Antiaging effects of *Bacopa monniera* Linn. (Brahmi) on submandibular glands of d-galactose induced experimentally aged female mice

Vishalmati Appasaheb Mankapure¹, Appasaheb Gangaram Mankapure¹, Meena Madhavan Pillai²

¹Department of Zoology, Jaysingpur College, Jaysingpur, Maharashtra, 416101, India; ²Department of Biotechnology, Kolhapur Institute of Technology, Kolhapur, Maharashtra, 416004, India; Email: vmankapure@gmail.com

ABSTRACT

Four groups of 5-6 months old female mice were maintained. Group I mice were normal (control) animals not receiving any drug. Aging was induced experimentally in group II mice (D-gal) by giving 5% D-galactose/day for twenty days. Experiments were also carried out to study the antioxidant and antiaging properties of well known protective Ayurvedic drug, *Bacopa monniera* (Brahmi) by providing co-treatment of Brahmi extracts (40 mg/kg body weight) along with D-galactose to the group III (D-gal + *B. monniera*) mice of the same age. D-galactose affected the histological as well as histochemical nature of the salivary glands considerably. The salivary acini showed considerable decrease in the number as well as cellular secretions; intercalated ducts and striated ducts got distorted showing degenerative changes in D-gal stressed animals. The salivary gland acini were least affected in the Brahmi co-treated (D-gal + *B. monniera*) groups along with D-galactose. The cellular elements and the integrity of the striated ducts as well as intercalated ducts were also retained proving its protective effect in these mice. The recovery tests were also carried out in the group IV animals which were D-galactose stressed aged mice by giving similar doses of *B. monniera* for 20 days (D-gal f. by *B. monniera*) immediately after termination of D-gal treatment. No considerable recovery was noticed in the histological structure of the glands; but a little glycoprotein recovery was found in the secretary activity of the glands as observed in their glycoprotein staining intensity which was retained to some extent in the salivary glands of aging induced mice of D-galactose f. by *B. monniera* recovery group.

Keywords: antiaging effect, submandibular glands, antioxidants, *Bacopa monniera* (Brahmi)

INTRODUCTION

Salivary glands are not only the organs of digestion and protection of oral health but their secretions also have an effect on the development and maintenance of various other organs in the body by secreting enzymes, glycoprotein and growth factors [1]. Salivary glands are much affected during aging with decrease in the salivary flow rates and protein secretions in older [2]. Regressive changes in the glandular ducts and inflammatory changes in the aged submandibular (SM) salivary glands were reported [3,4]. Considerable increase in the connective tissue and intralobular ducts during aging, associated with corresponding decrease in the acinar tissue were also observed at the cost of acinar cell loss but salivary production remained age-stable in healthy adults [5]. Considering the role of salivary glands in physiology of various organs by secreting several enzymes and growth
factors, it is necessary to either prevent further alterations due to aging or reverse these changes in the salivary glands.

A large number of indigenous medicinal plants rich in antioxidant properties are being used in geriatrics to help and to replenish the vital processes of the body for heightened memory and intelligence for restoration of youth and for resistance to decay and degeneration of body tissues due to free radical attack during aging. Among these *Bacopa monniera* Linn. (Brahmi) is one of the prime Ayurvedic herbs, a glabrous, creeping herb known as ‘Jalamimba’ or ‘Nir-brahmi’ in local languages (Sanskrit and Kannada). The chemical constituents of the plant extracts are found to be mainly the alkaloids, brahmin, herpestine, and bacosides [6]. In addition to bacoside A and B, two new saponins and glucopyranosides were isolated from *B. monniera*. Ethanolic extracts of *B. monniera* were shown to have antioxidant effect against lipid per oxidation in rat liver and a dose dependant increase in superoxide dismutase (SOD), catalases and glutathione peroxidase activity in all the regions of brain in aging rats [6]. It is also proved to have a wide range of potent promotive therapeutic abilities such as improvement of cognition enhancing activity [7], anxiolytic effect [8], bronchovasodialator activity [9], nootropic activity [10], anticancer activity and antioxidant activity [11], etc.

D-galactose (also called as brain sugar) is known to elevate age levels that accelerated aging process and induced aging within 20 days and its role was confirmed as a reliable mimetic aging agent (drug) in mice [12,13]. Therefore, the antiaging and antioxidant effects of this wonder drug, (*B. monniera*) on the histological structure as well as on the histo-chemical (glycoprotein) nature of the SM glands of D-galactose induced aged female mice were studied in the present study.

**MATERIALS AND METHODS**

Ethanolic extracts of shade dried fresh leaves of *Bacopa moniera* Linn. (Brahmi) were prepared. Female albino mice of about 5-6 months age weighing about 50 gm was selected for the experimental purpose because, oestrogen levels in adult female mice protect the body from oxidative stress [14]. All the female mice selected were seen to be present in diestrus phase because, oestrogen levels are much stable during this phase and highest lipid peroxidation was found to occur with lower activity of lysosomal enzymes in diestrus. Therefore it could help to detect the protective effect of ‘Brahmi’ in animals under oxidative stress. The experimental animals were divided into four groups of five each such as Group I (control): which were injected with 0.5 ml sterile water/day behind the neck subcutaneously. Group II (D-gal): these animals received 0.5 ml of 5% D-galactose/day through the same route and vehicle for 20 days to induce quick aging. Group III (D-gal + *B. monniera* co-treated): these animals received 0.5ml D-galactose containing *Bacopa* extracts – (40 mg/kg/day) for 20 days and Group IV (D-gal followed by *B. monniera*): these animals were treated with 5% D-galactose similar to those in group II to induce quick aging. Such aging induced females were given injections of ethanolic extracts of *B. monniera* (40 mg/kg/day) for next 20 days immediately after terminations of D-gal treatment to study the recovery of aging if any. All the animals were maintained under normal conditions of light and temperature and care was taken as per the guidelines of PCSEA, Government of India.

After completion of 24 hrs of each treatment, animals were weighed and sacrificed by cervical dislocation; salivary glands were excised and fixed in 10% NBF for 20 hrs at 4°C. Thin paraffin sections (6 m) of the tissues were cut on a rotary microtome and processed further. The sections were stained with routine hematoxyline-eosin (H/E) technique for histological studies. Glycoproteins in the tissues were stained by PAS technique17 for neutral glycoproteins and by AB (pH 2.5) (Alcian Blue) for acidic glycoproteins. The microphotographs of the treated salivary glands were compared with those of the control groups.
RESULTS AND DISCUSSION

Histology

Histo-architecture of the SM glands in control mice (Plate I) showed well formed acini (AC), intercalated ducts (ID), striated ducts (SD) and granular convoluted tubules (GCT). The acinar cells were pyramidal with a basal nucleus (Nu) in each while the ductal cells were cuboidal with a centrally placed nucleus. Cytoplasm of acinar cells was loaded with secretory granules (SG) darkly stained with eosin but the cytoplasm of ductal cells was devoid of secretion granules. D-galactose treated mice revealed many degenerated and denatured secretory acini (DeSA) and ductal epithelium with distorted and displaced pyknotic nuclei (PN) and vacuolated cytoplasm (V). The AC underwent atrophy with decrease in the total volume resulting into considerable increase in the surrounding interstitium. The GCT and acini showed very poor eosin positivity but there was a considerable increase in tubular structures of the gland suggesting the role of D-galactose that had affected the overall architecture as well as the secretory nature of aged SM glands.

In the (D-gal+Bac) co-treated group of animals, the glandular architecture was retained to more or less normal, without any shrinkage of the acini and GCT; so that the gap between the acini disappeared. The nuclei were properly placed in the acinar and ductal cells. Amount of ductual structures was not found to increase with reversal of distorted picture of ductual epithelium, even though a few cells retained vacuolated cytoplasm. The SM glands of mice treated with (D-gal followed by Bacopa) for 20 days also revealed degenerative changes in majority of the acini and ducts. The acini underwent shrinkage. The intercalated striated ducts (ISD) showed considerably enlarged lumina (Lu) with degenerated epithelium and displaced pyknotic nuclei (PN). The ductual structures (DS) and interstitial spaces (IS) were considerably increased with contemporary atrophy of the salivary acini which got filled with vacuolated spongy cells (VSC) stating that the B. monniera extracts supplied to the mice after D-gal induced aging could not recover the changes that have already occurred in the SM glands of these animals.

Glycoprotein histochemistry

Histochemical nature of the SM glands of all the four groups of mice was observed after staining the sections with PAS technique (Plate II) and with AB (pH 2.5) technique (Plate III). The secretory acini in control mice showed strong PAS and AB (pH 2.5) positivity while the GCT cells, SD and ICD (intercalated ducts) showed very poor, insignificant PAS (periodic acid Schiff’s) reaction (Plate IIa) and were absolutely not stained at all by AB at pH 2.5 (Plate IIIa) in the D-gal induced aged mice. The staining nature of the acini was found to be decreased considerably showing very poor purple color to PAS reaction (Plate IIb) and moderate staining with AB at pH 2.5 (Plate IIIb). The SM glands of (D-gal+Bac) co-treated group of animals interestingly showed a significant increase in the PAS reaction (Plate IIc) to become dark magenta in color as well as in the AB at pH 2.5 (Plate IIc) to become dark blue in color. But the secretory acini in the SM glands of mice receiving (D-gal followed by Bac) showed a considerable decrease in the PAS positivity as compared to those of normal controls as well as (D-gal+ Bac) co-treated group (Plate IId). The AB (PH 2.5) reactivity could also reveal similar decrease in the cells of secretory acini which stained very poorly showing faint blue color(Plate IIIId); the remaining structure as GCT,SD and ICD as well as the interstitial connective tissue spaces showed absolutely no staining at all.

In the present study, changes were found in both parenchymal and stromal components of the gland including degenerative decrease in the acinar cell populations and cellular elements resulting into shrinkage of secretory acini; followed by corresponding increase in the ductual structures and surrounding interstitial connective tissue spaces. Similar speculative morphological changes in the SM glands of aging rats were observed [15]. Parallel atrophy of acini and GCT with a concomitant
hyperplasia of ID and significant increase in the parenchymal ductual components as well as in the stromal inflammatory infiltrate and adipose tissue were revealed by several workers [16]. Such structural implications in the age related functions of salivary glands [17] have also resulted into functional decline. Several studies observed decreased salivary flow rates in the elderly with normal aging as well as in dementia and Alzheimer’s disease [18-20] resulting into dry mouth, which is more usual in geriatrics and increased oral infections [21].

Plate I. The sections of submandibular glands of control and treated mice ×200 stained with H/E technique. a: TS of control submandibular (SM) gland showing normal vascularisation (NbV), well developed serous acini (SaA), and mucous acini (MuA), compact connective tissue (CCT) and normal intercalated ducts (Isd); b: TS of D-galactose induced aged submandibular (SM) gland showing dilated blood vessel (DbV), increased interstitium (IICT), dilated mucous acini (McA) with pycnotic nuclei (PN) and disturbed serous acini (SeA); c: TS of Bacopa treated submandibular (SM) gland along with D-galactose showing normal blood vessel (bV), normal mucous (MuA) and serous acini (SeA), with again compact connective tissue (CCT); d: TS of submandibular glands of Bacopa treatment followed by D-galactose group showing degenerated and dilated serous acini (SeA) with pycnotic nuclei (PN), normal striated duct (Sd) and intercalated ducts (ISD). The mucous acini (MuA) were not much recovered with still oedematous connective tissue in between (OCT).

Age related changes in the DNA synthesis [21,22], protein synthesis, enzyme activity [23,24] and those in the expressivity of the genes [25] were also observed in the salivary glands of aging animals which may be the primary cause of the decline in the parenchymal cell populations that ultimately have resulted into changes in cell division indices and considerable increase in the rate of apoptosis, selectively in the acinar cells of aging mice [26,27]. This may be the second important reason for the decline of acinar cell populations followed by the appearance of intracellular and intercellular spaces and vacuoles (v) as seen in the senescent SM glands of mice in present
investigation. D-gal induced aging could observe a significant decrease in the glycoprotein synthesis of the SM are secretory acini in our study which were evident by decreased PAS and AB (pH 2.5) activity in these cells. The acinar components revealed solid masses of cells (SMC) whose staining decreased considerably in aging induced mice salivary glands. Similar observations were found in normal as well as in induced aging [28] and in neoplastic cells [29]. The salivary mucins contain oligosaccharide moieties [30] which in turn consist mainly of neutral glycoproteins [31], while the protein core of the saliva include repetitive sequences rich in D-galactosylated serine and threonine containing many helix breaking proline residues and smaller proteins called hisatins [32], having antifungal properties. These glycoprotein concentrations were shown to decline with increasing age. Some of the findings showed that quantitatively glycoprotein concentrations remained age stable during normal aging but their qualitative nature is disturbed probably due to change in the synthesis of DNA, m-RNA and proteins, due to slowing down of protein turn over and metabolic defects in the pathways of macromolecular synthesis resulting into failure of homeostasis during aging [33]. This might be the root cause of decreased number and nature of glycoprotein moieties.

Plate II. The sections of submandibular glands of control and treated mice ×200 stained with PAS technique. a: TS of control submandibular (SM) gland showing normal vascularisation (Nbv), well developed unstained serous acini (SeA), and dark magenta coloured mucous acini (MuA), compact connective tissue (CCT) and normal intercalated ducts (Isd); b: TS of galactose induced aged submandibular (SM) gland showing increased interstitium (IICT), dilated and degenerated mucous acini (MuA) with pycnotic nuclei (PN) and degenerated serous acini (SeA); c: TS of *Bacopa* treated submandibular (SM) gland along with D-galactose showing normal sucous (MuA) and serous acini (SeA), with again a little compact connective tissue (CCT, intercalated striated ducts are well defined but poorly stained (Isd); d: TS of Submandibular glands of *Bacopa* treatment followed by D-galactose group showing degenerated & dilated serous acini (SeA) with pycnotic nuclei (PN), normal striated ducts (Sd) and intercalated ducts (ISD). A few mucous acini (MuA) remained still degenerated with displaced basal membrane (BM) and the connective tissue remained still oedematous (OCT).
Plate III. The sections of submandibular glands of control and treated mice ×200 stained with AB pH (2.5) technique. a: TS of control SM gland showing dark blue coloured, normal mucous acini (MuA); well formed unstained Serous acini (SeA) and normal ductular elements (SD) and (lSD) which also remained unstained with a thin luminal lining of blue color; b: TS of D-galactose stressed aged SM glands showing degenerated very poorly stained mucous acini (MuA), with increased interstitium (lICT), degenerated serous acini (SeA) and increased ductular elements which are not stained at all; c: TS of Bacopa protected SM glands along with D-galactose showing normal, mucous (MuA) and serous (SeA) acini which are more darkly stained with AB at pH (2.5), decreased intestitium and ductular elements which remained unstained except a faint blue coloured luminar lining; d: TS of SM glands given D-galactose followed by Bacopa treatment showing again degenerated SeA as well as MuA. The enhanced ductal elements like GCT, SD as well as ISD which were not stained at all. A few MuA moderately stained even though degenerated at places.

The synthesis of glycoproteins in the salivary glands is found to be governed by sex hormones [34-36]. This study shows that salivary glands are sensitive to estrogen action which may act via ER-beta in oral tissues and salivary gland secretions in healthy aged women [37]. Thus, the decreased glycoprotein contents of SM are acinar cells in the aging induced (Group II) mice might be attributed to the low estrogen levels in the experimental (D-gal) stressed mice, which might have added to the decline in the SM gland function. In conclusion, D-gal exerts oxidative stress on the salivary glands also and it has induced aging in Group II mice with degenerated acinar and ductal epithelium with pyknotic nuclei, shrunken acini and increased oedematous interstitium. B. monniera being a potent antioxidant could protect the SM glands when aging was inducing in the group III
mice. But practically there is no recovery by giving *B. monniera* after induced aging in group IV mice.

**REFERENCES**