Histological study on effect of *Nigella sativa* on aged olfactory system of female albino rat

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Abstract

*Nigella sativa* (NS) has wide-ranging healing properties, neuroprotective and antioxidant effects. Aging process is commonly associated with a decline in the chemical senses including smell. To detect a possible improvement effect of NS on the aging of the olfactory system we used 15 female albino rats that equally divided into three groups: group I (control adult), group II (control aged), group III (treated aged) received 40 mg/kg/day NS orally for two months. Specimens from the olfactory epithelium (OE), main olfactory bulb (MOB) and piriform cortex (PC) were processed for light and electron microscopy. Aging in OE revealed reduction in thickness, vacuolations, an increase in PAS reaction and lipofuscin autofluorescence. Aged MOB and PC exhibited a reduction in basophilia and accumulation of neurofibrillary tangles (NFTs) in mitral and pyramidal cells respectively. NS treatment improved the structure and the thickness of the OE and reduced the lipofuscin autofluorescence. It also attenuated the reduction in cytoplasmic basophilia and the accumulation of lipofuscin pigment and the NFTs in both mitral and pyramidal cells and the lipofuscin autofluorescence. These observations indicate that use of NS, could be of value in improving the structural changes of the peripheral and central main olfactory organs, which occurred in association with aging.

Keywords: *Nigella sativa*, antiaging, olfactory system, structure.

Introduction

There is an increasing tendency for traditional medicine in the world. Many people prefer herbal products to chemical medicines. One of the traditional medicines is *Nigella sativa* (NS), which is a member of the buttercup (Ranunculaceae) family. It has been employed for thousands of years as a spice and food preservative. In addition, it has extraordinary and wide-ranging healing properties. NS has antifungal, anti-inflammatory and antibacterial activities, and also allegedly strengthens the immune system, cleanses the body, purifies the blood, improves blood circulation, and helps us live longer [1]. Many of these activities have been attribute to its quinone constituents [2]. The chemical composition of NS is diverse and contains amino acids, proteins, carbohydrates, alkaloids, saponins, and fixed and essential oils. There are four main components in NS, which have radical scavenging property and possessed variable antioxidant activity: thymoquinone, carvacrol, *trans*-anethole and 4-terpineol [3].

The olfactory system is a special part of the nervous system that has unique structural characteristics, as the olfactory mucosa, which contains the olfactory receptor cells, is the only part of the nervous system that is present in direct contact with the external environment [4]. Studies of aging especially in the nervous system have become an important focus of study in recent years. The neuronal physiological aging process is commonly associated with a decline in the chemical senses including smell and taste. The prevalence of measured olfactory impairment among elderly people is 70% [5]. Olfactory threshold is likely to be influenced by the most peripheral part of the olfactory system, while discrimination and identification are partly cognitive tasks, which are influenced by the central olfactory structures [6]. Olfactory decline usually begins in the second decade of life [7]. This may have an impact on the safety and quality of life of old people, as olfaction help to protect us from harmful substances such as environmental contaminants and spoiled food [8, 9]. Therefore, the aim of this work was to investigate a possible protective effect of NS on the histological aging changes of the olfactory system.

Materials and Methods

Fifteen female albino rats were used and divided into three groups, each group included five rats. Group I (control adult): three months old. Group II (control aged): 20 months old. Group III (NS-treated): 18 months old, received *Nigella sativa*, given in capsules at a dose of 40 mg/kg/day [10] for two months (until the age of 20 months). The capsules were dissolved in tap water and given orally by a gastric tube. *Nigella sativa* capsules were purchased from Pharco Pharmaceuticals, Egypt. The animals were maintained in the animal house under normal day and night cycles and appropriate temperature, fed rat chow, *ad libitum* and allowed free access of water. The experiment approved by the Institutional Ethics Committee of Assiut University, Egypt. The rats were anesthetized with ether and their thorax opened to expose the heart, which was used to perfuse the appropriate fixative.

Light microscopy

Three rats from each animal group were perfused with 10% formaldehyde solution intracardiac. Specimens were taken from the olfactory epithelium (OE) lining the dorsal part of the nasal septum, the main olfactory bulb (MOB)
and the posterior piriform cortex (PC). The specimens immersed into 10% formaldehyde to continue fixation two more days. Then, specimens were processed for the preparation of paraffin blocks. Serial coronal sections (5–7 μm) were cut, mounted on glass slides, and every 10th section was stained with Hematoxylin–Eosin stain. In addition, selected sections were processed for histochemical demonstration of polysaccharides using Alcian Blue (AB), at pH 2.5, for acid mucosubstances and Periodic Acid–Schiff (PAS) method for neutral mucosubstances in OE [11]. Bielschowsky silver impregnation method was used for demonstration of neurofibrillary tangles (NFTs) in neural cells in MOB and PC [12, 13]. To evaluate the amount of autofluorescent lipofuscin, a marker of (primarily) undegraded oxidized protein, paraffin sections were examined through a fluorescent microscope with UV filter (Olympus, BXSI, Japan) and UV lamp (Olympus, U-RFL-T) [14].

Electron microscopy

Two rats from each animal group were used and perfused intracardially with 4% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 hours and postfixed in 1% osmium tetroxide in phosphate buffer for two hours. Tissues were rinsed in the same buffer, dehydrated with alcohol, cleared with propylene oxide and embedded in Epon 812. Semithin sections (500–800 Å) were cut from selected areas of the blocks and contrasted with uranyl acetate and lead citrate [16]. These sections were observed with the transmission electron microscope (Jeol E.M.-100 CX11; Japanese Electron Optic Laboratory, Tokyo, Japan) and photographed at 80 kV.

Morphometry

Using computerized-assisted image analysis, the following parameters were measured on each 10th HE-stained section. The thickness of the OE in μm was measured by the arbitrary distance method, using a 40× objective lens (on measuring the thickness, three measurements were taken along the length of the OE/field and the mean value of these measures was taken [17]. The mitral cells body area [μm²] in mitral cell layer of the MOB was measured by the arbitrary area method [18] using a 100× oil immersion lens. The number of light and dark pyramidal cells per field, in layer II of the PC, was counted by the modified touch method [19] viewed using a 100× oil immersion lens.

Statistical analysis

The morphometric data of each animal group were statistically analyzed. The ANOVA test was employed to compare the studied animal groups: p<0.05 was considered significant.

Results

Olfactory epithelium (OE)

In light microscopy, the adult rats OE was pseudo-stratified columnar epithelium. Its mean thickness is 100.47±10.11 μm, ranging from 80 to 114.1 μm (Table 1, Figures 1 and 2).

| Thickness [μm] of the olfactory epithelium in the three studied groups |
|--------------------------|------------------|-----------------|--------------------|
| Group I                  | Group II         | Group III       |
| Mean±SD                  | 100.47±10.11     | 55.46±11.46     | 70.76±11.92        |
| Range                    | 80–114.1         | 34.3–76.5       | 50.1–93.2          |

In aged rats, the OE revealed a reduction in thickness to a mean of 55.46±11.46 μm (range: 34.3 to 76.5 μm) (Table 1, Figure 2); a decrease in the cellular populations, particularly in the intermediate and basal parts and the surface cellular projections (Figure 1B). The apical third revealed supranuclear vacuolations, whereas the intermediate and basal two thirds revealed dense nuclei and wide spaces (Figure 1B). An unstained area was observed under the basement membrane and parallel to it (Figures 1B and 5C). The fila olfactoria in the lamina propria showed degenerated nerve fibers.
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In NS-treated aged rats, the OE was thicker than that of group II, ranging from 50.1 to 93.2 μm (mean 70.76 ± 11.92 μm) (Table 1, Figure 2); contained numerous cellular population especially in the intermediate part and a few vacuoles (Figure 1C). The fila olfactoria in the lamina propria exhibited compact bundles of nerve fibers.

**Figure 2** – The mean thickness [μm] of the olfactory epithelium in the three studied groups.

PAS+ reaction is observed intraepithelially in the apical and basal parts of the epithelium of adult rats (Figure 3A). Whereas a strong AB-PAS+ reactions was observed in Bowman’s glands in the lamina propria and the ducts appeared as thin PAS+ lines passing through the epithelium (Figure 3A). The basement membrane was PAS+ (Figure 3A). In aged rats, the intraepithelial PAS reaction in the apical third of the epithelium increased, while AB-PAS+ reactions in Bowman’s glands decreased (Figure 3B). A weak AB+ reaction was observed under the basement membrane. In NS-treated aged rats, the intraepithelial PAS reaction in the apical part of the epithelium was less than that in aged non treated group, while AB-PAS+ reactions in Bowman’s glands was stronger (Figure 3C).

Autofluorescence for lipofuscin was negative in the epithelium of adult rats (Figure 4A), intense in the epithelium of aged rats (Figure 4B) and faint in the epithelium of NS-treated aged rats (Figure 4C).

In ultrastructure, the adult rat olfactory receptor cells had flask shape. Their nuclei appeared rounded with peripheral heterochromatin clumps. The supranuclear cytoplasm contained numerous RER cisternae with electron dense matrix populated by ribosomes, mitochondria and multivesicular bodies (Figure 5A). The apical dendrites contained mainly numerous longitudinally arranged mitochondria (Figure 5A).

**Figure 3** – (A) Photomicrograph, group I OE, paraffin section. PAS+ reactions in basement membrane (Λ), AB-PAS+ reactions in Bowman’s gland (Bg). AB-PAS staining, ×400. Scale bar = 25 μm. (B) Photomicrograph, group II OE, paraffin section. Intrapithelial PAS+ reaction (↑), AB-PAS weak reactions in Bowman’s gland, apical vacuoles (*), wide empty space (V). AB-PAS staining, ×400. Scale bar = 25 μm. (C) Photomicrograph, group III OE, paraffin section. AB-PAS+ reaction in Bowman’s glands (Bg). AB-PAS staining, ×400. Scale bar = 25 μm.

**Figure 4** – (A) Photomicrograph, group I OE, histological autofluorescence. Negative lipofuscin autofluorescence, ×400. Scale bar = 25 μm. (B) Photomicrograph, group II OE, histological autofluorescence. Intense lipofuscin autofluorescence (A), ×400. Scale bar = 25 μm. (C) Photomicrograph, group III OE, histological autofluorescence. Faint lipofuscin autofluorescence, ×400. Scale bar = 25 μm.
In aged rats, some olfactory receptor cells had irregular shaped nuclei with peripheral clumps of heterochromatin (Figure 5B). The supranuclear cytoplasm contained numerous lipofuscin granules. The mitochondria had destroyed cristae, some dilated RER cisternae and multivesicular bodies (Figure 5B). Their apical dendrites contained mitochondria and occasional lipofuscin granules (Figure 5B).

In NS-treated aged rats, the olfactory receptor cells had ovoid nuclei with peripheral clumps of heterochromatin. The supranuclear cytoplasm contained numerous RER cisternae and lysosomes (Figure 5C). Collagen fibers were deposited in the OE under the basal lamina of the aged rats (Figure 5D).

**Main olfactory bulb (MOB)**

In light microscopy, the adult rat MOB consisted of six distinct layers, from surface inwards these were: The olfactory nerve layer (ONL), contained the olfactory nerve fibers. The glomerular layer (GML) consisted of one row of glomeruli. Glomeruli were round or oval masses of varying sizes composed of fine fibrils. Periglomerular cells were observed around the glomeruli (Figure 6A). The external plexiform layer (EPL), a thick layer consisted of fine nerve fibers and few granule cells (Figure 6A). The mitral cell layer (MCL), a thin layer contained the somata of mitral cells arranged in a row (Figure 6A). Their cell bodies were multipolar and ranged in size from 63.8 μm² to 207.4 μm² (mean 111.24 ±35.79 μm²) (Table 2, Figure 7).

| Arbitrary area [μm²] of mitral cells in the main olfactory bulb in the three studied groups |
|-----------------------------------------------|----------------|----------------|----------------|
| Arbitrary area | Group I | Group II | Group III |
| Mean±SD | 111.24±35.79 | 70.91±18.79 | 89.28±28.64 |
| Range | 63.8–207.4 | 34–107.4 | 45.3–156.3 |

They had ovoid large nuclei with prominent single deeply stained nucleoli. Their cytoplasm contained deeply stained basophilic granules (Figure 6A), very faint lipofuscin autofluorescence was observed in mitral cells (Figure 8A) and it was minimally stained by Bielschowsky silver impregnation method (Figure 9A). The internal plexiform layer (IPL), a thin layer composed of fine nerve fibers and some granule cells (Figure 6A). The granule cell layer (GCL), contained large number of granule cells arranged in clusters (Figure 6A).

In aged rats, the olfactory nerve fibers were widely separated. The glomeruli were distorted in shape with marked reduction in their size (Figure 6B). The EPL contained some misplaced cell bodies of mitral cells.
Most of the mitral cells in MCL were reduced in size ranging from 34 to 107.4 μm² (mean 70.91±18.79 μm²) (Table 2, Figure 7) and usually present in rounded form containing darkly stained nuclei with ill-defined nucleoli and acidophilic cytoplasm (Figure 6B). Autofluorescent lipofuscin in mitral cells increased compared to adult (Figure 8B). Their cytoplasm also revealed numerous positively stained neurofibrillary tangles (Figure 9B).

In NS-treated aged rats, the olfactory nerve fibers were still widely separated in some areas. There was an increase in the size of the glomeruli compared to non-treated aged but their nerve fibrils were not compactly packed (Figure 6C). The EPL also contained some misplaced cell bodies of mitral cells (Figure 6C). The mitral cells in MCL were multipolar. Their arbitrary area was increased, ranging from 45.3 to 156.3 μm² (mean 89.28±28.64 μm²) (Table 2, Figure 7). Their cytoplasm contained more deeply stained basophilic granules (Figure 6C). Autofluorescent lipofuscin in mitral cells decreased compared to non-treated aged (Figure 8C) and the neurofibrillary tangles were reduced (Figure 9C).

In ultrastructure, the adult mitral cells had euchromatic nuclei with prominent nucleoli and few peripheral clumps of heterochromatin. The cytoplasm was abundant and contained aggregates of long cisternae of RER and ribosomes formed the Nissl substance, numerous mitochondria and occasional lipofuscin granules (Figure 9A).

In aged rats, mitral cells had euchromatic nuclei with prominent nuclear membrane. The cytoplasm contained aggregates of lipofuscin granules, mitochondria, few scattered short cisternae of RER and Golgi bodies (Figure 9B).

In NS-treated aged rats, the mitral cells had euchromatic nuclei. The cytoplasm contained aggregates of long cisternae of RER, mitochondria and some lipofuscin granules (Figure 9C).
Figure 8 – (A) Photomicrograph, group I MOB, histological autofluorescence. Very faint lipofuscin autofluorescence in mitral cells, ×400. Scale bar = 25 μm. (B) Photomicrograph, group II MOB, histological autofluorescence. Intense lipofuscin autofluorescence in mitral cells, ×400. Scale bar = 25 μm. (C) Photomicrograph, group III MOB, histological autofluorescence. Moderate lipofuscin autofluorescence in mitral cells, ×400. Scale bar = 25 μm.

Figure 9 – (A) TEM, group I MOB, MCL. Mitral cell, RER, lipofuscin granule (L), ×5000. Scale bar = 2 μm. Inset: Mitral cell with minimal staining of the cytoplasm. Bielschowsky silver method, ×400. Scale bar = 25 μm. (B) TEM, group II MOB, MCL. Mitral cell, free ribosomes (Rib), lipofuscin granules (L), ×5000. Scale bar = 2 μm. Inset: Mitral cell with positively stained neurofibrillary tangles. Bielschowsky silver method, ×400. Scale bar = 25 μm. (C) TEM, group III MOB, MCL. Mitral cell, RER, lysosomes (Ly), ×5000. Scale bar = 2 μm. Inset: Mitral cell with a few neurofibrillary tangles. Bielschowsky silver method, ×400. Scale bar = 25 μm.

Piriform cortex (PC)

In light microscopy of the adult rat piriform cortex, layer II revealed numerous packed light pyramidal cells ranged from 21 to 34 (mean 27.88±4.55) (Tables 3 and 4, Figure 10).

Table 3 – Number of light pyramidal cells in the piriform cortex in the three studied groups

<table>
<thead>
<tr>
<th>Light pyramidal cells</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>27.88±4.55</td>
<td>11.3±2.31</td>
<td>10.2±3.82</td>
</tr>
<tr>
<td>Range</td>
<td>21–34</td>
<td>7–15</td>
<td>5–18</td>
</tr>
</tbody>
</table>

Table 4 – Number of dark pyramidal cells in the piriform cortex in groups II and III

<table>
<thead>
<tr>
<th>Dark pyramidal cells</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>5.3±1.64</td>
<td>4.44±1.81</td>
</tr>
<tr>
<td>Range</td>
<td>3–8</td>
<td>2–8</td>
</tr>
</tbody>
</table>

Figure 10 – The mean number of light and dark pyramidal cells in the piriform cortex in the three studied groups.
Most of them showed apical and/or basal processes (Figure 11A). They had large rounded nuclei with prominent nucleoli. Their cytoplasm contained deeply stained basophilic granules (Figure 11A). In Bielschowsky silver stained sections, the cytoplasm was minimally stained (Figure 12A).

While, in aged rats pyramidal cells in layer II were less packed and widely separated. There was a reduction in their cytoplasmatic basophilia (Figure 11B). The number of light pyramidal cells ranged from 7 to 15 (mean 11.3±2.31) (Table 3, Figure 10). Dark pyramidal cells characterized by darkly stained hardly delineated nuclei and thin long apical processes were also observed (Figure 11B). They ranged from 3 to 8 (mean 5.3±1.64) (Table 4, Figure 10). In Bielschowsky silver stained sections, the cytoplasm of pyramidal cells revealed positively stained neurofibrillary tangles (Figure 11B).

In NS-treated aged rats, pyramidal cells in layer II were less packed and widely separated. They revealed apical processes (Figure 11C). There was an increase in their cytoplasmatic basophilia compared with group II (Figure 11C). The number of light pyramidal cells ranged from 5 to 18 (mean 10.2±3.82) (Table 3, Figure 10). Dark pyramidal cells ranged from 2 to 8 (mean 4.44±1.81) were also observed (Table 4, Figure 10). In Bielschowsky silver stained sections, the cytoplasm of pyramidal cells revealed less accumulation of neurofibrillary tangles compared with group II (Figure 11C).

In ultrastructure, the adult rats’ pyramidal cells had euchromatic nuclei containing prominent nucleoli and a few peripheral heterochromatin clumps. The cytoplasm contained RER, mitochondria and occasional lipofuscin granules (Figure 12A). Bergmann astrocytes were observed closely to the pyramidal cell bodies (Figure 12A). Their nuclei were oval with thin peripheral rim of heterochromatin. Their cytoplasm contained mainly mitochondria and few scattered short cisternae of RER (Figure 12A).

In aged rats, the pyramidal cells exhibited an increase in heterochromatin associated with the nuclear membrane, numerous cytoplasm lipofuscin granules, a few scattered short cisternae of RER, Golgi bodies and mitochondria (Figure 12B). The nucleus of Bergmann astrocytes contained more peripheral heterochromatin compared to adult. Their cytoplasm contained lipofuscin granules, mitochondria and few scattered short cisternae of RER (Figure 12B).

In NS-treated aged rats, the pyramidal cells had euchromatic nuclei with no remarkable peripheral clumps of heterochromatin. The cytoplasm was abundant and contained aggregates of RER and ribosomes formed the Nissl substance, Golgi bodies, multivesicular bodies, mitochondria and occasional lipofuscin granules (Figure 12C). Bergmann astrocytes were observed closely to the pyramidal cell bodies. Their nuclei contained more heterochromatin clumps compared to adult. Their cytoplasm contained few scattered short cisternae of RER and mitochondria (Figure 12C).
Figure 12 – (A) TEM, group I PC, layer II. Pyramidal cell nucleus (N), nuclear membrane (O), astrocyte (As), ×4000. Scale bar = 2.5 μm. Inset: Pyramidal cell with minimal positive staining for neurofibrillary tangles. Bielschowsky silver method, ×1000. Scale bar = 10 μm.

Figure 12 (continued) – (B) TEM, group II PC, layer II. Pyramidal cell nucleus (N), nuclear membrane (O), lipofuscin granules (L), free ribosomes (rib), ×4000. Scale bar = 2.5 μm. Upper inset: Astrocyte (As) containing lipofuscin granules (L), ×4000. Scale bar = 2.5 μm. Lower inset: Pyramidal cell with positively stained neurofibrillary tangles. Bielschowsky silver method, ×1000. Scale bar = 10 μm. (C) TEM, group III PC, layer II. Pyramidal cell nucleus (N), nuclear membrane (O), astrocyte (As), ×4000. Scale bar = 2.5 μm. Inset: Pyramidal cell with a few positively stained neurofibrillary tangles. Bielschowsky silver method, ×1000. Scale bar = 10 μm.

5 Discussion

The olfactory pathway consists of olfactory mucosa, nerves, bulb, tract and the olfactory cortex. The OB is the first central relay in the pathway processing odor information. It receives afferent input from olfactory sensory neurons (OSNs) located in the OE and is responsible for detecting odors in the nasal cavity. OSN axons synapse on mitral/tufted cell dendrites in OB glomerular neuropils.

Degeneration of the olfactory neuroepithelium in human may occur with or without exposure to infection or toxic substances [20]. These included a reduction in surface area and an increase in susceptibility to neuronal apoptosis in the OE, and a reduction of OB size and cell number. The aging changes reported in this work in OE are similar to those reported by other investigators [18, 21]. The increase in PAS reaction, which was accompanied by a decrease in the secretory activity of Bowman’s glands, could be due to the process of respiratory metaplasia known to occur in aged OE [22]. Several changes detected in this work in aged OE have been attenuated following treatment with NS. The latter prevented the reduction in the thickness of the olfactory epithelium and its cellular populations, the vacuolations, the increase in PAS reaction in the apical parts of the OE and the reduction in the secretory activity of Bowman’s glands which occurred in aged rats.

The significant decrease in the size of mitral cells in MOB of aged group detected in the present investigation is in accordance with findings of [18], who reported changes in the size of the cell body and the nucleus of the mitral cell in OB. Bhatnagar et al. [23] reported that the mitral cell size and concentration per unit area decreased with age. Use of NS preserved the mitral cells structure, which had statistically significant higher arbitrary area, maintained the number of light pyramidal cells in olfactory cortex and reduced the number of dark pyramidal cells. These findings coincide with findings of other investigators who recorded protective effects of NSO against injury of the rat frontal cortex and brain stem after chronic toluene exposure [24] and the efficacy of NSO in improving the regeneration of the sciatic nerve injury of the rat [25].

An in vitro study stated that pretreatment with NSO has drastically improved neuronal cell viability as compared to untreated cerebellar neurons cell culture before beta-amyloid protein intoxication [26]. NSO and thymoquinone have been recognized as neuroprotective agents for hippocampal cells of rats subjected to transient cerebral ischemia via four-vessel occlusion procedure for 20 minutes, an ischemia-reperfusion model of brain insult [27]. The elevated cellular deposits of lipofuscin, a so-called “age pigment” of post-mitotic cells in aged olfactory and mitral cells is a marker of primarily undegradable oxidized protein [28, 29], in the OE and the mitral cells of the OB in aging rats. The accumulation of lipofuscin has been previously observed in aging mice in OE and in OB mitral/tufted cells [30, 31], and in normally aging Harlequin mutant mice [14], and in mitral cells, glomerular and granule cell layers of the OB in normally aging in Harlequin mutant mice [14]. The protein accumulates as a fluorophore in lipofuscin granules in the brains of aging rats [32]. These lipofuscin deposits were found to be reduced in this work in aged treated group.

Reduced basophilia with dispersion of Nissl substance, which was observed in aged mitral and pyramidal cells, is considered as a known early morphological evidence of degeneration in neurons [33]. Treatment with NS revealed a remarkable preservation of basophilia and the cisternae of rough endoplasmic reticula responsible for protein synthesis in the neuronal cells. The neurofibrillary tangles observed in aged mitral
and pyramidal cells are accumulation of pairs of filaments in the neurons that become wrapped around each other. These structures are commonly reported to occur in the brains of aged individuals, even without clinical criteria for Alzheimer disease [34]. Similarly, [35] reported that 86% of normal aged subjects have neurofibrillary tangles in the OB. NS reduced their formation in aged treated group. In support of our finding, a neuroprotective effect of NS oil and its fractions against beta amyloid (Ab)-induced cell death in primary rat cerebellar granule neurons have been reported [26].

Although the fundamental mechanisms are still poorly understood, a growing body of evidence points toward reactive oxygen species (ROS) as one of the primary determinants of aging. Oxidative stress generated by ROS is a major mediator of the progressive decline in cellular function during aging in various tissues [36]. Literature clearly substantiates the involvement of oxidative stress in the olfactory system in aging. In the aging brain, free radical-mediated oxidative stress results in the oxidation of proteins, nucleic acids, and lipids, altering the structure and function of these macromolecules [37, 38]. Subtle molecular changes appear to have a significant impact on neuronal functional decline in aging brain [39]. Oxidatively damaged macromolecules may either be repaired or removed from the cell by various cellular processes, including DNA repair mechanisms and the ubiquitin-proteasome system. Oxidative stress-associated macromolecular damage can be detrimental to the cell as a result of undesirable and potentially catastrophic consequences, including mutations in DNA and RNA, which could predispose the cell to transformation and/or result in translation of aberrant/missfolded proteins; disruption of active sites of enzymes and misfolding of structural proteins; dysfunction of the ubiquitin-proteasome mechanism of protein disposal resulting in an accumulation of oxidatively damaged proteins as aggregates; and disruption of cellular membranes due to lipid peroxidation [28, 36, 39, 40].

Cells have in-built mechanisms to sense redox imbalances and either arrest their cell cycle and undergo senescence or trigger self-destruction by programmed cell death [41]. The beneficial effect of NSO had been attributed to its cytoprotective and antioxidants action [42] and its main active component, thymoquinone have been shown to possess antioxidant activities by decreasing the oxidative stress [42–45]. NS and thymoquinone have been found to reduce cytotoxicity induced by serum-glucose deprivation in rat pheochromocytoma cells (PC12) after 6 and 18 hours. A significant increase in intracellular ROS production was seen following serum-glucose deprivation [46]. NS acts by inhibition of eicosanoid generation and membrane lipid peroxidation [27, 47].

Thymoquinone is reported to prevent oxidative injury in various in vitro and in vivo studies in rats [43, 48, 49]. Thymoquinone has been suggested to act as an antioxidant and was reported to prevent membrane lipid peroxidation in tissues [48]. Reactive oxygen species (ROS) is presumably involved in neurodegenerative disorders [50, 51]. A considerable promising approach to neuroprotection is the use of antioxidants, which suppress the effects of ROS [52].

Using proteomics, Vaishnav [14] demonstrated that olfactory aging was accompanied primarily by increased oxidative stress-, mitochondrial metabolism- and synaptic/transport-associated changes. This author showed that oxidative stress associated protein damage is a key player at the molecular level in the cellular pathology underlying olfactory aging. He hypothesized that the olfactory system accumulates oxidative stress-mediated macromolecular damage over time, predisposing it to neurodegeneration. While damaged molecules can be repaired, replaced or removed from the affected cells in most tissues, the aging brain has been shown to be particularly susceptible oxidative damage, partly due to the lack of neuronal replacement and a decline in proteasomal activity in aging [28, 32, 39]. Protein, DNA/RNA and lipid oxidation and consequent cellular damage can occur due to elevated oxidative stress.

Conclusions

Use of Nigella sativa could be a valuable role in improving the structural changes of the peripheral and central main olfactory organs, which occurs in association with aging and results in deficit in the sense of smell.

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References

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