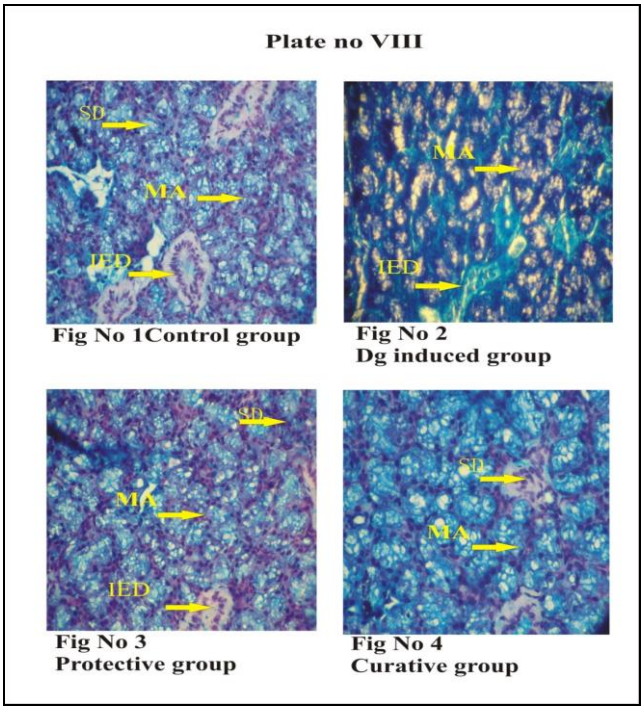

PLATE NO –VIII

Effect of Fenugreek seed extract on sulphated glycoproteins from sublingual gland of D-galactose induced aged male mice stained with AB pH-1. Figure no. 1: T.S. of sublingual gland of control group mice stained with AB- pH 1 (400X)

Figure no. 2: T.S. of sublingual gland of aging accelerated mice stained with AB- pH 1 (400X) Figure no. 3: T.S. of sublingual gland of D-galactose treated protective group mice stained with AB- pH 1 (400X) Figure no. 4: T.S. of sublingual gland of D-galactose treated curative group mice stained with AB- pH 1 (400X)

MA- Mucous acini; SD- Serous demilunes; IED- Interlobular duct

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