Effect of Glycowithanolides on Fucose Content in Salivary Glands of Aged Mice

Radhika N Mote[1] and Pillai M[2]

[1*]*Department of Zoology, Shivaji University Kolhapur -*[416004] *(MS) India.*
[2]*Department of Biotechnology, Kolhapur Institute of Technology, Kolhapur-* [416234] *(MS) India.*
dr.moteradhika@rediffmail.com*

Abstract

Glycowithanolides (WSG) is the extract of *Withania somnifera* leaves was tested to find its effect on fucose content in salivary glands of D-galactose(Dg) stressed adult and old male mice (*Mus musculus*). Adult and old male mice were divided in to protective group and curative group. Both the groups were further divided into four batches viz. 1st is the control batch received 0.5 ml 0.9 % saline per day for 20 and 40 days for protective and curative group respectively. Mice from 2nd, 3rd and 4th batches of protective group received 0.5 ml D-galactose (Dg), Dg+ centrophenoxine(CPH) and D-galactose (Dg) + (WSG) respectively for 20 days. Mice from 2nd, 3rd and 4th batches of curative group received 0.5 ml D-galactose (Dg) for 20 days then followed by 0.5ml saline, centrophenoxine and WSG for further 20 days respectively. Fucose content (µg/mg proteins) in salivary glands was estimated. In D-galactose stressed adult and old mice it was decreased significantly, but restored by the treatment of WSG and centrophenoxine. The restoration was not exactly up to the normal level but was near to the normal level in adult. In D-galactose stressed old mice there was restoration in fucose content but it was not like that of adult. Restoration was significantly higher in WSG treatment. Thus WSG can be used as a powerful natural antistresser.

Keywords: Antioxidants, D-galactose, Fucose, glycowithanolides, salivary glands

1. Introduction

The aging process is one of the serious problems of the modern world. Due to various medicines against terminal diseases like cancer, diabetes, atherosclerosis, rheumatism and various infectious diseases, life is saved but ageing process is not stopped. Modern medicines are unable to solve the problems of old age and disability (Ship *et al.,,* 2002).

Salivary glands are affected due these medicines and also due to the old age. The root cause is free radicals formed during aging, due to stress or toxicity of various medicines. Free radicals have been implicated in etiology of several human diseases as well as aging (Halliwell & Gutteridge, 1997).

In old age free radicals are not removed efficiently by defense mechanism of the cell which includes Super Oxide Dismutase (SOD), Catalase CAT and Glutathion Peroxidase (GPx) and some other antioxidants. There is a long list of antioxidants, suggested by various scientists and flavonides are supposed to be very good antioxidants extracted from various plants.

Withania somnifera is an amazing and popularly used ayurvedic plant commonly called as 'Indian ginseng' (Bhatnagar *et al.,,* 2005). It acts as anti-stress, adaptogenic agent as well as increases life span and delay ageing (Satyavati, 1995). In present study glycowithanolides extracted from *Withania somnifera* leaves was tested to find out its effect on fucose content in salivary glands of mice during aging. Salivary glands play important role in growth, differentiation and development (Bodre & Pillai, 2007; Walvekar & Pillai, 2008).

Several biologically active polypeptides such as Epidermal Growth Factor (EGF), Nerve Growth Factor (NGF), and Transforming Growth Factor (TGF) etc. are secreted by salivary glands (Sporn *et al.,* 1982). During old age salivary glands undergo changes in morphology (Kim and Allen 1993), histology (Scott *et al.,* 1986), biochemistry (Denny *et al.,* 1991a&b; Mahay *et al.,* 2004) and ultrastructure (Bogart, 1970). Several studies reported diminished functions of salivary glands leads to various old age related diseases such as xerostomia, dental caries, Sjogren's syndrome, periodontal disease etc (Olver *et al.,* 2006).

There is a close relationship between oral and systemic health (de- Almeida *et al.,* 2008). Thus salivary glands are the biomarkers of aging as they are adversely affected during aging (Baum *et al.,* 1992). Salivary glands are rich in glycoproteins mainly sulfated hexoses, fucose and sialic acids (Nisizava & Pigman, 1959). 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 (Listinsky *et al.,* 1998, Staudacher *et al.,* 1999).

It is a powerful immune modulator. It has significant role in slowing the growth of cancer cells. Its deficiency is accompanied by a complex set of phenotypes both in human and mice. Fucosylated glycanss have been implicated in the pathogenesis

of several human diseases (Lee *et al.,* 1997, Miyake *et al.,* 1992; Kim & Varki, 1997) in rheumatoid arthritis patient (Flogel *et al.,* 1998; Gornik *et al.,* 1999) in cystic fibrosis (Scanlin & Glick, 1999).

Fucose deficiency in animal causes a large number of phenotypic consequences, underscores the crucial role of fucosylated glycanes to many physiological and developmental processes (Baker & Lowe, 2003). For fucosylation fucose is obtained from GDP- fucose. GDP-fucose is synthesized by de- Novo and salvage pathway (Tonetti *et al.,* 1998). In salvage path way free fucose required for GDP-fucose synthesis is derived from extracellular of lysosomal fucose or lysosomal catabolism of glycoproteins and glycolipids. GDP-fucose thus synthesized is then transported into lumen of the Golgy apparatus for fucosylation (Baker & Lowe, 2003). Thus lysosomes and Golgi apparatus play important role in fucosylation.

This shows that fucosylated glycoproteins play important role in salivary glands which are biomarkers of aging. It is essential to study the fucose in salivary glands and prevention of its loss during aging and stress. In the present study WSG extracted from *Withania somnifera* was used to prevent the loss of fucose from aged and stressed salivary glands. For comparison a known antioxidant CPH was used. CPH is an efficient free radicals scavenger described earlier by several researchers (Zs Nagy & Nagy, 1980; Zs Nagy & Floyd, 1984).

1.1 Materials and methods

Adult (5 to 6 months old weighing 50 to 55 ± 2 g body wt.) and old (16 to 18 months old weighing 40 to 45 ± 2 g body wt.) male mice (*Mus musculus*) were selected for the study. They were supplied with Amrut mice feed (Pranav Agro Industries Pvt. Ltd. Sangli) and water *ad libitum*. Both adult as well as old mice were divided into two group viz. protective group and curative group. Each group further divided into 4 batches.

Batch 1 – Control: Control batch received 0.5 ml 0.9% saline/day for 20 days and 40 days for protective and curative groups respectively.

Batch 2 – D-galactose (Dg) stressed: Mice received 0.5 ml 5% D-galactose (prepared in 0.9% saline) per day for 20 days (Song *et al.,* 1999, Deshmukh *et al.,*, 2006) for protective group. Curative group received D-galactose for 20 days and then saline for further 20 days subcutaneously. Protective batch denoted as Dg-stressed and curative batch as Dg → saline.

Batch 3 – Centrophenoxine (CPH) treated: Mice of protective group received 0.5 ml 5% D-galactose along with centrophenoxine–a synthetic antioxidant per day (80 mg/kg body wt.) for 20 days (Patro and Sharma 1984). Curative group received 0.5 ml 5% D-galactose for 20 days and then centrophenoxine alone for further 20 days, sacrificed on 41st day. Protective batch denoted as Dg + CPH and curative batch denoted as Dg → CPH.

Batch 4 – Glycowithanolides (WSG) treated: Mice received 0.5 ml 5% D-galactose along with WSG (20 mg/kg body wt.) (Bhattacharya *et al.,*, 1997) per day for 20 days in protective group and denoted as Dg + WSG. In curative group mice received 0.5 ml 5% D-galactose for 20 days and then followed by 0.5 ml WSG alone per day for 20 days. Protective batch denoted as Dg + WSG and curative batch denoted as Dg → WSG.

All treatments were given at 9.00am.After completion of the treatments animals were sacrificed by cervical dislocation between 9.00am to 12.00 noon. Submandibular and sublingual glands were pulled, weighed, homogenized and centrifuged at 5000 rpm for 10 minutes at 10^{o} C temperature to prepare sample and used for estimation of proteins and fucose. The protein contents in both the salivary glands of adult as well as old mice were estimated by Lowry et al. (1951) and fucose by Dische and Shettles (1948) method.

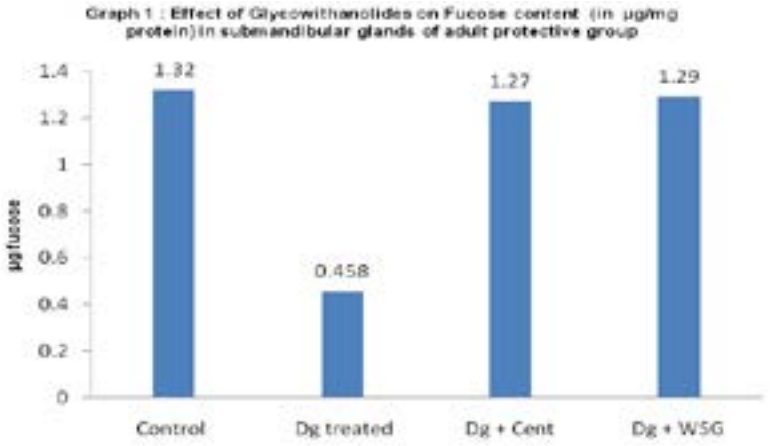
1.2 Preparation of plant extract

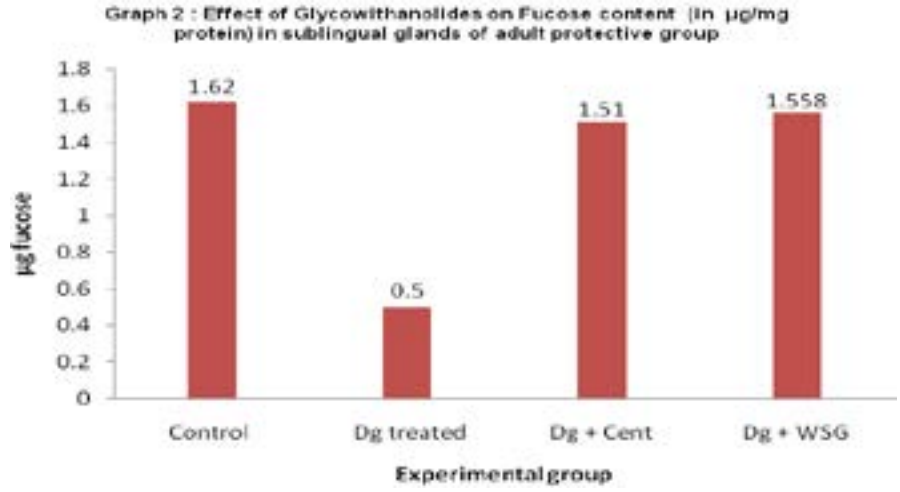
Glycowithanolides was extracted from fresh green leaves of *Withania somnifera.* Fresh leaves were shade dried, crushed and chloroform extract was prepared as described by Bhattacharya *et al.,* (1997). The aqueous concentrate of Withania *somnifera* leaves was exhaustively extracted with chloroform to remove fatty material and free withanolides. The aqueous solution was then spray dried and contained sitoindosides VII-X and withaferin collectively referred as glycowithanolides (WSG). The later was determined with the help of HPTLC as described by Bhattacharya et al. (1997). Glycowithanolides was freely soluble in water and saline. Plant extract was dissolved in sterile water and was given to the experimental mice subcutaneously (20 mg/kg body wt.)

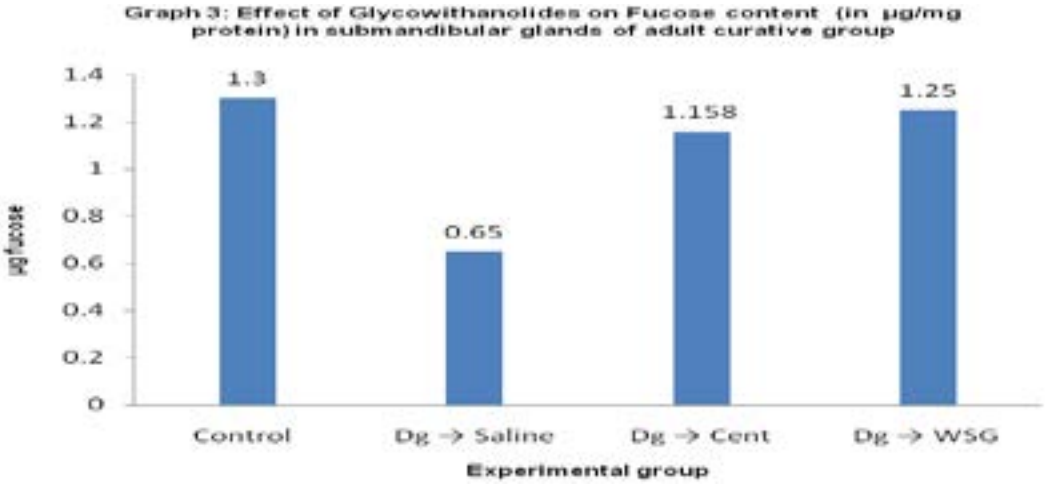
2. Results

In old mice fucose content in submandibular and sublingual glands was reduced significantly compared to adult. The fucose content in submandibular and sublingual glands of D-galactose stressed batches of both the adult (Table 1, graphs 1-4) and old (Table 2,

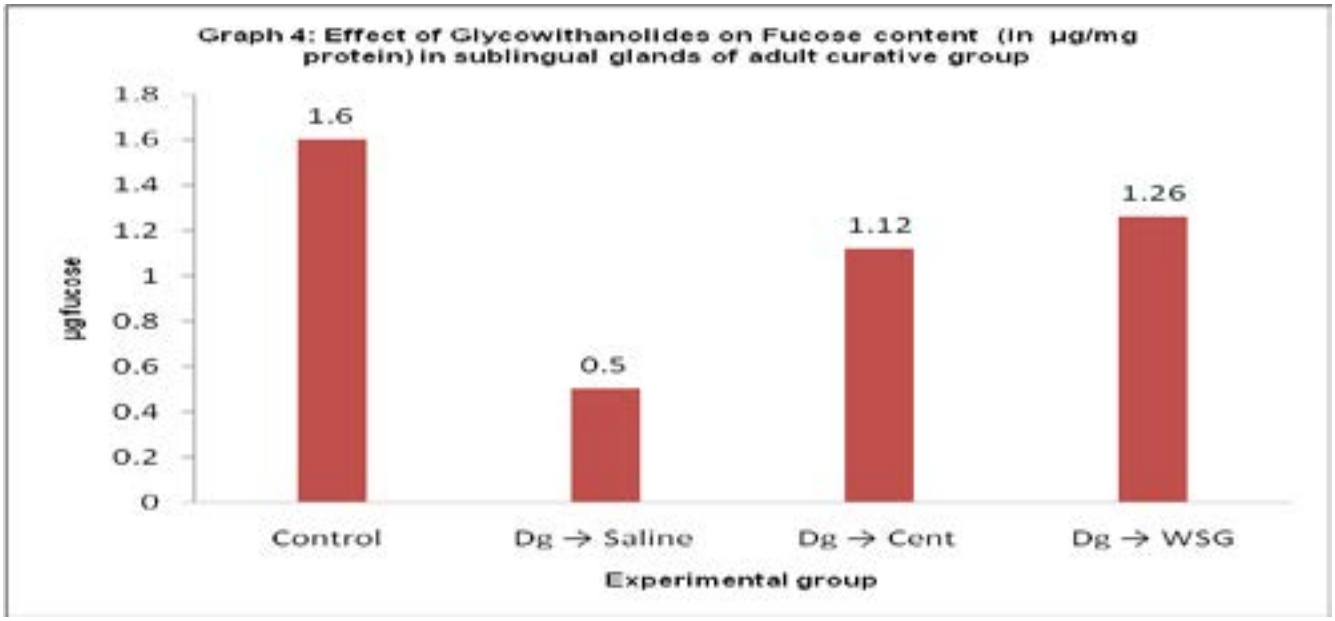
graphs 5-8) mice was decreased significantly (P<0.001) as compared to their respective control batches. But in both antioxidants i.e. WSG and CPH treatment there was increase in fucose content in both the salivary glands of adult as well as old mice as compared to the respective D-galactose stressed batches.

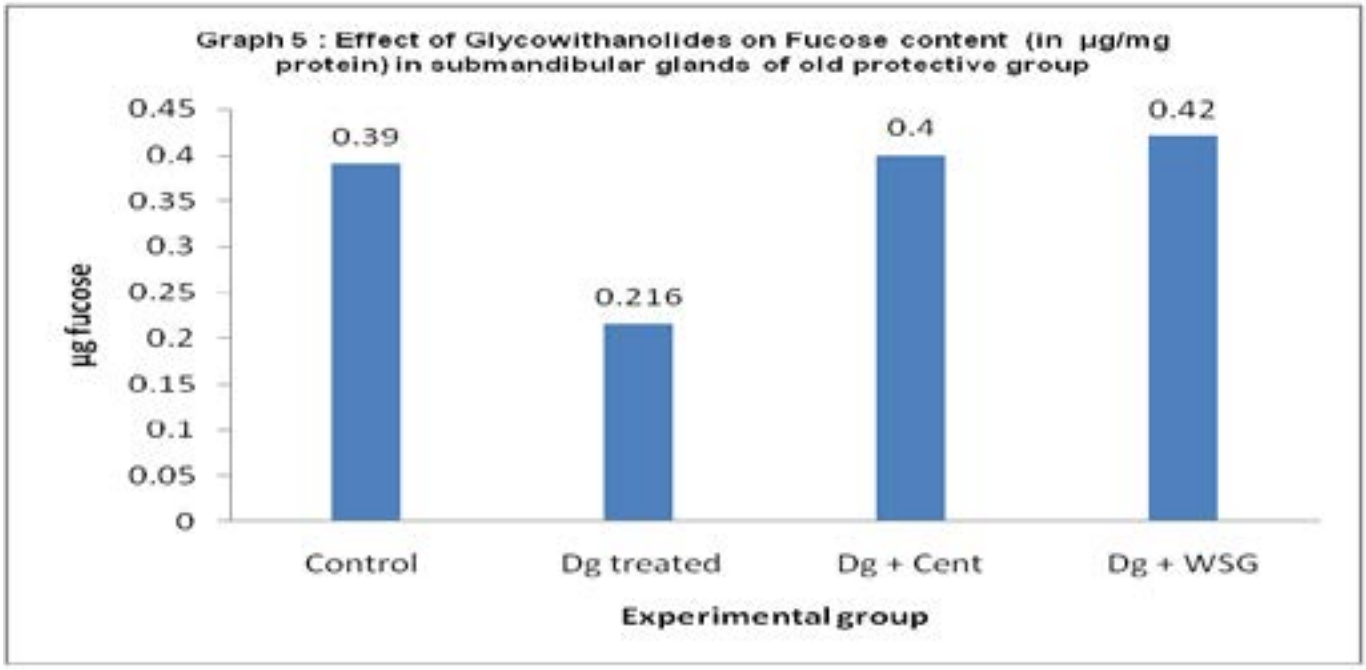






The fucose level was maintained to its normal level by both the antioxidants in adult. In case of WSG treatment, loss of fucose in both the salivary glands in protective batches of adult and old was prevented well than the curative batches. Similar effect was also observed in CPH treatment. When prevention of loss of fucose in case of WSG treatment was compared to CPH, the WSG seems to be more effective than CPH in both the glands of adult and old mice.

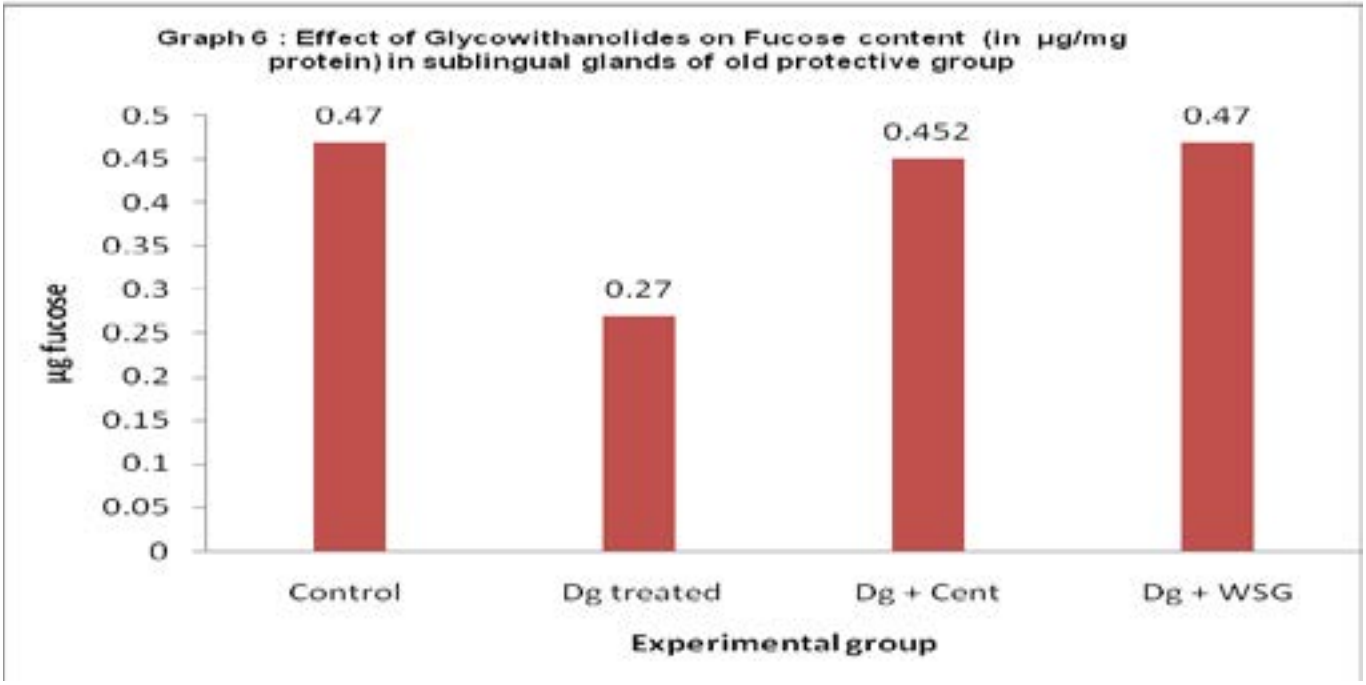


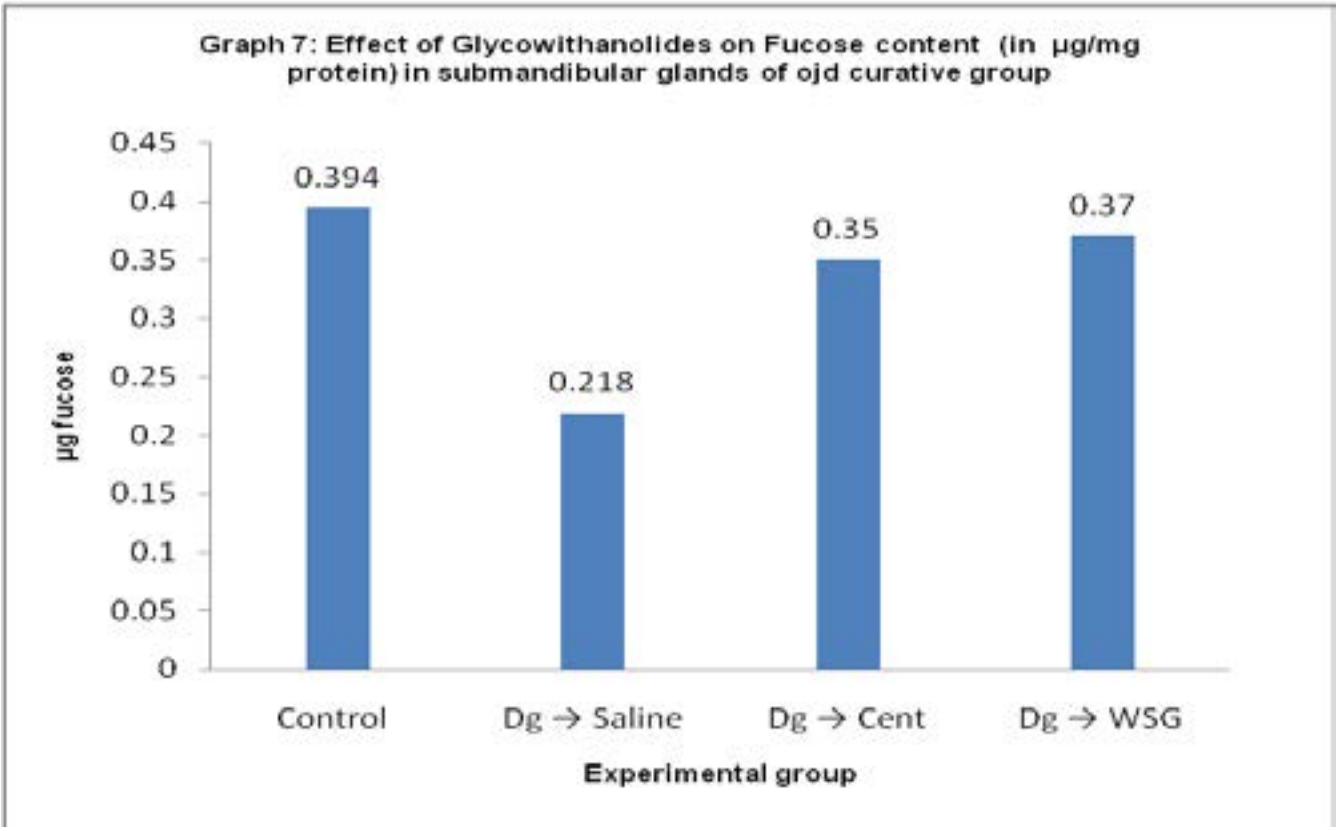


3. Discussion

The reduction in the fucose content of salivary glands may be due to the reduction in glycoprotein synthesis. The progressive decline in the rate of protein synthesis with age in the salivary glands was described in rat (Kuatt & Baum, 1981, Baum *et al.,* 1983, Rattan, 1996) in mice (Mote *et al.,* 2009). This decline in protein synthesis is due to free radicals induced structural damage in salivary glands cells (Scott,1977a; Azevedo *et al.,* 2005; Mote *et al.,* 2010). D-galactose induces oxidative stress followed by AGEs (Song *et al.,* 1999; Deshmukh *et al.,* 2006).

The changes observed in salivary glands of D-galactose stressed mice such as reduction in total proteins (Mote *et al.,* 2009), structural damage (Gresik, 2005) etc are similar to the changes observed in naturally aged animals (Brian *et al.,* 1981, Kim & Allen, 1993; Azevedo *et al.,* 2005). Similarly the increase in lipid peroxidation in brain (Lee *et al.,* 1997), in mitochondrial fraction of brain (Vora *et al.,* 2009), alterations in lysosomal enzymes (Vora *et al.,* 2005; Pillai *et al.,* 2003) were observed in D-galactose stressed mice.





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. Damage to the cell organelles during aging was reported by Sashima (1986), Ashour (1998) in rat salivary glands.

When D-galactose stressed adult and old mice were treated with CPH and WSG there was recovery in fucose content of submandibular and sublingual glands of both protective and curative groups of adult. The recovery was not up to the normal level in old. These antioxidants may help in removal of free radicals. CPH possess $OH^{\cdot}$ radical scavenging capacity (Zs-Nagy,

1989), which can help to protect the cellular damage. WSG is a powerful natural antioxidant described by many (Bhattacharya *et al.,* 1997; Naidu *et al.,* 2006; Kumar *et al.,* 2005; Harikrishna *et al.,* 2008; Rajasankar *et al.,* 2009). The antioxidant potential of *Withania somnifera* inhibit ROS induced lipid peroxidation (Gupta *et al.,* 2003; Kumar *et al.,* 2006; Palanyandi , 2006)which may prevent damage of Golgi, ER and other cell organelles and they remain intact to carry out cellular function. WSG increases cell's antioxidant enzymes i.e. SOD, CAT and GPx in Wistar rats (Gupta *et al.,* 2003; Naidu & Singh, 2006) and prevent free radical mediated cellular damage.

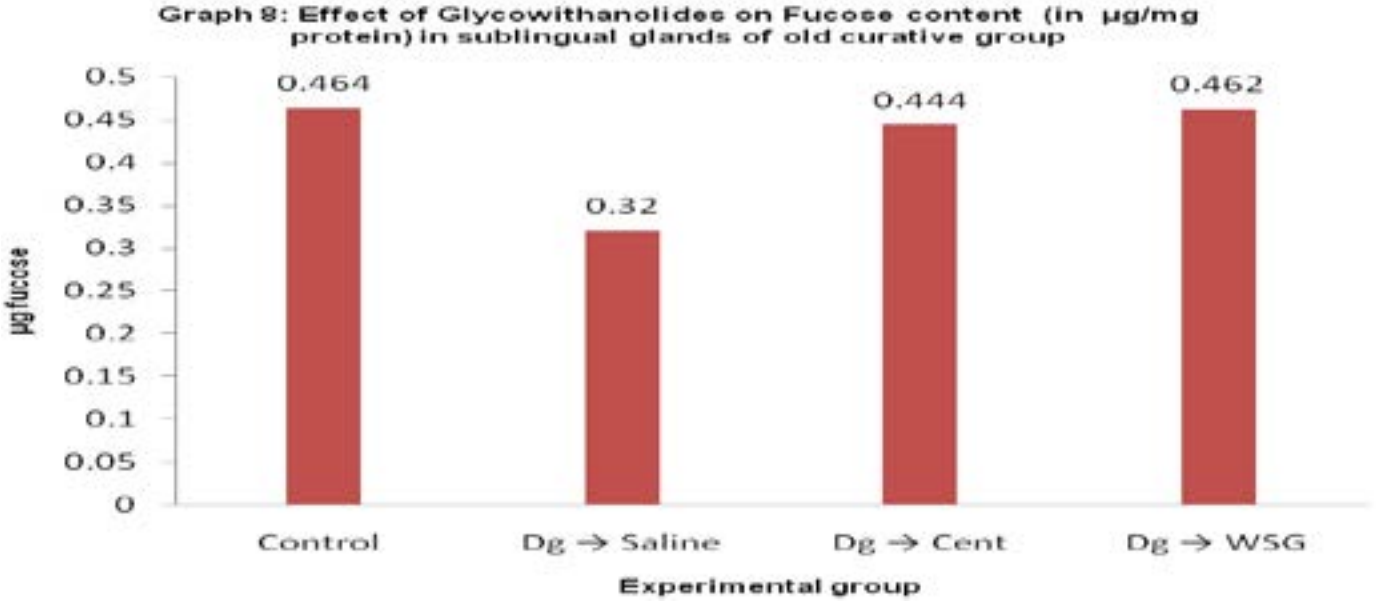


Table.1. Effect of Glycowithanolides (WSG) on fucose content (in µg/mg protein) in salivary glands of D-galactose stressed adult male mice (5 to 6 months age) of protective and curative groups

S. No.	Batches	Fucose content					
		Submandíbular gland			Sublingual gland		
I	Protective Group	Fucose (µg)	t-value	p-value	Fucose (µg)	t-value	p-value
A	Control (5)	1.32 ± 0.1303	A:B 5.2195	p<0.001	1.62 ± 0.1303	A:B 6.2415	p<0.001
B	Dg treated (5)	0.458 ± 0.0192	B:C 32.0470	p<0.001	0.5 ± 0.1581	B:C 5.0317	p<0.001
C	Dg + Cent (5)	01.27 ± 0.01581	C:D 0.9428	p>0.1	1.51 ± 0.0380	C:D 0.9954	p>0.1
D	Dg + WSG (5)	1.29 ± 0.01581	B:D 32.8364	p<0.001	1.558 ± 0.0319	B:D 5.2765	p<0.001
II	Curative Group	Fucose (µg)	t-value	p-value	Fucose (µg)	t-value	p-value
A	Control (5)	1.3 ± 0.0790	A:B 6.1282	p<0.001	1.6 ± 0.1	A:B 8.6908	p<0.001
B	Dg → Saline (5)	0.65 ± 0.0790	B:C 5.0537	p<0.001	0.5 ± 0.01	B:C 25.1030	p<0.001
C	Dg → Cent (5)	1.158 ± 0.0228	C:D 2.0162	p<0.1	1.12 ± 0.0474	C:D 2.3172	p<0.05
D	Dg → WSG (5)	1.25 ± 0.0790	B:D 5.6568	p<0.001	1.26 ± 0.01581	B:D 52.4449	p<0.001

values in the parenthesis denote the number of mice p>0.1 = non significant
values are mean ± s.d. p<0.001 = highly significant
p<0.01 = significant p<0.02 = significant p<0.05 = almost significant

Table.2. Effect of Glycowithanolides (WSG) on fucose content (in µg/mg protein) in salivary glands of D-galactose stressed old male mice (16 to 18 months age) of protective and curative groups

S. No.	Batches	Fucose content					
		Submandibular gland			Sublingual gland		
I	Protective Group	Fucose (µg)	t-value	p-value	Fucose (µg)	t-value	p-value
A	Control (5)	0.39 ± 0.0169	A:B 0.1057	p<0.001	0.47 ± 0.0187	A:B 7.9056	p<0.001
B	Dg treated (5)	0.216 ± 0.002	B:C 5.9497	p<0.001	0.27 ± 0.02	B:C 7.0103	p<0.001
C	Dg + Cent (5)	0.36 ± 0.0689	C:D 0.2291	p>0.1	0.452 ± 0.0130	C:D 1.0357	p>0.1
D	Dg + WSG (5)	0.38 + 0.01	B:D 39.7037	p<0.001	0.47 + 0.0122	B:D 7.7266	p<0.001
II	Curative Group	Fucose (µg)	t value	p value	Fucose (µg)	t value	p value
A	Control (5)	0.394 ± 0.0270	A:B 5.1192	p<0.001	0.464 ± 0.0207	A:B 5.3007	p<0.001
B	Dg → Saline (5)	0.218 ± 0.0083	B:C 10.3709	p<0.001	0.32 ± 0.0158	B:C 5.6247	p<0.001
C	Dg → Cent (5)	0.35 ± 0.0158	C:D 0.9428	p>0.1	0.444 ± 0.0207	C:D 0.6698	p>0.1
D	Dg → WSG (5)	0.37 + 0.0158	B:D 11.9422	p<0.001	0.462 + 0.0130	B:D 6.8162	p<0.001

values in the parenthesis denote the number of mice p>0.1 = non significant
values are mean ± s.d. p<0.001 = highly significant
p<0.01 = significant p<0.02 = significant p<0.01 = significant

Though with CPH and WSG there is recovery of fucose content in salivary glands both in D-galactose stressed adult and old mice it is more significant in WSG treatment.Though CPH and WSG are capable of recovery of fucose content and the structure of salivary glands, this is remarkable in the adult mice treated with D-galactose but in old mice (16 to 18 month old) this recovery is not like that of adult. This shows that during normal aging there may be permanent loss of certain cellular structures due to free radicals which are not removed or regenerated afterwards. This shows that WSG can be useful in treatment of alterations in salivary glands due to certain diseases like xerostomia, cancer or other medicines. But physiology of old salivary glands can't be changed up to satisfaction.

4. Acknowledgement

The authors thank to Department of Zoology, Shivaji University, and Kolhapur for providing the all facilities to carry out this work.

5. References

1• Ashour M (1998) Long term effects of melatonin on submandibular salivary glands in old rats. *East Medit. Health J*. 4(2), 324-331

2• Azevedo L, Damante J, et al. (2005) Age related changes in human sublingual glands: a post mortem study. *Arch. oral Biol*. 50, 565-574

3• Baum B, Kuatt B et al. (1983) Protein production and processing in young adult and aged rat submandibular gland cells. *InVivo. Mech. Aging Dev.* 23(2), 123-136

4• Baum B, Ship J et al. (1992) Salivary gland function and aging a model for studying the interaction of aging and systemic diseases. *Crit. Rev. Oral Biol. Med*. 4, 53-64

5• Becker D and Lowe J (2003) Fucose: biosynthesis and biological functions in mammals. *Glycobiology* 13(7), 41R-53R

6• Bhatnagar M, Jain C, et al. (2005) Antiulcer and antioxidant activity of *Asparagus racemosus* WILLD and *Withania somnif-*

era DUNAL in rats. *Ann. NY. Acad. Sci.* 1056, 261-278

7• Bhattacharya S, Kalkunte S, et al. (1997) Antioxidant activity of glycowithanolides from *Withania Somnifera.* Indian *J. Expt. Biol.* 35, 236-239

8• Bodare R and Pillai M (2007) Effect of salivary secretion on testis and associated organs in male mice. *J. Comparative animal physiol.* 25(2), 19-26

9• Bogart BI (1970) The effect of aging on the rat submandibular gland: An ultra structural Cytochemical and Biochemical study. *J. Morph*. 130, 337-352

10• Brian L, Kuyatt S, et al. (1981) Characteristics of submandibular glands from young and aged rats. *J. Dental Res.* 60(5), 936-941

11• de-Almeida P, Gregio A, Machado M et al. (2008) Saliva composition and functions. A Comprehensive Review. *J. Contemp. Dent. Pract*. 9(3),72-80

12• Denny P, Klauser D et al. (1991b) The effect of aging on mucin contents in mouse submandibular glands. *Arch. Oral Biol*. 36(7), 477-481

13• Denny P, Denny P, et al. (1991a) Age related changes in mucins from human whole saliva. *J. Dent. Res.* 70, 1320-1327

14• Deshmukh A, Gajare K, et al. (2006) D-galactose induced ageing in short duration: A quick model of accelerated ageing in mice. *J. Cell & Tissue Res.* 6(2), 753-756

15• Dische Z and Shettles (1948) Specific colour reaction of methyl pentose and a specific spectrometric micro method for their determination. *J. Biol. Chem.* 175, 595-603

16• Flogel M and Lauc G (1998) Fucoxylation and galactosylation of IgG heavy chains differ between acute and remission phases of juvenile choronic arthritis. *Clin. Chem. Lab Med.* 36, 99-102

17• Gornik I, Maravic G *et al.,* (1999) Fucosylation of IgG heavy chains is increased in rheumatoid arthritis. *Clin. Biochem.* 32, 605-608

18• Gresik EW (2005) Changes with senescence in the fine structure of the granular convoluted tubule of the submandibular gland of the mouse. *American J. Anatomy* 184(2),147-156

19• Gupta S and Dua A et al. (2003) *Withania somnifera* (Ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications. *Drug Metabol. Drug Interact*. 19(3), 211-222

20• Halliwell B and Gutteridge J (1997) Free radicals in biology and medicine. Oxford University Press, Oxford.

21• Harikrishnan B, Subramanian P et al. (2008) Effect of *Withania somnifera* root powder on the levels of circulatory lipid peroxidation and liver marker enzymes in chronic hypermmonemia. *E. J. Chem.* 5(4), 872-877

22• Kim S and Allen E (1993) Structural and functional changes in salivary glands during aging. *Microscopy Res. Technique.* 28(3), 243-253

23• Kim Y and Varki A (1997) Perspectives on significance of altered glycosylation of glycoproteins in cancer. *Glycoconj. J*. 14, 569-576

24• Kuatt B and Baum B (1981) Characteristic of submandibular glands from young and aged rats. *J. Dent. Res.* 60(5), 936-941

25• Kumar A and Kulkarni S (2006) Effect of BR-16A (Mentat) a polyherbal formulation on drug induced catalepsy in mice. *Indian J. Exp. Biol* 44, 45

26• Kumar V, Murthy KN, *et al.* (2005) Genetically modified hairy roots of *Withania somnifera* Dunal: a potent source of rejuvenating principle. *Ed. Rejuvenation Res. Spring* 8(1), 37-45

27• Lee C, Veindruch R et al. (1997) Age related alterations of the mitochondrial genome. *Free radical Biol. Med*. 22(7), 1259-1269

28• Listinsky J, Siegal G, *et al.,* (1998) Alpha-L-fucose a potential critical molecule in pathological processes including neoplasia. *Am. J. Clean Pathol*. 110, 425-440

29• Lowry O, Rosenbrough N, et al. (1951) Protein measurement with the Folin- phenol reagent. *J. Biol. Chem*. 193, 265-275

30• Mahay S, Pariente J, *et al.* (2004) Effect of aging on morphology, amylase release, cytosolic Ca^2 signals and acyl lipids in isolated rat parotid gland tissue. *Mol. Cell Biochem.* 266(1-2), 199-208

31• Miyake M, Taki T, *et al.* (1992) Correlation of expression of H/Le(y)/Le(b) antigen with survival in patients with carcinoma of the lung. *N. Engl. J. Med*. 327, 14-18

32• Mote R, Pillai M, et al. (2010) Protective effect of glycowithanolides on submandibular glands of D-galactose stressed mice *The Biosca.* 5(2), 295-299

33• Mote R, Pawar B, *et al.* (2009) Amylase activity in salivary glands of D-galactose stressed mice and protection by glycowithanolides. *Electronic J. Pharmacol. Therapy*. 2, 25-28

34• Naidu P, Singh A, *et al.* (2006) Effect of *Withania somnifera* root extract on reserpine- induced orofacial dyskinesia and cognitive dysfunction. *Phytother. Res*. 20, 140

35• Nisizawa K and Pigman W (1959) The composition and properties of the mucin in the caftle submaxillary glands. *Arch. Oral Biol.* 1, 161-170

36• Olver I (2006) Xerostomia: a common adverse effect of drugs and radiation. *Aust. Preser*. 29, 97-98

37• Palaniyandi S, Radhakrishanan P et al. (2006) Stabilization of membrane bound enzyme profiles and lipid peroxidation by *Withania somnifera* along with paclitaxel on benzo (a) pyrene induced experimental lung cancer. *Molecular and cellular Biochemistry*. 292, 13-17

38• Patro I and Sharma S (1984) Cytochemical interaction of nucleolus in the Purkinje cells of senile white rats under the influence of centrophenoxine. *Expt. Gerontol.* 19, 241-252

39• Pillai M, Pawar S, *et al.,.* (2003). Protective effects of *Hydrocotyle asiatica* extracts on brain during aging. *Indian J Comp Animal Physiol*. 21: 77 – 85.

40• Rajashankar S, Manivasagam T et al. (2009) Ashwagandha leaf extracts a potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. *Neuroscience Letters*. 454(1), 11-15

41• Rattan S (1996) Cellular and molecular determinants of aging. *Indian J. Exp. Biol*. 34, 1-6

42• Sashima M (1986) Age related changes of rat submandibular gland a morphometric and ultrastructural study. *J. Oral pathol*. 13(10), 507-512

43• Satyavati G (1995) Leads from Ayurveda on medicinal plants acting on the nervous system. In: Koslow SH, Srinivas S, Murthy R, Coelho GV, editors. *Decade of the Brain: India/US research in mental health and neuroscience*. Rockvillie, MD. *Nat. Inst. Mental Health*. pp: 185-189

44• Scanlin T and Glick M (1999) Terminal glycosylation in cystic fibrosis. *Biochim. Biophys. Acta*. 1455, 231-253

45• Scott J, Bodner L, et al. (1986) Assessment of age related changes in the submandibular and sublingual salivary glands of the rat using stereological analysis. *Arc. Oral boil.* 31, 69-71

46• Scott J (1977a) Degenerative changes in the histology of human submandibular salivary glands occurring with age. *J. Biol. Buccade*. 5(4),311-319

47• Scott J (1986) Structure and function in aging human salivary gland. *Gerontol.* 5, 149

48• Ship J, Pillemer S, et al. (2002) Xerostomia and the geriatric patient. *J. AM. Geriatr. Soc.* 50, 535-543

49• Song X, Bao M, et al. (1999) Advanced glycation in D-galactose induced mouse aging model. *Mech. Aging Dev*. 108(3), 239-251

50• Sporn M, Roberts A, *et al.* (1982) Polypeptide transforming growth factor isolated from bovine source and used for wound healing. *Sci.* 219, 1320-1333

51• Staudacher E, Altmann F, *et al.* (1999) Fucose in N-glycans from plant to man. *Biochim. Biophys. Acta* 1473, 216-236

52• Tonetti M, Sturla L et al. (1998) The metabolism of 6-deoxyhexoses in bacterial and animal cells. *Biochimie*.80, 923-931

53• Vora S, Patil R, et al. (2009) Protective effects of *Petroselium crispum* (Mill) Numan exA. W. Hill leaf extract on D-galactose induced oxidative stress in mouse brain. *Indian J. Experimental Biol.* 47(5), 338-342

54• Vora S (2005) Protective effects of *prtroselinum* crispum on the mouse brain and heart during aging. *Ph. D. thesis submit-*

ted to Shivaji University, Kolhapur, (MS), India

55• Walvekar M and Pillai M (2008) Endocrine relation between submandibular gland and testis. *J. Cell and Tissue Res.* 8(2), 1411-1416

56• Zs-Nagy I and Floyd R (1984) Electron spins resonance spectroscopic demonstration of the hydroxyl free radical scavenger properties of dimethylaminoethanol in spin tapping experiments confirming the molecular basis for biological effects of centrophenoxine. *Arch. Gerontol. Geriatr.* 3, 297-310

57• Zs-Nagy I and Nagy K (1980) On the role of cross-linking of cellular proteins in aging. *Mech. Ageing Dev*. 14, 245-251

58• Zs-Nagy I (1989) On the role of intracellular physicochemistry in quantitative gene expression during aging and effect of Centrophenoxine – A review. *Arc.h Gerontol. Geriatr* 9, 215-229

59•