

*Full Length Research Paper*

# Antioxidative effect of curcumin (*Curcuma longa*) on lipid peroxidation and lipofuscinogenesis in submandibular gland of D-galactose- induced aging male mice

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**Extract of turmeric (*Curcuma longa*) that is, curcumin was examined for antioxidant activity. The study deals with the effects of curcumin on lipid peroxidation and fluorescence product in the control, D-galactose-induced aged, D-galactose aging protected and D-galactose aging cured mice. The level of lipid peroxidation in terms of malondialdehyde and the level of lipofuscin granules in term of fluorescence product increased in submandibular gland of D-galactose-induced aged mice. After curcumin (30 mg/kg body weight) administration the levels of both the parameters reduced significantly. Thus, results suggest that curcumin prevent the formation of malondialdehyde and lipofuscin granules which are indicators of aging.**

**Key words:** Curcumin, antioxidant, lipid peroxidation, lipofuscin granules, submandibular gland.

## INTRODUCTION

Oxidative stress is a state of imbalance between generation of reactive oxygen species and levels of antioxidant defense system. Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals (Guner et al., 1996). Oxidative stress plays an important role in the chronic complications of many diseases.

Studies on natural products of potential therapeutic values are of immense importance not only because of the medicinal applications of the active principles involved but also in understanding nature's process and the homeostatic mechanisms operating in living organisms. Because of their safety and less side effects natural products have long been a topic of interest and targets for developments as anti-aging agents. The present study aims at understanding the effect of administration of curcumin, a plant polyphenol from *Curcuma longa* during D- galactose induced oxidative stress with respect to lipid peroxidation and lipofuscinogenesis in submandibular gland of male mice.

Up till now in our laboratory, age-related changes in the salivary glands and protective effect of different plant extracts like glycowithanolides (Mote et al., 2010) *Petroselinum crispum* extracts (Sonawane, 2007) etc. on salivary glands during aging were studied.

Thiyagarajan and Sharma (2004) has demonstrated the neuroprotective effects of curcumin against the effects of middle cerebral artery occlusion. In the last decade a large number of reports have been published on the beneficial effects of curcumin, and it has repeatedly been claimed that this natural product is efficient and safe for the prevention and treatment of several diseases including cancer (Anand et al., 2008; Goel et al., 2008; Ravindran et al., 2009).

So the present study was designed to elucidate the antiaging effect of curcumin in salivary gland of male mice.

## MATERIALS AND METHODS

Male Swiss albino mice (*Mus musculus*) of age six months, weighing about 50 to 55 g were used for the present investigation. Mice were maintained in the plastic cages in AC animal house (CPCSEA/233) under 12:12 h L: D cycle. The mice were provided with pelleted food from 'Pranav Amrut food' Sangli and water *ad*

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**Table 1.** Effect of curcumin on total and mitochondrial lipid peroxidation in submandibular glands of aging induced male mice (Lipid peroxidation in n mol MDA /mg wet tissue). Values are mean + S.D (Numbers in parenthesis denotes number of animals).

S/N	Groups	Treatment	Total lipid peroxidation	Statistical significance	Mitochondrial lipid peroxidation	Statistical significance
1	Group I	Control mice (5)	11.518 ± 0.013		28.83 ± 0.01	
2	Group II	Aging induced mice (5)	34.602 ± 0.0084	1:2, P<0.0001	69.234 ± 0.0114	1:2, P<0.0001
3	Group III	Aging protected mice (5)	17.312 ± 0.013	2:3, P<0.0001	57.682 ± 0.0084	2:3, P<0.0001
4	Group IV	Aging cured mice (5)	11.52 ± 0.01	2:4, P<0.0001	23.072 ± 0.0084	2:4, P<0.0001

**Table 2.** Effect of curcumin on fluorescence product in submandibular gland of aging induced male mice. Values are mean + S.D (Numbers in parenthesis denotes number of animals).

S/N	Group	Treatment	Fluorescence product	Statistical significance
1	Group I	Control mice (5)	0.00184 ± 0.000089	
2	Group II	Aging induced mice (5)	0.00572 ± 0.000084	1:2, P<0.0001
3	Group III	Aging protected mice (5)	0.001956 ± 0.000011	2:3, P<0.0001
4	Group IV	Aging cured mice (5)	0.00195 ± 0.00001	2:4, P<0.0001

*libitum*. Twenty mice were divided into following four groups of five animals each:

**Group I:** Control mice: Mice were injected subcutaneously with 0.5 ml sterile water/day /animal for 30 days.

**Group II:** Aging induced mice: The mice were injected subcutaneously with 5% D-galactose 0.5 ml /day/animal for 30 days to induce aging.

**Group III:** Aging protected mice: The mice were injected subcutaneously with 0.5 ml of 5% Dgalactose /day/animal along with, curcumin dissolved in honey at the dose of 30 mg/kg body wt /day/animal (Reddy and Lokesh, 1996) was fed orally for 30 days. The co-treatment of curcumin was carried out to study its effect on induced aging and oxidative stress.

**Group IV:** Aging cured mice: The mice were injected with 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 wt /day/animal, to study the effect of curcumin as antiaging agent and to study the recovery by curcumin.

After completion of the dose, the animals were sacrificed by cervical dislocation; submandibular glands were dissected out, blotted and weighed. The submandibular glands were homogenized by using mixture containing 75 mM phosphate buffer (pH 7.04), 1 mM ascorbic acid, 1 mM ferric chloride and 0.001 ml chlortetracycline (10 ppm).

#### Determination of lipid peroxidation

Tissue homogenate was prepared in chilled mortar using 75 mM potassium phosphate buffer pH 7.04 containing 1 mM ascorbic acid and 1 mM ferric chloride and the total lipid peroxidation and mitochondrial lipid peroxidation was estimated (Wills, 1966).

#### Measurement of fluorescence product

The lipofuscin granules were extracted using chloroform: methanol mixture (2:1 v/v). The fluorescence was measured on photofluorometer calibrated with Quinine sulphate (Dillard and

Tappel, 1971).

All values are expressed as mean ± S.D. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's Post Hoc test. A value of P<0.01 was considered statistically significant.

## RESULTS

The total lipid peroxidation and mitochondrial lipid peroxidation in submandibular gland was increased in mice with induced aging (group II) as compared to control (group I) and increase was highly significant (P<0.0001), while there was decrease in total as well as mitochondrial peroxidation in protective (group III) and curative (group IV) mice as compared to aging induced mice (P<0.0001) (Table 1).

The fluorescence product in the submandibular gland was increased in mice with induced aging as compared to control (P<0.0001), while it was decreased significantly in protective group and curative group as compared to induced aging group (P<0.0001) (Table 2).

## DISCUSSION

Free radicals have been implicated as the main source of organ damage and hence compounds capable of negating such damage will have potential benefits in the therapy of many diseases. Free radical generation and resultant oxidative stress results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates.

In present study, we investigated the effects of administration of a plant polyphenol curcumin during D-galactose-induced aging with respect to lipid peroxidation

and lipofuscinogenesis. D-galactose is a reducing sugar which interacts with free amino groups of proteins and amino acids to produce advanced glycation end products (AGEs) (Munch et al., 1996; Schmidt et al., 1996; Wautier and Schmidt, 2004). The accumulation of lipofuscin granules in submandibular gland of D-galactose-induced aging accelerated group is because of formation of advanced glycation end products. Curcumin has decreased the lipofuscin granules in submandibular glands and thereby resulted in highly significant decrease in fluorescence. This might be due to decrease in AGEs. Lipid peroxides are presumptive markers for free radical generation and oxidative stress (Sreepriya et al., 1998). This is evident from the statistically significant increase in group II. The reduced level of peroxidase in groups III and IV demonstrate the inhibitory effect of curcumin on lipid peroxidation chain reaction and hence an indication about their anti-oxidative effects.

Thus, we conclude that treatment of the animals with curcumin during D-galactose induced aging exhibited considerable beneficial effects as antioxidant and anti-oxidant.

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