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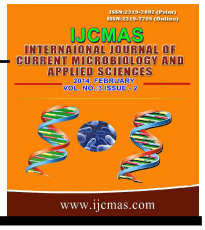

Original Research Article

Effects of dietary curcumin on protein profile in mice submandibular glands during aging

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Keywords

Curcumin; Submandibular glands; Aging; D-galactose; Protein.

A B S T R A C T

Aging is accompanied by declines in cellular proteolytic capacity. Decrease in protein synthesis is a general phenomenon in aging, the effect of such a decrease is expected to be very pronounced in exocrine glands which synthesize a large quantity of proteins for export, such as salivary glands. However, there is no information available regarding the changes in protein synthesis with aging in these glands. The aim of this study was to examine the effect of curcumin, on oxidative stress induced by D-galactose as well as during natural aging in the submandibular glands (SMG). Male Swiss albino mice, *Mus musculus* was used for the present investigation, animals were divided in to 9 experimental group as control-I and II, Dg- treated, Dg –protective, Dg- curative – I and II, natural aging (NA), NA curative –I and II. The effect of curcumin (30 mg/kg body weight) was evaluated against aging. The biochemical and electrophoretic alterarations in protein content in the SMG in was recorded. The protein content was significantly reduced during aging, after curcumin treatment in protective and curative groups it was again increased significantly. Thus, curcumin is able to ameliorate the stress induced changes in protein profile during aging. Ameliorative effect of curcumin in SMG of mice might be due to its antioxidative property.

Introduction

Aging is characteristically described as a time dependent functional decline, leading to cells incapacity to withstand external as well as internal challenges. According to this, aging is consequences of two independent biological processes: the loss of functionality and the loss of resistance or adaptability to stress. The consensus among researchers broadly views the concept of biological aging as an organism's failure to maintain homeostasis (Gutteridge, 1992). The free radical theory of aging was proposed in November, 1956, by Harman, which proposes that aging results from imperfect protection against tissue damage brought about by free radicals.

Oxidative stress is classically defined as a redox unbalance with an excess of

oxidants or a defect in antioxidants (Sies, 1985) . Free radicals are known to attack the structure of cell membranes, which create metabolic waste products. Such toxic accumulation interferes with cell communication, disturbs DNA, RNA and protein synthesis, lower energy levels generally impair vital chemical processes. ROS induced oxidation of proteins can lead to changes in the proteins three-dimensional structure or cross linking of the proteins (Farr and Kogoma,1991).

Gradual loss of reverse capacity is considered normal physiological aging (Fries,1980). Normal aging process has been suggested as etiological factor for salivary dysfunction (Perderson *et al.*, 1985; Cowman *et al.,* 1994; Yeh *et al*., 1998). Salivary dysfunction in older adults is likely due to systemic diseases, prescription and non-prescription medications, chemotherapy and head and neck radiations (Ship *et al*., 2002).

The aim of this study was to examine the modulatory effect of submandibular gland (SMG) functions in aged mice by stimulating salivary response by oral administration of curcumin.

Studies on natural products of potential therapeutic values are of important not only because of the medicinal applications of the active principles involved but also in understanding natures processes and homeostatic mechanisms operating in living organisms.

Curcumin is the major pigment from dried rhizome of the plant *Curcuma longa,* that has been used as spice and traditional medicine in Asia for centuries to treat gastrointestinal upset, arthritic pain, parasitic infections etc., Curcumin extends life span in *Drosophila* by reducing oxidative stress and increasing locomotive activity (Lee *et al.,* 2010). Several *in vivo* studies show the neuroprotective effect of curcumin that protects against neurodegenerative disorders including Alzheimer's disease.

Materials and Methods

Male Swiss albino mice *Mus musculus* Linn. were used for present investigation. Animals were grouped into 9 (n = 6)

Control- I group: Six months adult mice, weighing 50-55 gm, received subcutaneous injection of 0.5 ml of distilled water and were orally fed with 0.5ml honey as vehicle, for 30 days.

Control- II group: Six months adult mice, weighing 50-55 gm, orally fed with 0.5ml honey as vehicle, for 30 days.

D-galactose treated group: Received subcutaneous injection of 0.5 ml of 5% D - galactose for 30 days (Song *et al.,* 1999).

Dg-treated protective group: Received subcutaneous injection of 0.5 ml of 5% D-galactose and then along with injection, curcumin dissolved in honey (30 mg/kg body weight/day/animal) (Reddy and Lokesh, 1996) was fed orally for 30 days.

Dg-treated curative group I: Received subcutaneous injection of 0.5 ml of 5% D - galactose for 30 days and then for next 30 days were orally fed with curcumin dissolved in honey (30 mg/kg body weight/day/animal).

Dg-treated curative group II: Received subcutaneous injection of 0.5 ml of 5% D-galactose for 30 days and then for next 45 days were orally fed with curcumin dissolved in honey.(30 mg/kg body weight/day/animal),

Natural aging group: Mice were kept as it is without any drug or extract treatments up to the age of 24 months for natural aging.

Natural aging curative I: To naturally aged mice (20-22 months) curcumin dissolved in honey at a dose of 30mg/kg body weight was fed orally daily for 30 days.

Natural aging curative II: To naturally aged mice (20-22 months) curcumin dissolved in honey at a dose of 30mg/kg body weight was fed orally daily for 45 days.

Animals were supplied with pelleted mice food (Pranav Amrut food 'Sangli') and drinking water *ad libitum.* Animals were maintained in plastic cages in AC animal house (CPCSEA /233) under 12:12 hr L: D cycles.

Curcumin was obtained from Sigma chemicals CO. USA. All animals were sacrificed after 24 hrs of completion of treatment by cervical dislocation. The submandibular glands were dissected out, rinse in distilled water, blotted on blotting paper, weighed and kept in freezer for freezing.

Estimation of protein by Lowry's method (1951) using Bovine serum as standard. Electrophoretic separation of protein by Laemmli method (1970).

Statistical analysis

All values are expressed as mean ± S.D. The statistical analysis was performed using student's't' test. A value of P< 0.001 was considered statistically highly significant.

Results and Discussion

Table no. 1 shows effect of curcumin supplementation on protein content (µg/mg tissue) in SMG of D-galactose treated aged male mice. Protein content in SMG from control – I group was 800.833 ± 32.3136 µg/mg tissue which was significantly reduced in D-galactose treated group mice SMG (1:2, P<0.001). While in Dg-treated protective group mice SMG, the protein content was again increased and the increase was significant as compared to D-galactose treated group (2:3, P<0.01). In Dg-treated curative group–I and II, the protein content was significantly increased as compared to D-galactose treated group (2:4, P<0.01; 2:5, P<0.01). In that also, Dg-treated curative group –II group showed significant increase as compared to Dg-treated curative group –I (4:5, P<0.01).

Table no. 2 shows effect of curcumin supplementation on protein content (µg/mg tissue) in SMG of naturally aged male mice. In natural aging group the protein content was reduced to 227.5 ± 17.24 µg/mg tissue and was highly significant as compared to control - II group (1:2, P<0.001). In natural aging curative group – I and II the protein content in SMG was again significantly increased as compared to natural aging group (2:3, P<0.01; 2:4, P<0.01). In natural aging curative group – II the protein content was increased as compared to natural aging group – II (3:4, P<0.01).

The electrophoretic separation of proteins from all experimental groups show some what similar banding pattern,

Table.1 Effect of curcumin supplementation on protein content (µg/mg tissue) in submandibular glands of D-galactose induced aged male mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sr. No	Treatment (n=6)	Protein content	Statistical significance
1	Control- I	800.8333 ± 32.3136	1:2, P<0.001
2	D-galactose treated	280 ± 17.0294	2:3, P<0.01
3	Dg- treated protective	325 ± 16.7332	2:4, P<0.01
4	Dg-treated curative-I	627.5 ± 17.2482	2:5, P<0.01
5	Dg- treated curative -II	650.833 ± 32.3136	4:5, P<0.01

P<0.01= significant, P<0.001= highly significant.

Table.2 Effect of curcumin supplementation on protein content (µg/mg tissue) in submandibular glands of naturally aged male mice. Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Sr. No.	Treatment (n=6)	Protein content	Statistical significance
1	Control – II	800.8333 ± 32.3136	1:2, P<0.001
2	Natural aging	227.5 ± 17.2482	2:3, P<0.01
3	Natural aging curative- I	369.1667 ± 21.5445	2:4, P<0.01
4	Natural aging curative- II	470.00 ± 14.1421	3:4, P<0.01

P<0.01= significant, P<0.001= highly significant.

but the staining intensity from all groups was different (Plate.1). In case of electrophoretic separation of SMG from D-galactose treated group, the staining intensity of all bands was much reduced as compared to control group. The bands of high molecular weight mucins, lactoferin and peroxidase were totally disappeared. In Dg-treated protective group not much difference in banding pattern was observed when compared with D-galactose treated group. While in Dg- treated curative group- I and II, there was much more increase in band staining intensity as compared to D-galactose treated group was observed. Staining intensities of glycosylated PRPs, proline rich glycoproteins, amylase, lactoferin and peroxidase remarkably increased.

In case of natural aging group the electrophoretic separation of proteins from SMG shows decrease in the staining intensity of all the bands as compared to control group. In natural aging curative group- I and II, the staining intensity of all the bands was increased as compared to natural aging group. Especially, staining intensity of bands of lactoferin, peroxidase, amylase, proline rich glycoproteins, glycosylated PRPs was increased as compared to natural aging group (Plate. 2).

In the present study, the protein content in SMG of D-galactose treated induced aging mice as well as in natural aging mice was significantly reduced as compared to control groups.

Plate.1 The electrophoretic separation of proteins from all experimental groups

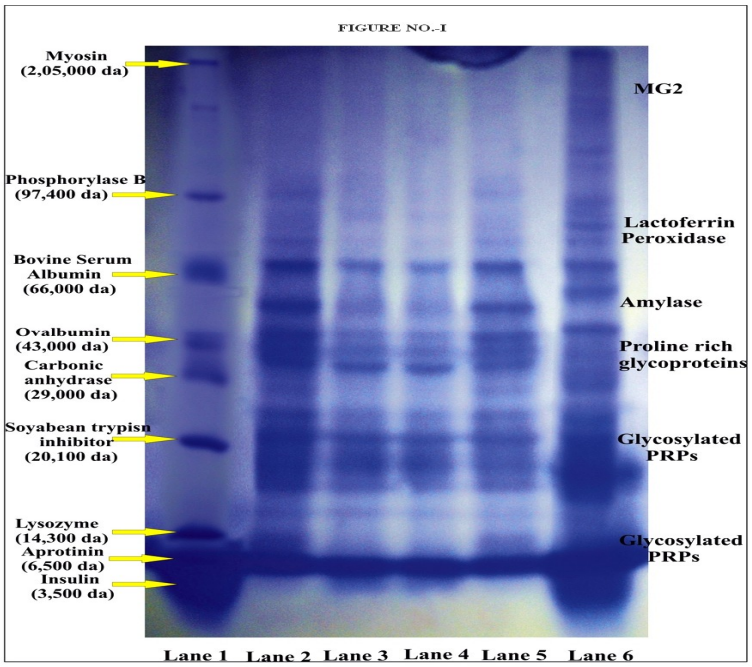
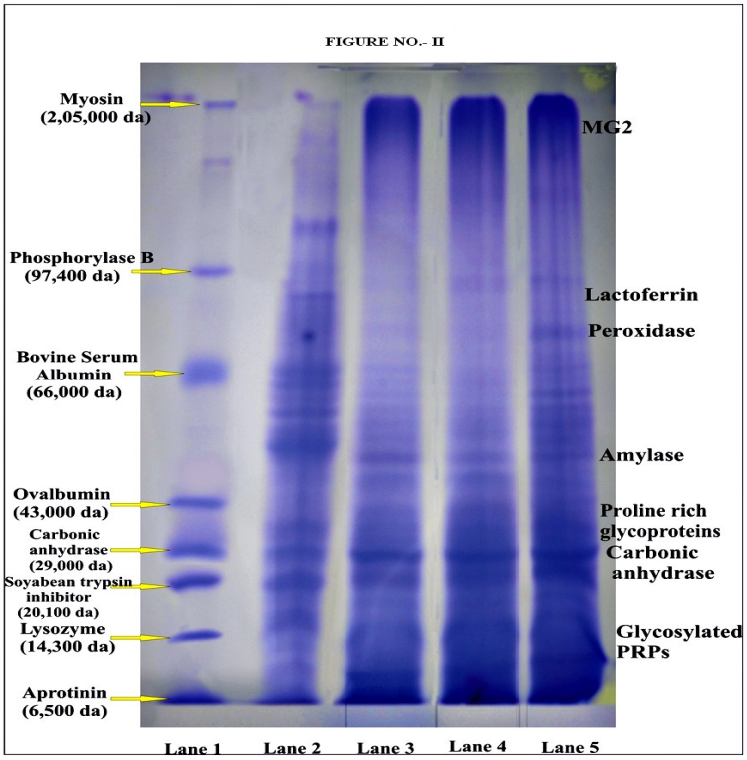


Plate.2 Electrophoretic separation of proteins from SMG



Both physiologically and pathologically, D-galactose treated animals resemble their aged control counterparts of 16-24 months old (Gong and Xu, 1991; Li *et al.,* 1995; Tian *et al*., 1996; Zhang *et al.,* 1996). Recent reports implicate the chronic administration of D-galactose for accelerating aging, influences age related cognitive decline in mice (Lei, 2008).

Efficient macromolecular turnover is integral to the normal function and survival of a biological system. Although there are larger variations in the rates of degradation of individual proteins, it is generally observed that overall protein turnover slows down during aging (Rattan 1996; Van *et al.,* 1995). However it should be pointed out that age related slowing down of bulk proteins synthesis does not mean that the synthesis of each and every protein becomes slower uniformly during aging. The physiological consequences of decreased protein turnover include the accumulation of altered and abnormal proteins in the cell, an altered pattern of post translational modification due to increased dwell time and a disruption of the organization of the cytoskeleton and extracellular matrix (Hipkiss, 2006).

Age related decline in the protein turnover is generally due to decrease in the proteolytic activity of various lysosomal and cytoplasmic proteases. Other reasons for age related change in the activities of various proteases leading to a decrease in the rate of protein turnover include slower transcription, reduced rates of protein synthesis and altered pattern post synthetic modifications.

But the supplementation of curcumin in Dg- treated protective group significantly increased the protein content in SMG. Also a significant increase was observed in the SMG protein content after curcumin treatment in the curative groups of both accelerated aging as well as natural aging groups, indicating curcumin is best antioxidant neutralizing the damaging effects caused by aging.

The increase in staining intensity of bands in protective and curative groups especially staining intensity of bands of amylase, proline rich glycoproteins, glycosylated PRPs, lactoferin and peroxidase was increases as compared to D-galactose - treated group. This confirms the results of biochemical estimation. Curcumin changes protein and glycoprotein turnover due to increase in transcription .This proves that curcumin turnover the proteins and glycoproteins including faster transcription which increases the rate of protein synthesis.

In conclusion, our study demonstrated that D-galactose induces marked oxidative stress in mice. Biochemical estimations and electrophoretic separation of proteins suggests that curcumin effectively protect proteins in SMG against oxidative damage during both induced aging as well as natural aging. Curcumin scavenges free radicals and therefore be associated with reduced protein damage/ oxidation/ oxidative damage in aging tissues.

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