NEURONAL CELL COUNT AND MORPHOMETRIC STUDY OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS AFTER CHRONIC NIGELLA SATIVA ADMINISTRATION

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Abstract

Nigella sativa, a commonly consumed natural supplement, is known for its antioxidant and neuroprotective effects. Following the previous studies which showed its positive effects on spatial memory performance, the current study focused on the neurohistological effect of Nigella sativa on hippocampal cornu ammonis 1 (CA1) pyramidal neurons. The hippocampus is important for memory formation; thus, it is essential to elucidate the characteristics of its constituent neurons. Male Sprague Dawley rats were chronically force-fed for five days a week for 20 consecutive weeks (100 days) with Nigella sativa oil (NSO) (6.0 μl/100 g body weight) and corn oil (CO) (0.1 ml/100 g body weight) as control. Dorsal hippocampal transverse sections (10 μm thick) from both groups were visualized under the light microscope and morphometric analysis was conducted using the Image-Pro Premier 9.1 64-bit software. Only cresyl-violet stained neuronal somas with each having whole visible nucleus and nucleolus were included in the morphometric analysis. Mean number of neuronal cell count in NSO group was found to be higher than the controls. Significant differences in somatic area/SA, somatic perimeter/SP, somatic aspect ratio/SAR, somatic circularity index/SCI, and somatic roundness/SRo were observed between the groups. Values of two parameters (SA and SP) of NSO group indicated significantly larger sized CA1 neurons, whereas values of the other three parameters (SAR, SCI and SRo) significantly indicated less round in shape CA1 neurons. These distinct effects on the morphological characteristics could be due to NSO various biochemical constituents.

Keywords: Morphometric, hippocampal neurons, Nigella sativa.

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INTRODUCTION

Nigella sativa is a dicotyledonous herbaceous plant belonging to the botanical family of Ranunculaceae. It is often found in the Mediterranean countries, Western Asia, Middle East and Eastern Europe. The plant produces fruits filled with capsules consisting of 3-7 follicles, where numerous black seeds are located [1]. It is also known as black cumin or Al-Habba Al-Sauda or Al-Habba Al-Baraka in Arabic [2].

In recent years N. sativa, which is commonly consumed as a natural supplement worldwide, has been extensively studied for its therapeutic effects in prevention and treatment of human diseases. Apart from being a well-known antioxidant, it has also been reported to have anti-cancer, anti-inflammatory, and immunomodulatory [1, 2]
properties, in addition to demonstrating neuroprotective properties such as preventing memory deficits [3, 4]. Chemical compositions of *N. sativa* that possibly allow it to possess all the healing properties mentioned include thymoquinone (TQ), thymohydroquinone, dithymoquinone, p-cymene, carvacrol, and 4-terpenol [5]. As a follow up to previous studies which showed its positive effects on spatial memory performance [6], the current study focused on its neurohistological effect on cornu ammonis 1 (CA1) pyramidal neurons of the hippocampus.

**METHODS AND MATERIALS**

A total of twelve male *Sprague Dawley* rats were used in this study; randomly allotted into two treatment groups (six rats per group): (a) *N. sativa* oil (NSO) (6.0 μl/100 g body weight), and (b) Corn oil (CO) (0.1 ml/100 g body weight) as control. They were chronically force-fed for five days a week for 20 consecutive weeks (100 days). All animal experiments were conducted in accordance with the guidelines as stipulated by the Institutional Animal Care and Use Committee (IACUC), University of Malaya [ISB/20/04/2012/DSHA (R)]. Rats were deeply anaesthetized before intracardially perfused. Brain tissues were harvested and fixed in 10% formalin. Once fixed, the tissues were processed, and finally embedded in paraffin, cut into 10 μm-thick sections, and Nissl’s stained with cresyl violet dye. Six dorsal hippocampal transverse sections of the brain hemispheres were randomly sampled for neuronal cell count and morphometric analysis. The slides were visualized under the light microscope (Olympus BX51) and images were captured using analysis software (Analyzer Life Science Software) (Figure 1). Morphometric analysis was then conducted using the Image-Pro Premier 9.1 64-bit software. Five morphological variables selected for this study were in accordance to the quantitative criteria outlined by Tsiola *et al.* [7]: (a) Somatic area (μm²): the area inside the soma; (b) Somatic perimeter (μm): the length of the soma’s boundary; (c) Somatic aspect ratio: the ratio between the major axis of the soma to the minor perpendicular axis; (d) Somatic circularity index, (4×π×area)/(perimeter)²: the circularity of the soma; (e) Somatic roundness, (4×area)/(π× maximum axis)²: the circularity of the soma but more specific; could meticulously distinguish differences in the cell outlines. Only stained neuronal somas with each having whole visible nucleus and nucleolus were considered (Figure 2). Statistical analyses for neuronal cell count and morphometric study were done using ANOVA test and two-tailed independent sample t-test, respectively. The results obtained were presented as mean±standard error (SE) and differences between groups were considered significant when p<0.05.
RESULTS AND DISCUSSIONS

Hippocampal CA1 neuronal cell count

Although no significant effect of the hippocampal CA1 neuronal cell count was found between the treatments (Table 1), there was slightly more pyramidal neurons in NSO group (87.08±1.71) compared to CO group (83.61±3.56). The results demonstrated that NSO had a tendency to increase the neuronal number instead of having a toxic effect which could cause a reduction in the neuronal population.

Table 1: Hippocampal CA1 neuronal cell count

<table>
<thead>
<tr>
<th>Group</th>
<th>Neuronal Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSO</td>
<td>87.08±1.71</td>
</tr>
<tr>
<td>CO</td>
<td>83.61±3.56</td>
</tr>
</tbody>
</table>

Morphometric analysis of CA1 pyramidal neurons

There were significant effects of NSO treatment on hippocampal CA1 neuronal morphology compared to CO treatment (Table 2, Figure 3).
Table 2: Measurements of the morphological size and shape of CA1 pyramidal neurons

<table>
<thead>
<tr>
<th>Group</th>
<th>Somatic Parameters</th>
<th>Shape Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Somatic Area/SA ((\mu m^2))</td>
<td>Somatic Perimeter/SP ((\mu m))</td>
</tr>
<tr>
<td>NSO</td>
<td>76.98±23.25</td>
<td>32.05±4.72</td>
</tr>
<tr>
<td>CO</td>
<td>75.76±19.39</td>
<td>31.55±4.05</td>
</tr>
</tbody>
</table>

Size as a neuronal somatic feature could be analysed based on somatic area (SA) and somatic perimeter (SP) [8, 9]. For both parameters, neurons in NSO group exhibited significantly larger values compared to neurons in CO group. SA of neurons in NSO group was 76.98±23.25 \(\mu m^2\) while it was 75.76±19.39 \(\mu m^2\) for CO group. SP of NSO group was 32.05±4.72 \(\mu m\) whereas it was 31.55±4.05 \(\mu m\) for CO group. A study on neurodegeneration of rats’ hippocampal neurons chronically exposed to toluene also showed morphological improvements with NSO treatment [10]. Since larger somas might indicate greater cellular information integration [11]; consequently, an increased amount of information processing by CA1 pyramidal cells could result in increased generation of hippocampal dependent memories [12, 13].

Morphological shape of cell bodies were analysed based on somatic aspect ratio (SAR), somatic circularity index (SCI), and somatic roundness (SRo). NSO group exhibited significantly higher SAR (1.35±0.28) than CO group (1.32±0.25), indicating shape of CA1 neuronal somas that were not so rounded [14]. Likewise, SCI and SRo emphasize on the roundness of the soma, with a value further from 1.00 indicates a less circular soma. However, value for SRo could distinguish pyramidal soma from round ones, while SCI only distinguishes circular-shaped neurons from irregular shaped ones [7]. The results demonstrated lesser roundness of NSO group, hence, possibly reflecting better retention of pyramidal shape of CA1 neuronal somas. The group showed a significantly lower SCI (0.70±0.12), further away from the value 1.00 compared to CO group (0.72±0.11). In comparison to the value of SRo of CO group (1.10±0.09), the value for NSO group (1.13±0.11) was significantly higher and further away from 1.00. Hence, hippocampal CA1 neurons in NSO group retained their pyramidal soma shape better than those in CO group. Therefore, findings from the selected measurements of the CA1 neuronal soma morphological characteristics indicated potential neuroprotective effect of NSO.

The antioxidant properties of NSO could most likely be the explanation for the observed pyramidal neuronal count, and also the significant development of the somatic size and somatic shape of the hippocampal CA1 neurons. Other findings by Alhebshi et al. [15] and Burits & Bucar [16] elucidated the potential of TQ in inducing conformational changes and altering the dynamics of protein, thus, preventing degeneration and most importantly, protecting the CA1 hippocampal neurons. These findings are in agreement with previous studies, which proved that NSO facilitated spatial memory [6, 17].
Figure 3: Morphological features of hippocampal CA1 pyramidal neurons (arrows): (a) NSO group, and (b) CO group. (200× magnification, 50 μm calibration)

CONCLUSION

Altogether these findings suggested that specific effects of the treatment on the morphological characteristics of hippocampal CA1 neurons were possibly due to various NSO biochemical constituents and their distinct neurological effects. Further studies are required to understand the specific role of N. sativa and to determine whether NSO specific constituents directly affect morphological characteristics of hippocampal neurons.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

DISCLOSURE

The authors report no conflicts of interest in this work.

REFERENCES


