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Effect of Parsley Extract on Protein Content and Histology of Submandibular Glands of Naturally Aged Male Mice

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Abstract: ***The aging is generally characterized by the declining in ability to respond to stress, increasing homeostatic imbalance and increased risks of diseases. Aging promotes free radical formation in the cell. The free radicals formed due to aging or various reasons are scavenged by antioxidants. In present studies antioxidant rich plant, viz- parsley (Petroselinum crispum. mill), was used to assess the free radicals generated during aging. Three groups of mice (Control, Naturally aged, Parsley receiving) were used during the experiments. Study was restricted to only submandibular glands. Histological sections of glands were stained with haematoxylene and eosine. Protein content was estimated in the experiment. Study shows damage in submandibular glands in naturally aged mice as compare to young control. Parsley corrected the histological structure of submandibular glands as well as protein content of submandibular glands in naturally aged animal.***

Keywords: Aging, submandibular glands, parsley, protein.

1. Introduction

The salivary glands are readily accessible and well characterized. Therefore, they are useful tools for the study of the normal aging process and the impact of stress on organ reserve and secretary functions (Baum *et al*, 1992). A common generalization associated with aging is that salivary gland function is altered (Storer, 1978) and diminished out put results in dental carries, altered mucosal integrity and impaired taste and agglutination. Salivary glands dysfunction has been traditionally attributed to old age (Thomson *et al*, 1999).Submandibular glands are termed as acinar cells, together formed called acini, which secrete mainly glycoproteins. Acini are surrounded by myoepithellial cells and supported by parenchyma. There are various ducts as intercalated ducts, striated duct and granular ducts, which are also called as granular convoluted tubules. These ducts have been considered to be the sites of formation of many enzymes like Kallikrein (Hojima *et al.* 1977) Proteases (Sreebny and Meger 1964), nerve growth factor (Schwah *et al*, 1976), epidermal growth factor (Cohen, 1962, Young and Van Lennep 1978) and various mesodermal growth factors (Weimer and Haraguchi 1975). Salivary proteins are mucins these are effective lubricant, they control permeability of mucosal surface, limit penetration of potential irritants and toxins to mucous cells, protect cell membranes against proteases generated by bacteria, and regulate colonization of oral cavity by bacteria and viruses (Mandel, 1992). Parsley is considered to be one of the highest sources of flavonol glycosides (Kreuzaler *et al*, 1973). It has been shown to possess remarkable histological correction in sublingual glands (Khandare *et al*, 2013); anti inflammatory, antioxidant and anti carcinogenic property (Patel *et al*, 2007).

2. Material and Methods

2.1 Animals

Adult male albino mice *(Mus musculus*) of 23 weeks were used for the present investigation. The breeding pairs were obtained from Hindustan Antibiotics, Pune. They were reared and kept in the animal house with the maintenance of constant temperature (about 25 to 30°C) and light and dark cycles. They were supplied with Amrut Mice feed (Pranav Agro Industries, Sangli) and water ad libitum. Animals were randomly assigned to the following three groups.

2.2 Control Group

Adult male mice (Age 23 week's old and weight 42 ± 0.6 gm) was treated as control.

2.3 Naturally aged group:

Naturally an aged male mouse (Age 76 week's old and weight 38.66 ± 1.032 gm) was used.

2.4 Parsley receiving old male mice group:

Old male mice (Age 76 week's old weight 38.66 ± 1.032 gm) were injected parsley 40 mg / kg body weight / day subcutaneously for 20 days.

2.5 Preparation of plant extract

Fresh Parsley (*Petroselinum crispum*) mill was obtained from the market. Green fresh leaves were separated and washed properly with water and rinsed with distilled water. Washed leaves were blotted properly with blotting paper and kept for drying in the shade. The dried leaves were crushed, powdered and sieved. The powder was soaked in double

distilled alcohol (ethanol) for 72 hours for extraction. The mixture of ethanol and powder of parsley leaves was filtered. The alcohol was evaporated by using high speed (Buchi Type) vacuum evaporator to obtain a thick paste like extract. This extract was collected by spatula and stored in a glass bottle. It was kept refrigerated (at 4°C) for further use.

2.6. Histology

The animals were sacrificed by cervical dislocation. The salivary glands were excised out. They were weighed and kept in the freezer for freezing and thawing and used for protein estimation. For the histology of sublingual glands, tissue were fixed in 2% CAF (Calcium acetate formalin) + 2% calcium acetate in 10% formalin fixative for 24 hours at 4°C. The tissue was washed in running tap water for 24 hours dehydrated through alcohol grades, cleared in xylene and embedded in paraffin. The sections were at a thickness of 6 microns and stained with eosin and haematoxylene.

2.7. Estimation of Proteins: (Lowry *et al*, 1931)

2.7.1. Preparation

1. Reagent A – 2% Na_2CO_3 in 0.1 N NaOH.
2. Reagent B – 0.5% CuSO4 in 1% freshly prepared Na^+, K^+ tartarate.
3. Reagent C – 50 ml A+1 ml B (made freshly at the time of use).
4. Reagent D – Folin c (90% Ciacolteu Phenol) reagent.

2.7.2. Procedure

The additions for the estimation of proteins were made as follows.

	Blank	Sample
Distilled Water	4 ml	3.5 ml
Sample		0.5 ml
Reagent C	5.5 ml	5.5 ml

The tubes were shaked well and kept for 10 min, 0.5 ml reagent D was added mixed well and kept for 30 min. The optical density was measured 660 nm. The final colour production is the result for biuret reaction of protein with copper ions in an alkaline medium at reduction of phosphomolybdic phosphotungstic reagent by the tyrosin and tryphophan present in treated protein.

2.7.3. Calculation:

The protein concentration 1 mg tissue was determined using standard graph of bovine serum albumin.

2.8. Statistical analysis: Statistical analysis was performed by Student't'-test., P<0.001

3. Result

Fig no. "a." describes the cross section of submandibular glands of adult mice (Age 23 weeks) was having well formed acini (AC) and convoluted granular tubules (GCT) and ducts were clear and darkly stained.

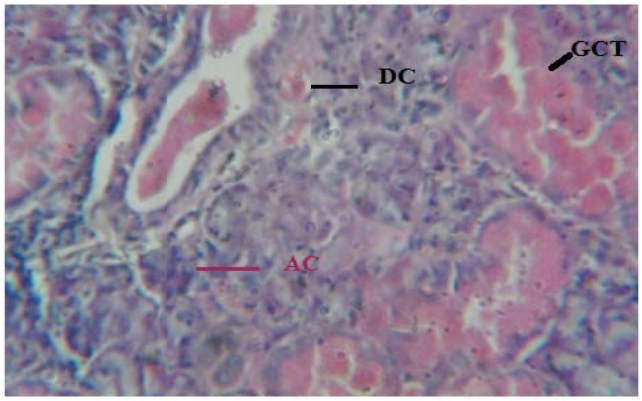


Figure (a): Control group, cross section of Submandibular gland]

In naturally aged mice, Fig no. "b." (Age 76 weeks) Submandibular glands, number and size of acini were reduced. Organization of acini and granular convoluted tubules with respect to one another was lost. Ducts were disorganized cell layer of the duct was thin, reduced staining reactivity of acini (AC) and GCT was lost and also their integrity.

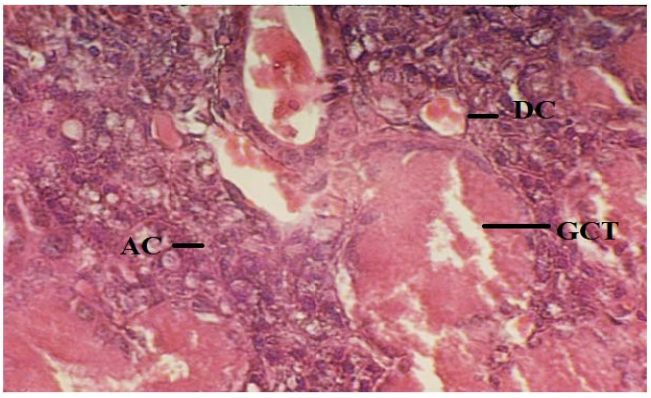


Figure (b): Naturally aged group, Cross section of submandibular gland]

Fig. no. "c" depicts the structure of submandibular gland of parsley receiving mice nuclear staining reactivity of both the granular cells (GC) and acini (AC) was increased as well as architecture acini became normal, but not of the granular convoluted tubules.

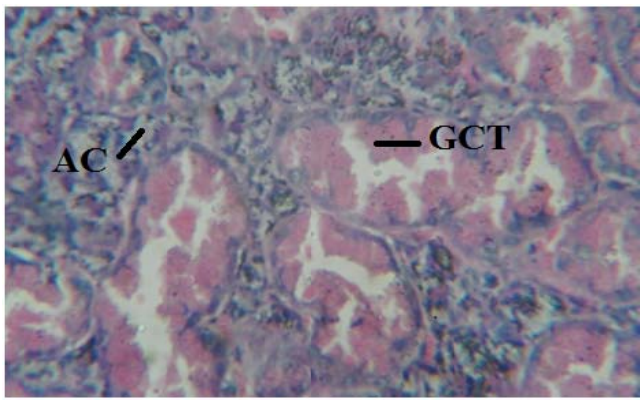


Figure (c): Parsley receiving group, Cross section of submandibular gland]

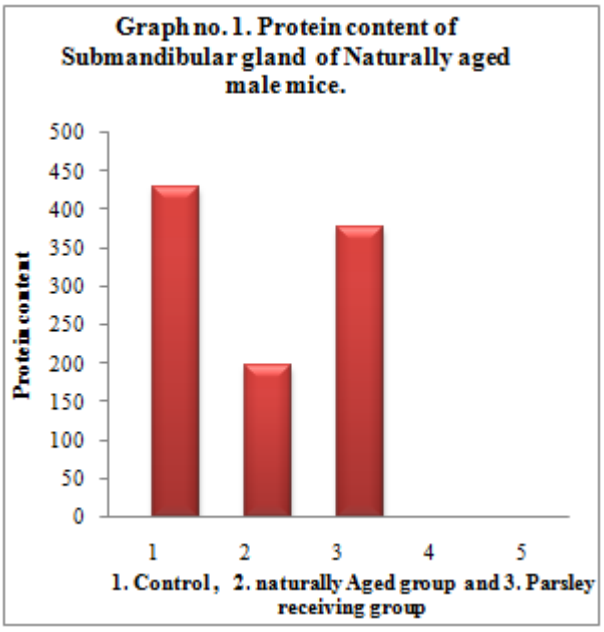
Table no. 1; graph.1, shows that, Protein content estimated in submandibular glands in control group was 430.18 ± 0.934 µg/mg weight tissue it was significantly (1:2 p<0.001) decreased in naturally aged mice. Naturally aged mice

receiving parsley group showed increase in protein content. The increase was significant (2:3 p<0.001) compared to naturally aged mice

Table 1: Effect of *Petroselinum crispum* extract on protein content (µg /mg) of submandibular gland of naturally aged male mice

Sr. No.	Animal Group	Age in weeks	Protein content µg/mg	
				Statistical significance
1	(6) Control	23	430.18 ± 0.934	1 : 2 t = 55.973
2	(6) Naturally aged	76	197.89 ± 2.2250	(p<0.001) 2 : 3
3	(6) Parsley receiving recovery	76	376.83 ± 1.9714	T = 39.509 (p<0.001)

Values are mean ± SD P<0.001, highly significant, Values in the parenthesis denote number of animals.



Graph 1: Protein content of submandibular gland of naturally aged male mice

4. Discussion

The purpose of this study was to examine the effect of dietary antioxidants (parsley) on stressed salivary glands. Dietary antioxidants are polyphenolic compounds which are ubiquitously present in foods of plants origin. Quercetin is a strong antioxidant studied against the aging and free radical toxicity in brain (Durate *et al*, 2001; Molina et al, 2003), heart (Mohanty *et al*, 2004) and other organs (Gurente *et al*, 2000). According to Giugliano *et al*, 2000; sufficient supply with antioxidants from diet might help to prevent the occurrence of pathological changes associated with oxidative stress. There are several antioxidants suggested in the literature to prevent or acceptable aging effect. Several attempts have been made to knock out antioxidants vitamin E (Zhang *et al*, 2002), vitamin C, melatonin (Reiter et al, 1996), lipoic acid (Packer *et al* 1997; Kalia *et al*, 2013) in mice and other animals. *Petroselinum crispum* possess strong antioxidant properties (Hirano et al 2001). It contains rich amount of vitamins C and A (Pattision et al, 2004). It is one of the richest sources of flavonoids, especially quercetin (Fejes *et al*, 1998). Petroselinum crispum improved the histological structure of sublingual glands in naturally aged male mice (Khandare *et al*, 2013) In present investigation the *Petroselinum crispum* shows promising results in proteins and corrected histological structure of submandibular glands.

5. Conclusion

Present experiments investigated that, Parsley (*Petroselinum crispum.*), improved the histological structure and showed recovery in protein contents of Submandibular glands in naturally aged male mice.

6. Abbreviations

GCT - Convoluted granular tubules
AC - Acinar cells
Dc - Duct

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